Th1/Th2 imbalance in schizophrenia:

An immunological exploration of etiology

Sonnig Sue Whei Chiang

Th1/Th2 imbalance in schizophrenia: An immunological exploration of etiology

Dissertation
Zum Erwerb des Doktorgrades der Humanbiologie
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

vorgelegt von Sonnig Sue Whei Chiang
aus Taichung Hsien, TAIWAN
2005

Contents

Introduction

Empirical part

Introduction

Schizophrenia is a chronic recurring illness and the most common psychotic disorder. The prevalence of schizophrenia is consistently about 1% throughout the world (Schultz and Andreasen, 1999). It characteristically begins in late adolescence or young adulthood (Carpenter, Jr. and Buchanan, 1994; Heaton et al., 1994) and lasts a lifetime with only occasional recovery (Braff et al., 1991). In most cases, schizophrenia first appears in men during their late teens or early 20s, while female schizophrenics often first onset during their late 20s or early 30s. The initial years of illness are often the most symptomatic and include severe psychosocial deterioration. Middle-aged years are more benign; in the elderly, symptom recovery has been described. Most commonly, people who have schizophrenia are unable to continue in employment or education.

Up to date, how schizophrenic symptoms arise remains unclear. After over century extensive research, still no agreement regarding the pathophysiology of schizophrenia has been reached among experts. Substantial contributions from multiple genes with small or moderate effects have been implicated. Nevertheless, various designs of genetic studies also indicate the essential role of environmental factors because the concordance rate for the monozygotic twins who grew up in a very similar surrounding is far less than 100% (Prescott and Gottesman, 1993). Epidemiological data further suggest a linkage between viral infection as an environmental factor and schizophrenia (Koponen et al., 2004; Limosin et al., 2003; Brown and Susser, 2002). Moreover, positive antibody titers against diverse viruses were detected in one part of schizophrenics (Dickerson et al., 2003; Chen et al., 1999; Yamaguchi et al., 1999; Waltrip et al., 1997; Adams et al., 1993; Barr et al., 1990; Pelonero et al., 1990; Bartova et al., 1987). Eliminating intra- and extra-cellular pathogens to defend the host is the responsibility of the immune system. Diverse immune dysfunctions have been reported in schizophrenia for over 3 decades (Muller et al., 2004). Immune aberrations possibly raised by viral infections during the pre-, peri- or postnatal phase have been thus described in schizophrenia (Munn, 2000). In various viral infections such as influenza virus, measles virus, rubella, herpes simplex virus 2 (HSV2), and Epstein-Barr virus (EBV), a shift towards the T helper type 2 system was indicated (Dhiman et al., 2004; Nakayama et al., 2004; Akaboshi et al., 2001; Oh and Eichelberger, 2000; Nakajima et al., 2000). Recently, Müller et al. and Schwarz et al. likewise suggested Th2 as a possible patho-mechanism in at least one subgroup

of schizophrenia (Schwarz et al., 2001a; Schwarz et al., 2001b; Muller et al., 2000). Direct empirical evidence evaluating the Th1/Th2 ratio in schizophrenia is, however, lacking.

Hence, this study attempted to explore Th2-shift in schizophrenia. This report contains mainly an introduction and an empirical section. The **introduction** is further divided into (1) Schizophrenia, (2) Th1/Th2 systems, and (3) Th1/Th2 cytokines in schizophrenia. The **empirical section** largely consists of the subsequent issues: (4) Questions and hypothesis, (5) Methods, (6) Results, and (7) Conclusion and discussion.

1 Schizophrenia

Schizophrenia has been deemed as a disorder in the central nervous system (CNS). Thus, distinct hypotheses or researches attempting to elucidate the biological etiology of schizophrenia assumed genetic- and/or environment-induced abnormalities in diverse neurotransmitter(s) within certain brain structure(s)/region(s). The most famous hypothesis of schizophrenia is the dopamine hypothesis. Originally, it postulates that the symptoms of schizophrenia are related to hyperactivity of central dopaminergic system (Meltzer and Stahl, 1976).

Schizophrenia includes a wide spectrum of "exophenotypes". The defining features of schizophrenia can be divided into three main categories: psychotic or positive symptoms, deficit or negative symptoms, and cognitive impairment (Kelly and Murray, 2000). Positive symptoms can be classified into three main groups, that is, hallucinations, delusions, and thought disorder/formal thought disorder. Formal thought disorder is described as a disturbance rather in the form of thinking than abnormality of content as occurred in hallucinations and delusions. The negative symptoms consist of severe disturbances in social interaction, motivation, expression of affect, ability to experience pleasure, and spontaneous speech (Rey et al., 1994; Schmand et al., 1994). Cognitive deficits in schizophrenia affect executive function, attention, memory, and general intellectual functioning (Weickert et al., 2000). The negative and cognitive symptoms are more persistent and chronic, whereas the positive symptoms have an episodic pattern. If the positive symptoms are active, then hospitalization is usually necessary (Andreasen, 1995). Negative symptoms are the least likely to improve over the course of illness and usually result in cognitive dysfunction; they contribute more strongly to overall psychosocial disability than do residual positive symptoms (Green, 1996). Both negative and cognitive symptoms together are referred to as prodromal symptoms; prodromal symptoms often precede the positive symptoms (Cornblatt et al., 1999).

To date exist two well-accepted diagnostic systems to facilitate an approximation of the entity of the disease. That is, the International Classification of Diseases, tenth edition (ICD-10) (World Health Organization, 1994) and the Diagnostic & Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (American Psychiatric Association, 1994). These two systems are criterion-based. Both ICD-10 & DSM-IV describe characteristic symptoms of

schizophrenia; they include delusions, hallucinations, disorganized speech (e.g., frequent derailment or incoherence), grossly disorganized or catatonic behavior, and negative symptoms (i.e., affective flattening, alogia, or avolition). In ICD-10, severe symptoms should have been present for at least 1 month, whereas in DSM-IV, a minimum of 6 months' duration is required (i.e., including less severe prodromal and residual symptoms). The DSM-IV criteria also require deterioration in social and/or occupational functioning, specified as dysfunction in work, interpersonal relations, or self-care. If during adolescence, then failure to reach level of interpersonal, academic, or occupational achievement is required.

According to DSM-IV, schizophrenia is divided into paranoid, catatonic, disorganized, undifferentiated, and residual schizophrenia (American Psychiatric Association, 1994). Nevertheless, apart from the 5 subgroups of DSM-IV, further subgroups such as hebephrenic, post-schizophrenic depression, simple, other, and unspecified schizophrenia are also described in ICD-10 (World Health Organization, 1994). Paranoid schizophrenia is characterized by a preoccupation with one or more delusions or frequent auditory hallucinations. For catatonic schizophrenia, presence of the following features is required, that is, psychomotor disturbances, such as stupor (lack of a motor response to stimulus), negativism, excessive motor activity, an absence of speech (alogia), peculiar movements, and repetitions of words and phrases (echolalia) or another's movements (echopraxia). Undifferentiated schizophrenia is defined as presence of schizophrenic symptoms without meeting any criteria for paranoid, disorganized, and catatonic schizophrenia. Residual schizophrenia is predominated by absence of prominent delusions, hallucinations, disorganized speech, as well as grossly disorganized and catatonic behavior despite continuing evidence of a disturbance. Hebephrenia is characterized by hallucinations, delusion, severe disintegration of personality including erratic speech, childish mannerisms, senseless laughter as well as bizarre, foolish, and regressive behavior; it usually becomes evident during puberty. Nowadays, hebephrenia is renamed as disorganized schizophrenia. The main features of disorganized schizophrenia contain disorganized speech and behavior as well as a flat or inappropriate affect. Simple schizophrenia is characterized by withdrawal, apathy, indifference, and impoverishment of human relationships without overt psychotic features. Post-schizophrenic depression is described as predominance by depressive symptoms and meeting the criteria of depression for at least 2 weeks as well as simultaneous presence of some schizophrenic symptoms with a schizophrenic history in the past one year.

Various subgroups stated above are classified according to preponderance of certain symptom complexes. They represent rather distinct symptom complexes than separable entities. They often merge together during the course of disease (Bondy, 2002).

2 Th1/Th2 systems

2.1 Innate and adaptive immunity

The innate and adaptive immunity are the basic functional units of the immune system which are involved in host defense and removing pathogens in viral infections (Rempel et al., 2004). Innate (natural/congenital/aspecific) immunity refers to antigen-nonspecific defense mechanisms. A host uses nonspecific mechanism *immediately or within several hours* after exposure to an antigen (Akpek and Gottsch, 2003). Innate immunity only recognizes a few highly conserved pathogen-associated molecular patterns present in many different microorganisms (Delclaux and Azoulay, 2003). In contrast, adaptive (acquired/specific) immunity refers to antigen-specific defense mechanisms. The antigen-specific mechanisms take *several days* to become protective and are designed to remove a specific antigen (Akpek and Gottsch, 2003). The adaptive immune system has the hallmarks of learning, adaptability, and memory. It possesses two main features which are absent in the innate immune system: (a) clonal selection and expansion of cells expressing antigen-specific receptors and (b) challenge memory facilitating faster and more rigorous responses to previously encountered antigens (Fabbri et al., 2003; Stenzel-Poore et al., 1988).

The adaptive immunity can be further subdivided into 2 branches – humoral and cell-mediated immunity (CMI)/cellular immunity. They are very important for proper immune responses (Woodland, 2003). Humoral immunity involves the production of antibody molecules in response to an antigen and is mediated by B-lymphocytes. Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigen; it is mediated by T-lymphocytes (Fabbri et al., 2003).

2.2 Th1/Th2 systems

T lymphocytes are the most important cells in coordinating the immune response and are a major source of cytokines. Cytokines participate in various aspects of adaptive and innate immunity (Fabbri et al., 2003; Stenzel-Poore et al., 1988). T cells have several functions executed by different subpopulations such as T helper (Th), T suppressor, and cytotoxic T

cells. Th cells direct other cells such as B cells or macrophages to carry out their tasks. Upon activation, naive CD4+ T helper cells differentiate into at least two distinct subpopulations, Th1 and Th2. Th1- and Th2-system which are originally defined on the basis of their cytokine profiles and effector functions, are effective against intracellular and extra-cellular pathogens, respectively (Mosmann and Coffman, 1989). The balance between both Th-subsets is thought to be pivotal in determining the outcome of an immune response towards an infectious organism (Breytenbach et al., 2001) and is therefore critical for host defense and the pathogenesis of immune-mediated diseases (Agnello et al., 2003; McGuirk and Mills, 2002).

Th1 cells mainly produce IFN-γ, IL-2, TNF-α, and IL-12, while Th2 lymphocytes predominantly release IL-4, IL-13, IL-10, and IL-6. However, both TNF-α and IL-10 can be secreted by Th1 and Th2 cells (Romagnani, 1999; Katsikis et al., 1995). The development of Th1 and Th2 cells from a common undifferentiated precursor is regulated at many levels (Agnello et al., 2003). Some examples for those are interactions of peptide antigen with the T cell receptor (TCR), cytokine signaling, actions of co-stimulatory molecules, induction of transcription factors, and antigen dose (Agnello et al., 2003; Rothoeft et al., 2003; Farrar et al., 2002; Ben Sasson et al., 2001; Murphy et al., 2000; Ausubel et al., 1997; Carballido et al., 1997; Kuchroo et al., 1995; Prabhu Das et al., 1995). IL-4 activates the Janus kinase 1 (JAK1) and JAK3, leading to activation of the signal transducer and activator of transcription 6 (STAT6), whereas IL-12 activates JAK1 and TYK2 conducting to STAT4 activation (Santana and Rosenstein, 2003). STAT6 and STAT4 are essential for the development of Th1 and Th2, correspondingly (Anderson et al., 2003). IL-4 drives the development of the Th2-system. IL-12 is in most cases not necessary for maintaining Th1 responses once Th1 responses are induced (Gazzinelli et al., 1994). Instead, IFN-γR signaling is required for Th1 further differentiation (Tau et al., 2000). Binding of IFN-γ to its receptor IFN-γR activates JAK1 and JAK2, leading to phosphrylation of STAT1 (Bach et al., 1997). In addition to STAT4 and STAT6, there are some other transcription factors specific for Th1/Th2 systems. They are GATA3 and c-Maf in Th2 cells as well as ERM and T-bet in Th1 cells (Murphy et al., 2000). IFN-γ and IL-4 were characterized as the key cytokines of the Th1 and Th2 system due to their roles in the differentiation and development of the Th1/Th2 system. The ratio between both major cytokines is thought to implicate the balance between both Th1/Th2 systems (Giannakoulas et al., 2004; Li et al., 2003; Sakami et al., 2002). Newly, the IFN-γ/IL-10 ratio is likewise regarded as an indicator of Th1/Th2 balance in various viral infections ((Avery and Hoover, 2004; McElhaney et al., 2004; Zhang et al., 2000)).

The Th1 system induces cell-mediated immune responses and is associated with inflammation and tissue destruction that leads to organ-specific autoimmune diseases (Chen et al., 2000; Golding and Scott, 1995). On the contrary, the Th2 system promotes humoral immune responses, allergic reactions to environmental antigens as well as anti-inflammatory activities (Chen et al., 2000; Mosmann and Sad, 1996). Activation of Th2 cells may inhibit the central nervous system (CNS) inflammation and limit the noxious effects of Th1-mediated immunity (Chen et al., 2000; Racke et al., 1994).

2.2.1　Th1 cytokines and their principal biological functions

2.2.1.1　IFN-γ: the major Th1 cytokine

IFN-γ is a *40-50 kDa* homodimer with 146 amino acids. The human IFN-γ gene contains four exons and maps to chromosome *12q24.1*(Naylor et al., 1983). IFN-γ is produced by Th1 cells, natural killer (NK) cells (Trinchieri, 1995), NK T cells, CD8+ T cells, T cells expression γδ T-cell receptors (TCRs) (Almanzar et al., 2004; Mocchegiani et al., 2004; Lertmemongkolchai et al., 2001), macrophages/monocytes, dendritic cells (DCs), and B cells (Della et al., 2004; Durali et al., 2003; Airoldi et al., 2000; Ohteki et al., 1999). The IFN-γ receptor (IFN-γR = CD119) is composed of a ligand binding α chain and a signaling β chain (Bach et al., 1997). IFN-γR signaling is required for Th1 differentiation (Tau et al., 2000). IFN-γR are expressed on all types of human cells except mature erythrocytes. The IFN-γR gene maps to human chromosome 6 (Pfizenmaier et al., 1988). Signaling through IFN-γR utilized the JAK/STAT pathway. Binding of IFN-γ to its receptor induces receptor dimerization and activation of JAK1 and JAK2 that recruit and phosphorylate STAT1 (Bach et al., 1997).

IFN-γ & CNS

In the CNS, IFN-γ is mainly responsible for the activation of microglial cells, induction and up-regulation of the major histocompatibility complex II (MHC-II) antigens in all three types of glial cells including astrocytes, oligodendrocytes as well as microglial cells (Neumann et al., 1996; Colton et al., 1992; Satoh et al., 1991; Vidovic et al., 1990). In addition, IFN-γ also exerts several impacts on astrocytes: induction of co-stimulatory molecules, conversion of astrocytes into effective antigen-presenting-cell (APC), promotion of inducible nitric oxide synthase (iNOS) transcription, elevation of nitric oxide (NO) production, induction of TNF-α

mRNA and protein (Dell'Albani et al., 2001; Nikcevich et al., 1997; Neumann et al., 1996; Hewett et al., 1993; Lee et al., 1993; Colton et al., 1992; Satoh et al., 1991; Vidovic et al., 1990; Chung and Benveniste, 1990; Pfizenmaier et al., 1988). All neurons seem to express IFN-γRα and exposure to IFN-γ induces cell surface expression of MHC-I protein (Neumann et al., 1997). IFN-γ appears to play a potential role in neuronal development since it promotes neurite outgrowth and matures neurofilament protein expression which are usually associated with neuronal differentiation on cultured hippocampal and cortical neuroblasts (Barish et al., 1991).

IFN-γ & PS

IFN-γ has antiviral and antiparasitic activities. However, the main biological activity of IFN-γ appears to be immunomodulatory. In contrast, the other IFNs are mainly antiviral (Schroder et al., 2004). IFN-γ stimulates the expression of CD4 in T-helper cells and is a modulator of T-cell growth and functional differentiation (Boehm et al., 1997). IFN-γ inhibits the growth of B-cells induced by IL4 and the production of IgG1 and IgE elicited by IL4 in bacterial lipopolysaccharides (LPS) stimulated B-cells (Collins and Dunnick, 1993; Snapper and Paul, 1987). IFN-γ regulates the expression of MHC-II genes in cell such as B cells, DCs, and professional APCs and is the only IFN that stimulates the expression of these proteins (Mach, 2002). In macrophages, IFN-γ stimulates the release of reactive oxygen species (Widner et al., 2000) and up-regulates IL-1, IL-1RA, IL-6, IL-8, IL-10, IL-12, TNF-α, IFN-α, IFN-γ, monocyte chemoattractant protein 1 (MCP-1), MCP-3, macrophage migration inhibitory factor (MIF), macrophage colony stimulating factor (M-CSF), granulocyte-CSF (G-CSF), granulocyte macrophage-CSF (GM-CSF), macrophage inflammatory protein 1 (MIP-1), MIP-2, leukocyte inhibitory factor (LIF), oncostatin M (OSM), and TGF-β (Cavaillon, 1994). Furthermore, IFN-γ also regulates a number of genes which contain IFN-stimulated response element (ISRE) or IFN response sequence (IRS) within their promoter regions; several of those genes are themselves components of transcription factors (Boehm et al., 1997).

2.2.1.2 *IL-2: T cell growth factor*

IL-2 was first described as "T-cell growth factor"; it is a protein of 133 amino acids with a molecular weight of *15 kDa* (Malek, 2003). The main secretory source of IL-2 is the T-helper cell, particularly naïve T cells and Th1 cells. The human IL-2 gene contains four exons and maps to human chromosome *4q26-28* (Sykora et al., 1984).

IL-2R

Three different types of IL-2Rs with high, intermediate, and low affinity are distinguished. They are expressed differentially and independently. The high-affinity IL-2R consists of subunits IL-2Rα (p55), IL-2Rβ (p75), and a γ chain (64 kDa). The intermediate-affinity IL-2R comprises IL-2Rβ and γ chain, while the low-affinity IL-2R contains solely IL-2Rα. IL-2Rα functions as a T-cell activation (TAC) antigen, IL-2Rβ as the ligand binding domains, and γ chain as a signaling component. The γ-subunit is required for the generation of high and intermediate affinity IL-2R, but does not bind IL-2 by itself (Minami et al., 1993). The genes encoding these three subunits map to human chromosome 10p14-15, 22q11.2-12, and Xq13, respectively. Besides, activated lymphocytes continuously secrete a *42 kDa/55 kDa* fragment of the TAC antigen, a soluble IL-2 receptor (sIL-2R), which circulates in the serum and plasma (Miska and Mahmoud, 1993; Pizzolo et al., 1992). Brain IL-2Rs are enriched in the hippocampal formation, an area critical for the acquisition and consolidation of spatial learning and memory (Petitto et al., 1999).

Producing cells of IL-2

IL-2 is produced mainly by activation of CD4+ T-cells (de Waal et al., 1993c; Ferrer et al., 1992). Resting cells do not produce IL-2. There are detectable levels of IL-2-like material in the hippocampus, striatum, and frontal cortex; however, specific IL-2 binding sites were observed only in the hippocampus (de Waal et al., 1993c; Ferrer et al., 1992; Araujo et al., 1989).

Biological activities

IL-2 is a growth factor for all subpopulations of T-lymphocytes (Abbas, 2003). It is an antigen-unspecific proliferation factor for T-cells that induces cell cycle progression in resting cells and thus allows clonal expansion of activated T-lymphocytes (Malek, 2003). This effect is modulated by hormones such as prolactin (Moreno et al., 1998). In addition, IL-2 mediates multiple biological processes including growth and differentiation of B cells, generation of lymphokine-activated killer cells, augmentation of NK cells (Wustrow, 1991), In the CNS, IL-2 stimulates the growth of oligodendroglial cells in vitro (Benveniste and Merrill, 1986), modulates N-methyl-D-aspartate receptors (NMDA-R) of native mesolimbic neurons (Ye et al., 2001), and influences mesocorticolimbic dopamine release (Ye et al., 2001). IL-2 damages the blood-brain-barrier (BBB) and the integrity of the endothelium of brain vessel (Ellison et al., 1987); however, it does not cross the BBB via a saturable transport system (Waguespack et al., 1994)

2.2.1.3   IL-12: guide of Th1 development

15

IL-12 is a heterodimeric *70 kDa* glycoprotein (IL-12p70) consisting of a 40 kDa subunit (IL-12β or IL-12p40, 306 amino acids) and a 35 kDa subunit (IL-12α or IL-12p35, 197 amino acids) (Liu et al., 2003; Li et al., 1996b). The two genes encoding IL-12p40 and IL-12p35 are unrelated and located on separate chromosomes (*5q31-33* and *3p12-13.2*) in humans; their expressions are regulated independently of each other (Sieburth et al., 1992). IL-12 is primarily produced by (1) phagocytic cells and (2) antigen-presenting cells (APC) such as monocytes, dendrite cells (DC), activated B lymphocytes, and to a lesser extent by (3) T-cells (Schultze et al., 1999; Heufler et al., 1996; Trinchieri, 1995).

IL-12R

The IL-12 receptor (CD212), about 110 kDa, is composed of IL-12Rβ1 and IL-12Rβ2 chains (Presky et al., 1996b). Binding of IL-12R activates the JAK-STAT pathway of signal transduction. Signaling transduction through IL-12R induces tyrosine phosphorylation, primarily of the Janus family kinases JAK2 and TYK2, which, in turn, phosphorylate and activate STAT1, STAT3, STAT4, and STAT5 (Presky et al., 1996a). STAT4 is particularly crucial for Th1 responses (Murphy et al., 1999; Kaplan and Grusby, 1998; Thierfelder et al., 1996). IL-12R is expressed mainly by activated CD4+ T cells, CD8+ T cells, and CD56+ NK cells, but also on other cell types such as (APC) DCs, B-cell lines, and certain subsets of T cell (Airoldi et al., 2000; Grohmann et al., 1999; Presky et al., 1996b). Activation of T cells through TCR up-regulates the transcription and expression of both chains of IL-12R; its up-regulation, in particular that of IL-12Rβ1 (19p13.1) (Yamamoto et al., 1997), is enhanced by IL-12 itself, IFN-α, IFN-γ, TNF, and co-stimulation through CD28. In T cells, the expression of IL-12Rβ2 (1p31.2-31.3) (Morton et al., 1997) is confined to Th1 cells and correlates with responsiveness to IL-12 (Rogge et al., 1997; Szabo et al., 1997).

Biological activities of IL-12

IL-12 has effects on both innate and adaptive immune systems (Stern et al., 1996). On the innate immune system, IL-12 induces IFN-γ (Sugimoto et al., 2003). However, repeated administrations of IL-12 are associated with persistently elevated plasma levels of IL-10 and declining IFN-γ, TNF-α, IL-6, and IL-8 responses (Portielje et al., 2003). IFN-γ operates in a positive feedback mechanism since IFN-γ in turn stimulates IL-12 synthesis by phagocytic cells (Cassatella et al., 1995). In addition, IL-12 activates NK cells, promotes NK cell cytotoxicity, cytokine production, in particular high levels of IFN-γ, and mediates NK cell chemotaxis (Aste-Amezaga et al., 1994; Naume et al., 1992).

On the adaptive immune system, IL-12 has impacts on both cellular immune responses and humoral immune responses. IL-12 is an important co-stimulus for proliferation and further activation of fully differentiated Th1 cells and IFN-γ secretion (Germann et al., 1993). However, animal models have revealed that IL-12 is not an absolute requirement for Th1 differentiation or IFN-γ production (Magram et al., 1996). On humoral immune response, a two-step model of humoral enhancement of IL-12 was proposed (Metzger et al., 1996). IL-12-induced IFN-γ by Th1 and NK cells mediates early switching of B cells towards IgG2 with temporal suppression of IgG1 production. Afterwards, IL-12 stimulates the switched B cells to secret more antibody, regardless of their isotypes (Metzger et al., 1996).

2.2.1.4 *TNF-α: crucial neuro-endo-immunological mediator*

Tumor necrosis factor-alpha (TNF-α), also called cachectin, is *17 kDa* and has a length of 157 amino acids (Perez et al., 1990). The gene encoding for TNF-α is found within the MHC on chromosome 6 (Schwab et al., 2003).

TNF producers

TNF-α is widely expressed in several cell types of the immune system (including B cells, T cells, basophils, eosinophils, DC, NK, neutrophils, and mast cells), microglia, and astrocytes (Gould et al., 2004; Nadeau and Rivest, 2000; Aggarwal, 1992).

TNF-R

There are two types of TNF receptors: TNF receptor type I (TNF-RI = 55 kDa = CD120a) and type II (TNF-RII = 75 kDa = CD120b) (Bazzoni and Beutler, 1996; Tartaglia et al., 1991). TNF-Rs are widely distributed throughout most cells and tissues, including the brain (microglia, astrocytes, and oligodendrocytes) (Wilt et al., 1995; Tada et al., 1994; Kinouchi et al., 1991). The expression of TNF-Rs was up-regulated by IL-1β, IFN-γ, and TNF-α in vitro (Wilt et al., 1995; Tada et al., 1994; Winzen et al., 1993; Pandita et al., 1992; Kinouchi et al., 1991). These two different TNF-Rs mediate distinct cellular responses; TNF-RII initiates signals for the proliferation of thymocytes and cytotoxic T cells, whereas TNF-RI initiates signals for cytotoxicity and the induction of the protective activity (Schottelius et al., 2004; Tartaglia et al., 1991). In addition, soluble TNF-RI was 30-fold more potent to interfere with TNF binding to its receptors and 5 to 10-fold more potent to inhibit TNF mediated cytotoxicity than sTNF-RII (Hale et al., 1995). Soluble TNF-R (sTNF-R) blocks the antiproliferative effects of TNF and, therefore, may modulate the harmful effects of TNF (Aderka et al., 1992).

The biologic effects of TNF-α are remarkably broad. For example, TNF-α has effects on (1) various immune cells including monocyte/macrophages (e.g. induction of cytokines, chemotaxis, inhibition of differentiation), polymorphonuclear leukocytes (e.g. increasing phagocytic capacity), and lymphocytes (e.g. activation of cytotoxic T-cell invasiveness) as well as (2) non-immune cells (e.g. vascular endothelial cells, fibroblasts, adipocytes, endocrine system) (Schottelius et al., 2004; Ellerin et al., 2003). On the endocrine system, TNF-α stimulates adrenocorticotrophic hormone (ACTH), corticotropin releasing hormone (CRH), and prolactin, nevertheless, inhibits thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), and growth hormone (GH) (Schottelius et al., 2004; Bernardini et al., 1990).

TNF-α acts on the CNS to cause fever and sickness behavior (Johnson, 1977). Pro-inflammatory activities of TNF-α in the brain may (1) alter BBB integrity by inducing expression of adhesion molecules on the surface of endothelial cells (Barten and Ruddle, 1994; Shrikant et al., 1994a), (2) stimulate glial cells (Ruedig and Dringen, 2004), and (3) trigger apoptosis of microvascular endothelium via TNF-RI signaling (Lou et al., 1997). TNF displays various effects on the vascular endothelium (Booth et al., 2004; Ferrero, 2004), including the release of pro-inflammatory cytokines, the increase of endothelial permeability, and up-regulation of adhesion molecules such as ICAM-1, VCAM-1, and E- selectin (Omari and Dorovini-Zis, 2003b; Lucas et al., 1997).

2.2.2 Th2 cytokines and their essential biological activities

2.2.2.1 IL-4: the key Th2 cytokine

IL-4 is a *20 kDa* glycoprotein with 129 amino acids (Kuhnle et al., 1996). The human IL-4 gene maps to chromosome *5q23-31* (Dolganov et al., 1996). The IL-4 gene is in close proximity to other genes encoding hematopoietic growth factors such as GM-CSF, M-CSF, IL-3, and IL-5 (van Leeuwen et al., 1989).

Two types of IL-4 receptors (IL-4R) exist, both using subunit IL-4Rα. Type I IL-4R comprises the IL-4Rα and the common γ chain (γc); signaling via this receptor activates JAK1 and JAK3. The γ subunit of the IL2R serves as a signaling component (Essner et al., 2001). Type II IL-4R comprises IL-4Rα and IL-13R; signaling via this receptor activates

JAK1, JAK2, and TYK2 (Murata et al., 1998). IL-4 binding to either of these receptors activates STAT6. Deficiency in IL-4Rα (140 kDa) impairs signaling via both types of IL-4 receptors (Jankovic et al., 2000). Mitogenic effects of IL-4 involve activation of IL-4-induced phosphotyrosine substrate (4PS) (Ryan et al., 1996), while IL-4-specific gene induction involved STAT6 (Quelle et al., 1995; Hou et al., 1994).

IL-4 producing cells

IL-4 is produced by mature Th2 cells, mast cells, and basophils (Gauchat et al., 1993; Brunner et al., 1993; Heller et al., 1983). The production of IL4 by non-B or non-T-cells like mast cells is stimulated, if these cells interact with other cells via their Fc receptors for IgE or IgG (Brunner et al., 1993; Plaut et al., 1989).

Biological activities of IL-4

The biological activities of IL-4 are species-specific in which murine IL-4 acts only upon mouse cells and human IL-4 only upon human cells (Morrison and Leder, 1992). Early secretion of IL-4 leads to polarization of Th cell differentiation toward Th2-like cells (Mosmann and Coffman, 1989). Th2 cells secrete their own IL-4 and subsequent autocrine production of IL-4 supports Th2 cell proliferation. The Th2-cell secretion of IL-4 and IL-10 leads to the suppression of Th1 responses by down-regulating the production of macrophage-derived IL-12 (Morrison and Leder, 1992) and inhibiting the differentiation of Th1 cells (Mosmann and Coffman, 1989). In activated B-cells, IL-4 stimulates the synthesis of IgG1 and IgE and inhibits the synthesis of IgG3, IgG2a, and IgG2b (Mathers and Cuff, 2004; Tangye et al., 2002; Grunewald et al., 1998; Honer et al., 1993; Roper et al., 1990). This Isotype switching induced by IL-4 in B-cells is antagonized by IFN-γ (Yssel et al., 1993; Thyphronitis et al., 1989). In addition, IL-4 has marked inhibitory effects on the expression and release of the proinflammatory cytokines. It is able to block or suppress the monocyte-derived cytokines, including IL-1, TNF-α, IL-6, IL-8, and MIP-1α (te Velde et al., 1990; Hart et al., 1989). In contrast to its inhibitory effects on the production of proinflammatory cytokines, IL-4 stimulates the synthesis of the cytokine inhibitor IL-1RA (Vannier et al., 1992). Furthermore, IL-4 inhibits NK cell activation induced by IL-2 and stimulates the proliferation of thymocytes (Che and Huston, 1994; Barcena et al., 1991).

2.2.2.2 IL-13: shares similarities with IL-4

IL-13 is a 132-amino-acid long protein of about *10 kDa*. The human IL-13 gene has been mapped close to the IL-4 gene on chromosome *5q31* (Izuhara and Arima, 2004; Smirnov et

al., 1995; McKenzie et al., 1993). IL-13R comprised three components: IL-13Rα, IL-4Rα, and the common γ chain of IL-2R (IL-2Rγ). IL-13Rα again contains IL-13Rα1 and IL-13Rα2 (Roy et al., 2002b). IL-13Rα chain alone has weak binding activity for IL-13. IL-13Rα1 binds IL-13 with subsequent recruitment of IL-4Rα to efficiently transduce a signal, whereas the IL-13Rα2 can bind IL-13 in the absence of IL-4Rα. IL-4R and IL-13R share more components including IL-4Rα, IL-2Rγ, and STAT6 (Blanchard et al., 2004; Terabe et al., 2004). JAK2 is associated with IL-4Rα and TYK2 is associated with the IL-13Rα1 component of the IL-13R complex (Roy et al., 2002a). Human B-lymphocytes and monocytes expressed a very small number of IL-13R, while resting or activated human T cells expressed little or no IL-13R (Obiri et al., 1995). IL-13 competes for IL-4 binding, while IL-4 does not compete for the IL-13 binding on some cell types (Obiri et al., 1995). IL-4 does not always bind well to cells that bind IL-13, but the reverse is also true. IL-4 can compete more effectively for IL-13R binding than IL-13 itself. IL-4Rα also participates in the formation of the IL-13R complex in some cell types (Obiri et al., 1997).

IL-13 producing cells

Murine IL-13 is produced primarily by activated Th2-cells, while in humans IL-13 is secreted by CD4+ and CD8+ T cells. In CD4+ T cell clones, all Th0, Th1-like, and Th2-like subsets released IL-13 following antigen-specific or polyclonal activation (de Waal et al., 1993b). Moreover, mast cells, basophils, eosinophils, NK cells, and DC were also reported to produce IL-13 (Izuhara and Arima, 2004; Hoshino et al., 1999; Saint-Vis et al., 1998; Peritt et al., 1998; Gibbs et al., 1996; Li et al., 1996a; Burd et al., 1995). In the CNS, although human microglia stimulated by LPS did not produce IL-13, human microglia did express mRNA transcripts for IL-13Rα2 (Lee et al., 2002; Wu and Low, 2002).

IL-13 biological activities

IL-13 and IL-4 share a common cellular receptor IL-4Rα; this accounts for many of the similarities between these two anti-inflammatory cytokines (Kotowicz et al., 1996; Callard et al., 1996; Zurawski et al., 1993). IL-4 and IL-13 share only 20% to 25% primary amino acid homology, but the major α-helical regions which are essential for their activity are highly homologous (de Waal et al., 1993a; de Waal et al., 1993b). Both IL-4 and IL-13 induce IgE class switching in B cells (Punnonen et al., 1993), enhance monocyte/macrophage antigen presentation ability, down-regulate inflammatory cytokine production by monocytes/macrophages, produce anti-inflammatory molecules (de Waal et al., 1993b), chemokines (Fujii-Maeda et al., 2004; de Waal et al., 1993b), adhesion molecules such as VCAM-1 (Bochner et al., 1995), and suppress apoptosis (Tangye and Raison, 1997). The

principal functional difference between IL-4 and IL-13 lies in their effects on T cells. IL-4 is a dominant mediator of Th2 cell differentiation, proliferation, and activity, whereas IL-13 has minimal effects on T-cell function (Wynn, 2003; Zurawski and de Vries, 1994). Surprisingly, both IL-4 and IL-13 are also potent enhancers of IL-12 production by human peripheral blood mononuclear cells (Bullens et al., 2001; D'Andrea et al., 1995). In addition, IL-13 was suggested to act as a proinflammatory cytokine in the brain since IL-13 could regulate LPS-induced sickness behavior (Bluthe et al., 2001).

2.2.2.3 IL-10: primary anti-inflammatory/inhibitory cytokine

IL-10 is a 18 kDa homodimeric protein having a length of 160 amino acids (Haddad et al., 2003; Powell et al., 2000). It is initially found as cytokine synthesis inhibitory factor (CSIF) (Haddad et al., 2003; Opal et al., 1998; Lalani et al., 1997; Howard et al., 1992). The human IL-10 gene contains four exons and maps to chromosome 1 (*1q31-3*2) (Haddad et al., 2003). Human IL-10RI (IL10RA) is a 90-110-kDa protein that is expressed on a limited number of cell types (Liu et al., 1994). Human IL-10RII (IL10RB) is a 60-kDa protein that contains 325 amino acids. IL-10RI is associated with STAT3/JAK1, while IL-10RII is associated with TYK2 kinase (Kotenko et al., 1997). The human IL-10RI and IL-10RII genes are located within chromosome 11 (*11q23.3*) and 21 (*21q22.1*), respectively (Reboul et al., 1999; Taniyama et al., 1995).

IL-10 producing cells

IL-10 is produced by murine Th2-cells, but not Th1-cells. In humans, IL-10 is predominantly secreted by activated CD8+ T cells, CD4+ T cells (resembling Th0, Th1, and Th2) after both antigen-specific and polyclonal activation (Reboul et al., 1999; Taniyama et al., 1995; Yssel et al., 1992), and secondarily also by macrophages/monocytes following activation by bacterial lipopolysaccharides (LPS) as well as by mast cells (Haddad et al., 2003). The synthesis of IL-10 by monocytes is primarily and effectively inhibited by IL-4 and IL-10 (Haddad et al., 2003).

Biological activities of IL-10

IL-10 is a pleiotropic cytokine with important immunoregulatory functions whose actions influence activities of many cell-types in the immune system (Gallagher et al., 2000). It exerts inhibiting effects on distinct immune cells, particularly on T, B, NK cells, and monocyte/ macrophages.

On T cells, IL-10 inhibits the synthesis of Th1 cytokines such as IFN-γ, IL-2, and TNF-β (De Smedt et al., 1997). The effect on IFN-γ release appears to result from the IL-12 suppression by accessory cell (Haddad et al., 2003; D'Andrea et al., 1993). Additional effects on T cells include: (1) inducing CD8+ T cell chemotaxis, (2) inhibiting CD4+ T cell chemotaxis towards IL-8 (Gesser et al., 1997), T cell apoptosis via Bcl-2 up-regulation (Alas et al., 2001) and IL-2 production following activation (Taga et al., 1993; de Waal et al., 1993c) as well as (3) interrupting T cell proliferation following low antigen exposure accompanied by B7/CD28 co-stimulation. Besides, IL-10 exerts various effects on B cells as well: (1) initiating B cell differentiation and growth (Weiss et al., 2004; Itoh and Hirohata, 1995; Rousset et al., 1992), (2) inducing/enhancing IgA and IgM (Austin et al., 2003; Armitage et al., 1993; Rousset et al., 1992), (3) directly switching IgG isotypes due to being able to induce IgG1 and IgG3 in humans in the absence of TGF-β (Beniguel et al., 2003), and (4) having divergent effects on IL-4-induced IgE secretion. If IL-10 is present at the time of IL-4 induced class switching, it reverses the effect; if it is present after IgE commitment, it augments IgE secretion (Jeannin et al., 1998). The synthesis of immunoglobulins induced by IL-10 is antagonized by TGF-β (Armitage et al., 1993). On NK cells, IL-10 (1) facilitates IFN-γ secretion in NK cells primed by IL-18 (Cai et al., 1999), (2) potentiates IL-2-induced NK cell proliferation (Carson et al., 1995) and (3) NK cell cytotoxicity, in concert with both IL-12 and/or IL-18 (Cai et al., 1999; Micallef et al., 1999). Nevertheless, IL-10 enhances NK cell production of IFN-γ, but inhibits macrophage production of IFN-γ-inducing factors (Shibata et al., 1998). On monocyte/macrophages, IL-10 (1) inhibits various cytokines and chemokines including IL-6, TNF-α, IL-12, MIP-1α, and MIP-2α (Tryzmel et al., 2003; Clarke et al., 1998; de Waal et al., 1991), (2) reduces cell surface MHC-II expression (Chadban et al., 1998), and (3) inhibits prostaglandin E2 (PGE2) stimulated by LPS (Niiro et al., 1994). Additionally, IL-10 and IFN-γ antagonize each other's production and function in human monocytes (Chomarat et al., 1993).

2.2.2.4   IL-6: essential neuro-endo-immunological mediator

IL-6, also called IFN-β2/B-cell stimulatory factor 2/hepatocyte stimulating factor (Ferguson-Smith et al., 1988), is a *26 kDa* protein with 185 amino acids (Conti et al., 2002). The human IL-6 gene contains five exons and maps to human chromosome *7p15-21*(Ferguson-Smith et al., 1988). The IL-6R is a protein of *80 kDa* (Fujisawa et al., 2002). IL-6R consists of 2 chains: IL-6Rα and IL-6Rβ. The IL-6/IL-6R complex associates with a 130-kDa

transmembrane glycoprotein (gp130). Glycoprotein 130 is involved in signal transduction. The IL-6R is expressed on various cell types such as lymphocytes, monocytes, fibroblasts, vascular endothelial cells, and pituitary cells (Barton, 1997). In addition, soluble IL6 receptor (sIL-6R) enhances the effect of IL-6 (Schobitz et al., 1995).

IL-6 producing cells

Many different cell types produce IL-6. The main sources in vivo are stimulated monocytes/macrophages, fibroblasts, and endothelial cells (Coil et al., 2004; Dalal et al., 2003; Ng et al., 2003; Soderquist et al., 1998; Yachie et al., 1990). Additionally, T-cells, B-lymphocytes, eosinophils, mast cells, astrocytes, and microglia also produce IL-6 after stimulation (Azzolina et al., 2003; Delgado et al., 2003; Inoue, 2002; Diehl and Rincon, 2002; Hoenstein et al., 2001; Lorentz et al., 2000; Frei et al., 1989). IL-6 mRNA was found to be generally low in the brain (Schobitz et al., 1993); it was present in the hippocampal formation with highest signal in the dentate gyrus, habenular nucleus, piriform cortex, hypothalamus, and striatum (Chen et al., 2003; Gadient and Otten, 1994; Schobitz et al., 1992).

Biological activities of IL-6

IL-6 is involved in regulating a wide variety of immune functions, such as B- and cytotoxic T-cell differentiation, induction of IL-2 production and IL-2R expression in T cells, T cell growth, acute-phase reactions, and hematopoiesis (Hirano, 1998; Taga and Kishimoto, 1997).

Newly, Diehl and Rincón (Diehl and Rincon, 2002) suggested that APC IL-6 promotes Th2 differentiation and simultaneously inhibits Th1 polarization through IL-12 independent molecular mechanisms. IL-6 activates transcription mediated by the transcription factor Nuclear Factor of Activated T cells (NFAT), leading to IL-4 production by naïve CD4+ T cells and their differentiation into effector Th2 cells. The induction of Th2 differentiation by IL-6 is dependent upon endogenous IL-4. In addition, IL-6 binds to IL-6Rα, leading to the dimerization of gp130/IL-6Rβ (Brakenhoff et al., 1995). Dimerization of gp130 by IL-6 causes the activation of two signaling pathways: (1) the JAK/STAT pathway and (2) the CCAAT/enhancer binding protein (C/EBP) pathway (Weihua et al., 2000; Heinrich et al., 1998). IL-6 inhibits Th1 differentiation via the JAK/STAT1 pathway by inducing the suppressor of cytokine signaling 1 (SOCS1) expression (Siewert et al., 1999). IL-6 up-regulates SOCS1 expression in activated CD4+ T cells, thereby interfering with signal transducer and activator of transcription 1 (STAT1) phosphorylation induced by IFN-γ. Inhibition of IFN-γR-mediated signals by IL-6 prevents auto-regulation of IFN-γ gene

expression by IFN-γ during CD4+ T cell activation, thus preventing Th1 differentiation. This pathway is IL-4- and IL-12-independent (Diehl and Rincon, 2002).

Furthermore, IL-6 exerts distinct effects on the CNS; they include activation of the hypothalamic-pituitary-adrenal axis (HPA), reduction of food intake, induction of fever, and neuronal growth (Godbout and Johnson, 2004; Path et al., 2000). IL-6 induces nerve growth factor (NGF) in astrocytes, enhances NGF-stimulated astrocyte proliferation (Levison et al., 2000; Marz et al., 1999; Schafer et al., 1999; Kossmann et al., 1996), promotes survival of the mesencephalic catecholaminergic and septal cholinergic neurons in vitro (Kushima and Hatanaka, 1992; Hama et al., 1991), and attenuates the neurotoxic effects of NMDA on striatal cholinergic neurons (Toulmond et al., 1992).

3　Th1/Th2 cytokines in schizophrenia

3.1　*Possibilities from peripheral viral infections to CNS disorders*

The concept that the central nervous system (CNS) is an immunological privileged site due to lack of immunocompetent cells and antigen presentation per se has dominated the relevant field for decades; the blood-brain-barrier (BBB) exists between the CNS and the peripheral system and prevents the entrance of large proteins and leukocytes into the CNS (Engelhardt et al., 1997). Lately, growing evidence supports the possibilities of peripheral viral infections to develop diseases in the CNS, despite of the blockage of the BBB.

3.1.1　Permeability of the blood-brain-barrier (BBB)

3.1.1.1　*Under certain pathological conditions*

The <u>BBB</u> is under certain pathological conditions <u>permeable</u> such as brain damage, infections or application of prostaglandins (PGE) (Jaworowicz, Jr. et al., 1998), other inflammatory mediators, viral, bacterial, parasitic compounds (Descamps et al., 2003), or products of tissue damage such as arachidonic acid (Unterberg et al., 1987). The most profound activator of inflammatory cytokine expression in the brain is damage to the CNS; that is, mechanical injury, inflammation, neurotoxins, ischemia or infection causing an increase in the concentration of several proinflammatory cytokines (e.g. IL-1, TNF-α, IL-6) in the CSF and in the brain (Merrill and Benveniste, 1996b; Hopkins and Rothwell, 1995a). Brain injury induced BBB dysfunction is mediated by intra-cerebral neutrophil accumulation, chemokine release (e.g., IL-8), and upregulation of adhesion molecules (e.g., ICAM-1) (Otto et al., 2000).

3.1.1.2　*No barrier in few places of the CNS*

In addition, the brain's three sensory circum-ventricular organs including the <u>subfornical organ</u>, <u>organum vasculosum of the lamina terminalis</u>, and the <u>area postrema</u> lack a functional BBB; they are the only regions in the brain in which neurons are exposed to the chemical environment of the systemic circulation (McKinley et al., 2003). Cytokines from the peripheral system may also affect the brain at those sites.

3.1.1.3 Changeability of BBB permeability

Furthermore, some cytokines can have an effect on the permeability of the BBB and/or act, probably indirectly, as vasomodulator agents of the cerebral microvessel endothelium. For instance, injection of IL-2 and IL-6 significantly enhances the permeability of the BBB, while injection of TNF-α reduces it (Saija et al., 1995). Neutrophils were newly found to be able to reduce or increase permeability of the BBB, depending upon their proximity and migration through the endothelium (Inglis et al., 2004).

3.1.2 CNS expresses cytokines and their receptors

The CNS expresses cytokines and their receptors per se. It can produce cytokines such as IL-1, IL-6, TNF-α, IFN-γ, and lymphotoxin-alpha (LT-α) during the inflammatory response or even during their normal development. Normal unstimulated human microglia expressed constitutively mRNA transcripts for IL-1β, IL-6, IL-8, IL-10, IL-12, IL-15, TNF-α, and IFN-γ (Lee et al., 2002; Morris and Esiri, 1998), while treatment with lipopolysaccharide (LPS) or amyloid β peptides (Aβ) led to increased expression of mRNA levels of IL-8, IL-10, IL-12, and TNF-α as well as elevated protein levels of IL-1β, IL-8, and TNF-α (Lee et al., 2002). Activated microglia, therefore, represents a source of cytokine producing cells in the CNS (Benveniste, 1997; Gehrmann, 1995). In addition, human microglia also expressed mRNA transcripts for IL-1RI, IL-1RII, IL-5R, IL-6R, IL-8R, IL-9R, IL-10R, IL-12R, IL-13R, and IL-15R (Lee et al., 2002). IL-1R, IL-2R, IL-6R, IL-7R, IL-12R, TGB-βR, TNF-R, and a number of growth factors have been localized in the brain being expressed at the highest levels generally in the hippocampus and hypothalamus (Mehler and Kessler, 1997; Hopkins and Rothwell, 1995b; Otero and Merrill, 1994). Furthermore, astrocytes express not only the mRNA of sIL-4R but also IL-4Rα; nevertheless, they do not secret IL-4 (Barna et al., 2001; Brodie et al., 1998).

3.1.3 Presence of APC equivalents in the CNS

Microglia and astrocytes are the APC equivalents in the CNS. Microglial cells are one type of highly differentiated and quiescent tissue macrophages; they are located within the CNS parenchyma (Kielian, 2004; Cosenza et al., 2002). Human microglial cells exhibit cell-type-

specific antigens for macrophage/microglia lineage cells including CD11b (Mac-1), CD68 (macrophage antigen), B7-2 (CD86), human leukocyte antigen-ABC (HLA-ABC), HLA-DR, ricinus communis aggulutinin lectin-1 (RCA-1), CD45 (leukocyte common antigen), CD64 (Fc receptor), CR3 (complement type 3 receptor), and MHC (major histocompatibility complex) class I and II (Lee et al., 2002; Kreutzberg, 1996; McGeer et al., 1993; Thomas, 1992). MHC-II molecules are essential for lymphocyte development, antigen presentation, the activation of APC and T-cells (Clark, 1995). Upon stimulation with Th1 supernatants or IFN-γ, microglia express CD80, CD86, MHC-II, CD40, as well intercellular adhesion molecule-1 (ICAM-1 = CD54) and efficiently present antigen leading to T cell proliferation and production of IL-2 and IFN-γ by Th1 as well as release of IL-4 by Th2 cells (Seguin et al., 2003; Aloisi et al., 1998).

Astrocytes are the most numerous cell type and the major glial cell type within the CNS (Croitoru-Lamoury et al., 2003; Dong and Benveniste, 2001). Astrocytes can be induced by TNF-α, IL-1, and IFN-γ to express ICAM-1, MHC-I, MHC-II, vascular cell adhesion molecule-1 (VCAM-1), and leukocyte function-associated antigen-3 (LFA-3 = CD58) (Ballestas and Benveniste, 1995; Hery et al., 1995; Weber et al., 1994; Shrikant et al., 1994b; Williams, Jr. et al., 1993). Activated lymphocytes and monocytes can bind to astrocytes in an ICAM-1 dependent manner (Hery et al., 1995). Despite that IFN-γ-treated astrocytes express MHC-II and ICAM-1 and present antigens less efficiently to Th1 cells, but they were as efficient as microglia in inducing IL-4 secretion by Th2 cells (Aloisi et al., 1998). So, astrocytes are also regarded as immunocompetent cells within the brain (Dong and Benveniste, 2001).

3.1.4 CNS produces adhesion molecules required for leukocyte migration

The CNS also expresses or produces the adhesion molecules required for leukocyte migration. The CNS resident glial cells such as microglia and astrocytes, neurons, and brain vascular endothelial cells were found to express ICAM-1 and VCAM-1 (Hery et al., 1995; Brosnan et al., 1995; Sobel et al., 1990). TNF-α, also produced by the brain itself, is the most important cytokine being able to up-regulate ICAM-1 and VCAM-1 and, thus, influences the adhesive properties of both astrocytes and brain endothelium; to a lesser extend, IFN-γ and IL-1 also showed such effects (Merrill and Benveniste, 1996a; McCarron et al., 1993). Cytokines initiate the inflammatory response first through up-regulation of several adhesion molecules

in the BBB endothelium such as selectin E, selectin P, ICAM-1, and VCAM-1. Particularly, VCAM-1 expression at the BBB is essential for leukocyte entry. ICAM-1 is involved in cell extravasation into inflamed tissue and is, therefore, crucial for antigen presentation to T cells and required for a complete activation of T cells (Sibson et al., 2004; Omari and Dorovini-Zis, 2003a; Bernardes-Silva et al., 2001; Engelhardt et al., 1997; Engelhardt et al., 1995). Moreover, induction of ICAM-1 and VCAM-1 by cytokines on the CNS resident glial cells such as astrocytes, microglia, and neurons may guide inflammatory leukocytes to express LFA-1/Mac-1 and/or late activation antigen-4 (VLA-4) into and through the brain, thereby further contributing to impairment of the BBB (Hailer et al., 1998; Hery et al., 1995).

3.1.5   Facts: migration of peripheral immune cells through the BBB

Recent evidence suggests that activated T cells cross the intact BBB and that a series of immunological events is initiated when T cells recognize antigens in the CNS (Toda, 2003). Monocytes were also shown to be able to migrate across human brain-derived endothelial cells (HBEC) in the absence of inflammatory conditions, at rates exceeding those of lymphocytes (Seguin et al., 2003). Another in vitro model demonstrated that Th1 and Th2 cells migration were differently regulated by HBEC. Migration of both lymphocyte subsets was dependent on LFA-1/ICAM-1 interaction on HBEC and the BBB; the BBB seemed to favor the migration of Th2 cells (Biernacki et al., 2001).

The BBB is thus no more a "forbidden city" for pro- and anti-inflammatory cytokines. It is, hence, possible to develop a disease in the CNS such as schizophrenia from a peripheral viral infection during the pre- or peri- or post-natal phase.

3.2   Th1/Th2 cytokines in schizophrenia

3.2.1   Th1 abnormalities in Schizophrenia

3.2.1.1   IFN-γ: reduced in diverse schizophrenic subgroups

So far, no report relating to Th1/Th2 ratios in schizophrenia was published. Findings regarding Th1/Th2 cytokines in schizophrenia are numerous, nonetheless, contradictory.

IFN-γ in vitro production

The vast majority of studies showed <u>reduced</u> in vitro IFN-γ production in schizophrenia (Kaminska et al., 2001; Rothermundt et al., 2000; Arolt et al., 2000; Rothermundt et al., 1998;

Arolt et al., 1997; Wilke et al., 1996; Hornberg et al., 1995; Katila et al., 1989; Moises et al., 1985). The decrease in IFN-γ in the study of Hornberg et al. (1995) simply exhibited a trend to be significant, while the reduction in the study of Katila et al. (1989) was not noticeable. As a matter of fact, most of the studies cited above investigated only a subgroup of schizophrenics. The studies of Kaminiska et al. (2001) and Wilke et al. (1996) included only or mainly paranoid schizophrenics, that of Arolt et al. (2000) contained schizophrenics having positive family schizophrenic history, and that of Rothermundt et al. (1998, 2000) merely consisted of acute schizophrenic patients. The schizophrenic subjects included in the study of Katila et al. (1989) were mixed with drug-naïve and chronic patients. Only Wilke et al. (1996) found the reduction observed not only in paranoid schizophrenics but also in the whole group of schizophrenic patients including paranoid and residual schizophrenia. Moreover, the decrease of IFN-γ in vitro secretion in acute schizophrenics remained clear even if compared to the first-degree relatives of schizophrenics (Arolt et al., 1997). Nevertheless, three further reports from Cazzullo et al. (Cazzullo et al., 2002; Cazzullo et al., 2001; Cazzullo et al., 1998) revealed different results; two studies (2001, 2002) showed <u>enhanced</u> IFN-γ in vitro production, while one (1998) revealed <u>no alteration</u>. The patients participated in those three studies of Cazzullo et al. (2002, 2001, 1998) were mostly paranoid schizophrenics, drug-free schizophrenic subjects, and chronic patients with schizophrenia, respectively.

Serum IFN-γ

To date, three studies investigating serum IFN-γ showed <u>no diversity</u> between the whole schizophrenic group or any schizophrenic subgroup and healthy controls (Kim et al., 2001; Gattaz et al., 1992; Becker et al., 1990), while one revealed a remarkable <u>increase</u> in paranoid schizophrenic patients (Kaminska et al., 2001).

3.2.1.2 IL-2: controversial

On the contrary, the findings in connection with another Th1 cytokine IL-2 that is often deemed as an indicator of T-cell activation were controversial.

IL-2 in vitro production

Some studies investigated drug-naïve schizophrenics and revealed <u>decreased</u> IL-2 in vitro production (Ganguli et al., 1995; Ganguli et al., 1992; Villemain et al., 1989). Nevertheless, some other studies examined drug-free schizophrenic patients and showed the same results as those from drug-naive patients (Zhang et al., 2002a; Kim et al., 1998). Similar findings were also reported in acute schizophrenics (Rothermundt et al., 1998; Ganguli et al., 1992). Other

studies without further characterizing their schizophrenic subjects demonstrated similar outcomes as well (Arolt et al., 2000; Hornberg et al., 1995; Yang et al., 1994; Ganguli et al., 1989). However, abundant findings showed no change in vitro IL-2 secretion in distinct schizophrenic subgroups such as paranoid, residual, chronic, drug-free schizophrenics, patients dominated with positive symptoms, and schizophrenics having positive family psychiatric history (Kowalski et al., 2000; Cazzullo et al., 1998; Arolt et al., 1997; Wilke et al., 1996). And almost equal number of studies demonstrated an increased IL-2 in vitro production in paranoid, residual, drug-free/drug-naïve, and acute schizophrenics (Cazzullo et al., 2002; Cazzullo et al., 2001; Rothermundt et al., 1998; O'Donnell et al., 1996).

Serum IL-2

Outcomes regarding serum/plasma IL-2 were also conflicting; some studies reported of increases, some showed no alteration, and only one demonstrated a reduction in schizophrenia or in a subgroup with a certain feature (Ebrinc et al., 2002; Zhang et al., 2002b; Kaminska et al., 2001; Theodoropoulou et al., 2001; Kim et al., 2001; Erbagci et al., 2001; Kim et al., 2000; Kim et al., 1998; Barak et al., 1995; Xu et al., 1994; Gattaz et al., 1992). The schizophrenic subjects in those studies stated above differed from one another not only in their ethnic groups but also in distinct clinical features.

CSF IL-2

Results concerning CSF IL-2 are rare; Barak et al. (1995) showed enhanced CSF IL-2, while two other studies failed to find any diversity between medicated or non-medicated schizophrenics and controls in this regard (Rapaport et al., 1997; el Mallakh et al., 1993).

IL-2R & sIL-2R

Serum IL-2R was found to be unaltered in drug-naïve schizophrenics (Villemain et al., 1989). The findings pertaining to serum sIL-2R were more positive (increase) than negative (no change) in schizophrenia or in various schizophrenic subgroups (Rothermundt et al., 1998; Maes et al., 1997; Arolt et al., 1997; Naudin et al., 1997; Muller et al., 1997; O'Donnell et al., 1996; Maes et al., 1996; Barak et al., 1995; Maes et al., 1995b; Rapaport and Stein, 1994; Maes et al., 1994; Rapaport and Lohr, 1994; Rapaport et al., 1993; Ganguli and Rabin, 1989; Rapaport et al., 1989). Newly, increased sIL-2R was also observed in siblings, but neither fathers nor mothers, of schizophrenics (Gaughran, 2002). Barak et al. (Barak et al., 1995) examined CSF sIL-2R in schizophrenics and Gaughran et al. (Gaughran et al., 1998) investigated sIL-2R α subunit in patients either with schizophrenia or schizophreniform disorders; both studies exhibited an elevation in CSF sIL-2R and sIL-2Rα. Soluble IL-2Rα was regarded as a marker of T-lymphocyte activation and proliferation (Lawn et al., 2001).

Only Kowalski et al. (Kowalski et al., 2000) found a <u>reduced</u> PHA-stimulated whole blood sIL-2R production in drug-free schizophrenics predominated with positive symptoms. It's noteworthy that the results from most of the relevant studies involved solely one schizophrenic subgroup, but not the whole group of schizophrenic patients.

3.2.1.3 Other Th1-related molecules

Plasma IL-12 levels were found to be higher in patients with major depression, but between schizophrenics and healthy controls no clear difference was shown in this respect (Kim et al., 2002; Kim et al., 2001). Nevertheless, medicated schizophrenic patients did exhibit higher serum IL-18 levels than their healthy counterparts (Tanaka et al., 2000).

Neopterin is an indicator of the activity in the Th1 cellular immune system (Gaughran, 2002). Baseline urine neopterin of schizophrenics was reduced and raised to the same level as those in controls after haloperidol or clozapine treatment (Sperner-Unterweger et al., 1992). However, Nikkilä et al. (Nikkila et al., 2002) reported of no alteration in CSF neopterin at admission and after treatment in schizophrenics. ICAM-1 is thought to be another marker for the Th1 activation (Gaughran, 2002). Schizophrenic patients were found to have more cells expressing CD54+ (ICAM-1) (Theodoropoulou et al., 2001). Nevertheless, soluble CD14 (sCD14), a monocyte activation marker, was shown to be unchanged in schizophrenia (Naudin et al., 1997).

3.2.2 Th2 alterations in schizophrenic patients
IL-4

The key Th2 cytokine, IL-4, was less well studied for its barely measurable level in most subjects by using many available analysis methods. Results published in this regard were limited. IL-4 in vitro production and serum level were shown to be <u>decreased</u> and <u>unaltered</u>, respectively (Kaminska et al., 2001). On the contrary, Wilke et al., (Wilke et al., 1996) demonstrated an <u>increased</u> IL-4 in vitro production; however, the increase was not significant. The only publication regarding CSF IL-4 showed that it was only detectable in some childhood-onset schizophrenia, but not patients with obsessive-compulsive disorder or attention deficit hyperactivity disorder (Mittleman et al., 1997).
IL-6

The outcomes concerning IL-6, produced by Th2 cells as well, showed a relative consistency. Most studies reported (1) underlined increased IL-6 in serum, plasma, and CSF (Garver et al., 2003; Zhang et al., 2002b; Kaminska et al., 2001; van Kammen et al., 1999; Lin et al., 1998; Naudin et al., 1997; Frommberger et al., 1997; Naudin et al., 1996; Maes et al., 1995a; Xu et al., 1994; Maes et al., 1994; Shintani et al., 1991), (2) enhanced plasma IL-6R (Maes et al., 1997) as well as (3) an augmentation in the endogenous agonist of IL-6, sIL-6R (Lin et al., 1998; Maes et al., 1995a). Furthermore, serum gp130 levels in schizophrenics also showed a trend to be higher, despite of the family histories of patients (Lin et al., 1998). Nonetheless, no change was shown in (1) IL-6 in vitro production in paranoid or drug-free schizophrenic patients (Kaminska et al., 2001; Kim et al., 1998), (2) plasma IL-6 in drug-free male schizophrenics and schizophrenics in remission (Kim et al., 2001; Kim et al., 2000; Frommberger et al., 1997) as well as (3) serum and CSF IL-6 in the whole schizophrenic subjects (van Kammen et al., 1999; Kim et al., 1998; Barak et al., 1995). In addition, increased serum IL-6 was found to be associated with duration of illness (Naudin et al., 1997) (Ganguli et al., 1994). It's worthy to note that the enhanced serum/plasma IL-6 in most of studies stated above involved only a certain schizophrenic subgroup, but not the whole group of schizophrenic patients.

Results pertaining to the association between schizophrenia and IL-5 are quite rare. The only one study showed that the CSF IL-5 levels of schizophrenics were undetectable (Mittleman et al., 1997). Leukaemia inhibitory factor (LIF) is related mainly to Th2 (Piccinni et al., 2001). LIF, essential for embryo implantation, can be up-regulated by IL-4 and progesterone (Piccinni et al., 2000). Serum LIF-R levels in schizophrenia were found to be similar to those in controls (Maes et al., 2002).

3.2.3   Schizophrenia and Th1/Th2-produced cytokines

Both TNF-α and IL-10 can be released by Th1- and Th2-cells. Few studies investigating IL-10 revealed that IL-10 in vitro production of schizophrenia was similar to that of controls (Cazzullo et al., 2002; Kaminska et al., 2001; Rothermundt et al., 1998). Only Cazzullo et al. found (1) that IL-10 in vitro release was apparently higher in schizophrenia and (2) that paranoid schizophrenics had the lowest IL-10 production among distinct schizophrenic clinical subgroups (Cazzullo et al., 1998). Two further studies examined serum IL-10 levels; Maes et al. demonstrated increased serum IL-10 in schizophrenia (Maes et al., 2002), while

32

Kaminiska et al. showed a reduction in paranoid schizophrenics (Kaminska et al., 2001). Solely Mittleman et al. (Mittleman et al., 1997) studied CSF IL-10 and found hardly detectable CSF IL-10 in children with schizophrenia.

Outcomes regarding TNF-α were contradictory. Serum or plasma TNF-α was found to be either increased or unaltered (Kaminska et al., 2001; Theodoropoulou et al., 2001; Erbagci et al., 2001; Monteleone et al., 1999; Naudin et al., 1997; Xu et al., 1994). Similar results were obtained in terms of TNF-α in vitro production (Kaminska et al., 2001; Kowalski et al., 2001).

3.3   Effects of anti-psychotics on Th1/Th2 cytokines

There are a number of studies examining the effects of various neuroleptics on different Th1/Th2 cytokines. However, none of them investigated the impacts of anti-psychotics on the ratio between IFN-γ and any of the major Th2 cytokines including IL-4, IL-10, and IL-13. In the following section, the findings concerning the influences of neuroleptic medication on diverse Th1/Th2 cytokines were summarized in order to offer an overview of the topics in this regard.

Atypical neuroleptics & Th1/Th2 cytokines

Atypical neuroleptic and Th1 cytokines

Although the results of Rothermundt et al. and Katila et al. did not support that neuroleptic medication might influence IL-2 and IFN-γ in vitro production in schizophrenia (Rothermundt et al., 2000; Katila et al., 1989), some others showed that neuroleptic medications differently affect IL-2 production (Cazzullo et al., 1998) and that the addition of clozapine induced sIL-2R secretion in peripheral blood mononuclear cells (Hinze-Selch et al., 1998).

Clozapine was shown to increase various Th1 cytokines such as IL-2, sIL-2R, IFN-γ, TNF-α, sTNF-Rp55, and sTNF-αp75 either in serum/plasma or in vitro production (Rudolf et al., 2002; Haack et al., 1999; Hinze-Selch et al., 1998; Muller et al., 1997; Maes et al., 1996; Hinze-Selch et al., 1996; Pollmacher et al., 1995; Maes et al., 1994). However, Monteleone et al. reported of clozapine treatment leading to a reduction in TNF-α (Monteleone et al., 1997), while Song et al. found a bi-modal effect of clozapine on IFN-γ in vitro production (Song et al., 2000). 10^{-6} M of clozapine significantly increased LPS/PHA-induced IFN-γ in vitro

production, whereas 10^{-4} M of clozapine led to a reduction in IFN-γ secretion in healthy subjects (Song et al., 2000).

Another atypical neuroleptic <u>risperidone</u> was shown to enhance sIL-2R (Maes et al., 1996) and IL-2 production; nevertheless, the increase was not statistically significant (Cazzullo et al., 2002). The effects of risperidone on IFN-γ were, nonetheless, dependent upon the length of treatment; it firstly reduced, but later increased IFN-γ release (Cazzullo et al., 2002). However, Kim et al. did not find any effect of risperidone on plasma IL-2 and IFN-γ levels; but after 4-week treatment, risperidone significantly enhanced plasma IL-2 levels in acute schizophrenics (Kim et al., 2001).

Atypical neuroleptic and Th2 cytokines

Reports regarding the effects of <u>clozapine</u> on Th2 cytokines are rare. Clozapine was shown to have no effect on the IL-6/sIL-6R ratios in schizophrenia (Maes et al., 1994). Monteleone et al. also failed to detect any effect of clozapine on plasma IL-6 (Monteleone et al., 1997). But Maes et al. found that clozapine led to increased serum/plasma IL-6 after short-term treatment, nonetheless, decreased IL-6R levels after prolonged treatment in acute schizophrenic patients (Maes et al., 2002; Maes et al., 1997). In healthy subjects, clozapine was found to reduce LPS/PHA stimulated whole blood IL-6 and IL-10 in vitro production (Song et al., 2000). It was, however, shown to increase serum IL-10 in subjects with schizophrenia, although the enhancement was not significant (Maes et al., 2002).

<u>Risperidone</u> was newly shown to increase plasma IL-6 (Maes et al., 2002), IL-10 production (Cazzullo et al., 2002), and serum IL-10 (Maes et al., 2002), while it exerted its effects on IL-4 secretion in a time-dependent manner (first reduction and later enhancement) (Cazzullo et al., 2002). However, Kim et al. found no effect of risperidone on plasma IL-6 in acute schizophrenics (Kim et al., 2001).

<u>Olanzapine</u>, a further atypical neuroleptic, shares with clozapine various similarities including its chemical structure and the binding profiles of diverse neurotransmitter receptors. Both olanzapine and clozapine were found to be able to induce transient agranulocytosis. To date, no report about the effects of olanzapine on diverse typical Th1/Th2 cytokines was published. Olanzapine was shown to increase CD8+ cells, an important source of various cytokines. However, no effect of olanzapine was observed on CD14, CD19, CD3, CD4, and CD45

(Bilici et al., 2003). Olanzapine could have influences on Th1/Th2 cytokines indirectly through its effects on CD8+ cells.

Typical neuroleptics & Th1/Th2 cytokines

Typical neuroleptics and Th1 cytokines

Results regarding the effects of typical neuroleptic <u>haloperidol</u> on Th1 parameters were conflicting.

Haloperidol was shown either to *increase* sIL-2R (Kowalski et al., 2000), IL-2 (Rudolf et al., 2002), and IFN-γ in vitro production (Rudolf et al., 2002) or to *decrease* TNF-α, IL-2 (Kowalski et al., 2000) in vitro production, and serum IL-2 (Kim et al., 2000) or to have *no effect* on IL-2 in vitro production (Rudolf et al., 2002; Boukhris et al., 1988), plasma sIL-2R, and TNF-α at a medium dosage (Pollmacher et al., 1997). The increase in sIL-2R levels was higher in patients with a predominance of positive symptoms compared with those whom dominated with both positive and negative symptoms (Kowalski et al., 2000). In addition, <u>perazine</u>, anther typical neuroleptic, was found to decrease TNF-α in vitro production (Kowalski et al., 2000).

Typical neuroleptics and Th2 cytokines

Reports regarding the effects of <u>haloperidol</u> on Th2 cytokines are rare. Only Pollmächer et al. found no influence of haloperidol on plasma IL-6 at medium dosage (Pollmacher et al., 1997).

The findings in this regard originated mostly from in vitro data gained from clinical studies or in less extent from in vivo data obtained from animal experiments. The problem of the clinical data from schizophrenic subjects is that most of the patients did not have the same experimental conditions. It occurred very frequently that different patients had been treated with different medicines for various lengths of period, some treated with different neuroleptics one after another, and some even under distinct neuroleptics simultaneously. The comparisons from the clinical data are often hard to explain. The in vivo data from animal experiments have advantages over those from schizophrenic patients in this regard; nonetheless, whether the results from animals can be totally transferred to humans remains as an unsolved issue.

4   Questions and hypothesis

Based on the findings regarding (1) the associations between the increased prevalence rates in schizophrenia and diverse epidemics of distinct viral infections a few decades ago, (2) positive antibody titers against distinct viruses in one part of schizophrenics, (3) the role of the immune system, especially Th2-shift in various viral infections, (4) distinct immune dysfunctions found in schizophrenia, and (5) the suggestions of two recent reviews (Schwarz et al., 2001b; Muller et al., 2000), the following questions were posed:

(1) Did a Th1/Th2 imbalance in favor of Th2-shift occur in any subgroup of schizophrenic patients?

(2) If yes, in which of the epidemiological and/or clinical schizophrenic subgroup(s) could a clear Th2-shift be observed?

(3) If yes, had any of those immunological and endocrinological parameter(s) measured in this study significantly contributed to the variances of diverse Th1/Th2 ratios in schizophrenia?

To the first question, we assumed that there was at least one schizophrenic subgroup having an apparent Th2-shift. The others were open questions, no hypothesis was supposed. Th2-shift was defined as significantly **reduced IFN-γ/IL-4** and/or **IFN-γ/IL-10** and/or **IFN-γ/IL-13 ratio** compared to healthy subjects.

Firstly, Th2-shift was examined in the whole group of schizophrenic subjects. Then, the schizophrenic patients were subdivided into diverse subgroups according to either **genders** (male/female) or distinct clinical features including (1) paranoid/non-paranoid schizophrenia, (2) drug-naïve/drug-free (pre-medicated) schizophrenia, (3) patients in the first-/other disease episode, (4) schizophrenics having different lengths of washout period ($\leq$ 1 week or $\geq$ 3 months), (5) schizophrenia with negative/positive family psychiatric history, (6) acute/chronic schizophrenia, (7) early/late onset schizophrenia, (8) schizophrenic patients with high/low scores on distinct PANSS subscales (negative, positive, and global), and (9) patients having different degrees of symptom severity. According to the scores on the Clinical Global Impressions (**CGI**: National Institute of Mental Health) at admission, at discharge, and the difference between both time points, schizophrenic patients were clustered into different

subgroups with distinct symptom severity. Moreover, the effects of <u>nicotine abuse</u> on Th1/Th2 cytokines were also scrutinized because it was believe to have impacts on cytokines.

In addition, despite of the well-known correlations between the immune and endocrine system (Egger, 1992), so far, no immunological investigation in schizophrenia has considered the influences from the endocrine system simultaneously. This is the first immunological study in schizophrenia research to have taken distinct <mark>endocrinological parameters</mark> into account. Diverse endocrinological parameters such as prolactin, cortisol, testosterone, estradiol, and the sex hormone binding globuline (SHBG) were measured. Those hormones were included into the analysis as <mark>co-variants</mark> in order to control their impacts on Th1/Th balance since they were found to have either promoting or inhibitory effects on Th1/Th2 cytokines (Protonotariou et al., 2004; Dimitrov et al., 2004; Iwata et al., 2004; Elenkov, 2004; Xie et al., 2002; Elenkov and Chrousos, 2002; Miyaura and Iwata, 2002; Angele et al., 2001). The inclusion of the endocrinological variables stated above into the analysis could further ensure that Th2-shift in schizophrenia was rather a result of disease process, but not just an interactive outcome of distinct hormones.

However, mainly due to the <u>space limit</u>, this <mark>report focus</mark>es on the findings from the <mark>whole schizophrenic group</mark> and both <mark>gender subgroups</mark>. The outcomes concerning various schizophrenic clinical subgroups were only briefly summarized in a table without presenting the detailed results from MANCOVA. The other reasons were that (1) there were a lot of missing data in many clinical variables and (2) that, therefore, different clinical parameters had different case numbers. In many circumstances, the case number of a schizophrenic clinical subgroup was so much smaller than that of healthy subjects. If including the significance tests of all demographical and endocrinological parameters of each single clinical subgroup, this report would become very complicated and confusing. Even if only the outcomes from multi-variance-analysis in all clinical subgroups presented, the content of this report would be too much.

However, even there was a big difference in case numbers, the diversities in Th1/Th2 ratios between many schizophrenic clinical subgroups and healthy controls reached a statistic significance level. They could, thus, offer a good insight into Th2-shift in distinct schizophrenic clinical subgroups. This was also one of our goals. Therefore, the results relating to distinct <u>clinical subgroups</u> were <u>briefly presented</u> in a table in order to give an

overview of "Th1/Th2 imbalance in diverse schizophrenic clinical subgroups" and simultaneously to **<u>reduce</u>** the **<u>complexity</u>** of this report.

5 Methods

5.1 Subjects

Totally, 114 schizophrenic patients, 36 psychiatric patients with schizophrenia-related disorders, and 101 healthy controls were included into the study. Ninety of the 114 schizophrenics, 36 patients with schizophrenia-related diseases, and 78 of the 101 healthy controls had complete serum data including serum Th1/Th2 cytokines/ratios, hormones, SHBG, age, and gender. Fifty-nine of the 114 schizophrenics, 25 of the 36 patients with schizophrenia-related disorders, and 78 control subjects had complete whole blood assay data which contained whole blood assay Th1/Th2 cytokines/ratios, hormones, SHBG, age, and gender. And 44 of the 114 schizophrenics, 14 of the 36 patients with schizophrenia-related disorders as well as 76 of the 101 healthy subjects had complete lymphocyte data regarding lymphocyte Th1/Th2 cytokines/ratios, hormones, SHBG, age, and gender. Altogether, 40 schizophrenic patients and 72 normal subjects had complete serum, whole blood assay, and lymphocyte data. The final 40 schizophrenics and 72 controls having complete data in serum, whole blood assay, and lymphocyte were included into the "multiple regression analysis" in order to compare data from diverse materials (serum, whole blood assay, lymphocyte) in the same subjects. The demographical data of both patient and control group as well as the clinical data of schizophrenic patients were described in "Results". All participants of this study had given their written informed consents to take part into the study.

The inclusion criteria for all subjects were: no severe medical disease, free of acute allergies, inflammatory disorders, autoimmune diseases, and clinically apparent infections. Further essential inclusion criteria for schizophrenic patients to fulfill were (1) the diagnosis of schizophrenia, (2) no history of psychotropic substance addiction or abuse except nicotine, and (3) no personality disorders according to DSM-IV (American Psychiatric Association, 1994). In addition, healthy controls were free of any psychiatric disorder and had no first-degree biological relative who had ever suffered or been suffering under any psychiatric disease.

5.2 Materials and preparation

Serum, whole blood as well as isolated lymphocytes of subjects were used to investigate the balance between both T-helper systems. The main analysis methods comprised Cytometric Bead Array (CBA), Enzyme-linked ImmunoSPOT (Elispot), and Enzyme-Linked Immunosorbent Assay (Elisa).

In order to prepare the blood samples for diverse methods, two different kinds of culture medium were needed. The first one (Medium I) contains RPMI 1640 medium with 2 g/l NaHCO3 as well as 0.532 g/l L-glutamate (Biochrom; Berlin, Germany) and 1% of sodium pyruvate (Biochrom; Berlin, Germany). The second kind of medium (Medium II) is Medium I supplemented with 10% of fetal bovine serum (Biochrom; Berlin, Germany).

All blood samples of subjects were drawn between 8 and 9 AM every morning. Two tubes of blood samples were collected in ethylene diamine tetra acetate (EDTA) tubes (Sarstedt Monovette; Nürmbrecht, Germany) and two further in anticoagulant citrate phosphate dextrose adenine (CPDA) tubes (Sarstedt Monovette; Nürmbrecht, Germany).

Preparation of blood samples

Right after collection, EDTA samples were centrifuged in an electronic centrifuge (Omnifuge 2.0 RS: Heraeus Sepatech, Germany) at 6°C with a speed of 4000 rpm for 10 minutes. Then the serum samples were transferred into distinct micro-tubes (Sarstedt; Nümbrecht, Germany) and stored at –80°C until use. CDPA blood samples were for the Whole Blood Assay and the Elispot analysis. For each subject, 200 μl of blood was firstly taken out of one CDPA tube, mixed with 3ml Medium II (Biochrom; Berlin, Germany) supplemented with 1% of penicillin streptomycin (Biochrom; Berlin, Germany), and kept in a 50ml-polypropylene falcon (Sarstedt; Nümbrecht, Germany). A final concentration of 5μg/ml phytohemagglutinin (PHA) (Biochrom; Berlin, Germany) was secondly added to the mixture of whole blood and culture medium. Then the mixture of whole blood, medium, and PHA was incubated at 37°C in 5% CO_2/95% humidity for 46 hours. After 46 hours, the stimulated whole blood was centrifuged at 6°C by 3270 rpm for 10 minutes; the resultant supernatant was distributed into several micro-tubes (SARSTEDT; Nümbrecht, Germany) and kept at –80°C until analysis. The leftover CDPA blood sample was mixed thoroughly with approximately 3 times as much Medium I for Elispot-analysis. About 30 ml of diluted whole blood was transferred slowly to a falcon containing 15 ml of biocoll separating isotonic solution (density = 1.077, Biochrom; Berlin, Germany) so that the diluted whole blood floated right on the surface of the separating solution. Afterwards, the blood sample with separating solution was centrifuged at 30°C by

1400 rpm for 40 minutes. This resulted in three clearly separable layers – the top one was the isolated lymphocytes. The lymphocytes were then washed with 50 ml of Medium I by centrifuging them twice at 1400 rpm for 10 minutes. After washing, 1400 µl of Medium II, 180 µl of dimethyl sulfoxide (DMSO) (Sigma; Steinheim, Germany), and additional 180 µl of fetal bovine serum were added to the isolated lymphocytes in a vial (Nunc, Denmark). It was firstly kept at –20°C for 2 hours and finally stored at –80°C until use.

5.3   Cytokine measurements

5.3.1   Cytometric Bead Array (CBA)

CBA is a newly developed Elisa-variant. The Human Th1/Th2 Cytokine CBA Kit-II (Becton Dickinson Pharmingen, USA) was used to quantify IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ protein levels in serum and PHA-stimulated whole blood. The BD CBA system uses the sensitivity of amplified fluorescence detection by flow cytometry to measure soluble analytes in a particle-based immunoassay. Flow cytometry allows discriminating different particles on the basis of size and color. The BD CBA employs a series of particles with discrete fluorescence intensities to simultaneously detect multiple soluble analytes. Each bead in CBA provides a capture surface for a specific protein and is analogous to an individually coated well in an ELISA plate. Compared to conventional ELISA, CBA has the following advantages: (a) the comparability among distinct cytokine productions of interest is much higher due to using the same sample to measure different cytokines. (b) Only a small volume of sample is required in order to measure several cytokines at one time. (c) The detectable ranges are much greater and thus particularly well suited to assessing in-vitro production of cytokines such as IFN-γ. (d) That the method could be conducted with falcons, in addition to microplates, and assessed by FACS flow cytometer allows flexibility of test volumes used. This is particularly advantageous for proteins having very low in vivo concentrations. CBA is thus more flexible than conventional ELISA. However, its precision remains at least as good as conventional ELISA. According to the manual of the manufacturer, the intra-assay coefficients of variation for IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ are 2-5%, 3-4%, 2-3%, 2-3%, 2-4%, and 3-4%, respectively. The inter-assay coefficients of variation for cytokines in the same order as stated above are 4-9%, 3-7%, 3-7%, 4-6%, 3-6%, and 5-11%, correspondingly. The sensitivities of each cytokine measured here in the same order as the above stated are 2.6, 2.6, 3.0, 2.8, 2.8, and 7.1 pg/ml, respectively.

Whole blood assay

The procedure consists of (1) preparation of human Th1/Th2 cytokine standards, (2) preparation of mixed human Th1/Th2 cytokine capture beads, (3) CBA, cytometer setup, (4) data acquisition and analysis. For each vial of lyophilized human Th1/Th2 cytokine standards, 200 µl of assay diluent was added; this was the original cytokine standard. Afterwards, both standards vials were agitated in order to mix thoroughly and then let stand for 30 minutes.

Preparation of cytokine standards

Sixteen 12×75 mm BD Falcons were labeled with a red marker from 0 to 15. Another 14 BD falcons were labeled with a blue marker from 1 to 14; in each of the blue #1 to #13 tubes, 200 µl of assay diluent was added. Three hundred µl of cytokine standards and 25 µl of assay diluent were firstly added into the falcon blue #14, and then mixed thoroughly; this was the top diluted cytokine standard. Secondly, 200 µl of the diluted cytokine standard from blue #14 was then transferred to the falcon "blue #13" and mixed with 200 µl of assay diluent in it. After thoroughly mixing, 200 µl of standard diluent from the falcon "blue #13" was added to the falcon "blue #12". So through transferring 200 µl of diluted standard to the next tube with a lower number, a series of standard diluents was done. In the final, 50 µl of diluted cytokine standard from each tube marked with blue labels was transferred into the red-labeled falcon with a corresponding number. The falcon "red #0" was filled with 50 µl of pure assay diluent and the one with "red #15" with 50 µl of the original cytokine standard without any further dilution.

Preparation of cytokine capture beads

Depending on the number of test samples, each falcon was labeled with a unique identification number in order to distinguish various samples, which were filled in the falcons. Fifty µl of the supernatant from each subject was transferred into the corresponding falcon. Then, all falcons filled with cytokine standards or supernatants were stored in a cool box until the next step.

Before each capture bead suspension was transferred into a new falcon, it must be vigorously mixed for a few seconds. The volume of each capture bead suspension depends upon the number of assay tubes, including all cytokine standards and test samples. Usually, except cytokine standards only maximum 50 samples can be measured; therefore, there were total 65 assay tubes. For each cytokine standard or test sample tube, 10 µl of capture bead for each cytokine was needed. So for 6 different cytokines and 65 assay tubes (cytokine standards plus test samples), it summed up to be 3900 µl ($10\mu l \times 6 \times 65 = 3900$ µl). The mixture of diverse cytokine capture beads was then mixed thoroughly.

To each cytokine standard and test sample tube, further 50 µl of the capture bead mixture and 50 µl of the PE detection reagent were added. The final mixture of standards/samples, capture beads, and PE detection reagent let stand at room temperature, protected from exposure to light, and incubated for 3 hours. Three hours later, each final assay tube was washed with 1 ml of wash buffer (centrifuged at 200 × g for 5 minutes) and then drained carefully. Afterwards, another 300 µl of fresh wash buffer was added to each assay tube in order to re-suspend the bead pellet. Right before analyzing standards/samples on a flow cytometer, each assay tube was agitated for 3-5 seconds.

Serum samples

The procedure to measure cytokine levels in sera was basically the same as that for whole blood assay. The only diversity was the volumes of serum, diverse capture beads, and PE detection reagent. In each case, 100 µl, instead of 50 µl, of serum, capture beads, and PE detection reagent were added to each assay tube due too low cytokine levels in serum in many subjects, especially IL-2 and IL-4, as well as due to lower sensitivity of IFN-γ capture antibodies. Nevertheless, for each standard tube, the volumes of cytokine standards, capture beads, and PE detection reagent were the same as those for the whole blood samples. After this minor modification, all 6 cytokine levels measured in serum were detectable in all subjects, including healthy controls and schizophrenic patients. So, the final resulted measures for distinct Th1/Zh2 cytokines were divided by 2 in order to obtain the original cytokine levels.

5.3.2 Enzyme-linked ImmunoSPOT (ELISPOT)

ELISPOT was used to assess the in vitro productions of IFN-γ, IL-4, IL-12, IL-13, and IL-10 by isolated lymphocytes. The antibodies against the cytokines stated above are from MABTECH (Sweden). The first/capture antibodies applied in the order mentioned above were Mab1-DIK, MabIL-4-I, Mab IL12-I, MabIL13-II, and Mab 9D7. The second/detection antibodies used were Mab-1-Biotin, Mab IL4-II Biotin, Mab IL12-II Biotin, Mab IL13-II-Biotin, and Mab 12G8-Biotin. And the enzyme applied was Streptavidin-Alkaline Phosphatase-PQ. The micro-plates used to perform ELISPOT were from Millipore (Molsheim, France) MultiScreen Sterile Clear Plates with 0.45 µm surfactant and had mixed cellulose ester membranes.

The ELISPOT technique was originally used to enumerate antibody secreting B cells. It is a variation of the ELISA. The ELISPOT assay is a sensitive assay for analysis of cell activation at the single-cell level. Compared to ELISA, ELISPOT is more suitable to detect cytokines with very low in vitro productions since the detection sensitivity can be improved through manipulating cell concentrations. In order to compare the balance of the main Th1/Th2 cytokines within the same length of period, the incubation duration for all Th1/Th2 cytokines was the same – 46 hours, although according to the instructions of the Mabtech and relevant literature, the Th1 cytokines require much shorter time to be released than the Th2 ones. In addition, due to the great variations in productions among distinct cytokines, diverse cell concentrations were use to detect different cytokines; for IFN-γ 40,000 cells/well and 80,000 cells/well were used, for IL-4 and IL-13 200,000 cells/well and 160,000 cells/well, and for IL-10 and IL-12 80,000 cells/well and 160,000 cells/well.

The assay was performed in 96-well microtiter plates. Firstly, the wells were coated with 100 μl of high affinity monoclonal capture antibodies with a concentration of 7.5μg/ml (diluted with coating buffer $NaHCO_3$ + Na_2CO_3, pH = 9.6) and incubated at 4°C overnight to let the capture antibodies bind to the plate membrane. The very next day, the plate was washed with 200 μl/well of filtered PBS (pH = 7.4) for 6 times. Then cells with various concentrations, depending on the cytokine detected, were added to each well and incubated at 37°C/5% CO_2/95% humidity for 46 hours. This procedure was performed under a sterile flow. Forty-six hours later, cells were removed by washing with PBS for 6 times as the previous description. Then 100 μl of the biotinylated detection antibodies (1μg/ml) were added to each well and incubated for 2 hours. During this period antigen-specific responding cells released the cytokine that was captured in the immediate vicinity of the cells. After 2-hour incubation and 6-time washing, 100 μl of streptavidin conjugated with enzyme alkaline phosphatase (1μg/ml) was added to each well and the plate was incubated for 2 hours until spots emerged at the site of the responding cells. The spots were examined and counted by KS ELISPOT release 4.4.11.2001 (Carl Zeiss, Germany).

5.3.3 Enzyme-Linked ImmunoSorbent Assay (ELISA)

The ELISA is a fundamental tool of clinical immunology. Based on the principle of antibody-antibody interaction, this test allows for easy visualization of results and can be completed without the additional concern of radioactive materials use. Purified antibodies against

proteins of interest were pre-coated onto an ELISA plate. If sera or supernatants tested contain the proteins of interest, those antibodies will bind to the proteins on the plate. Anti-human immunoglobulins, the second antibodies, are coupled to an enzyme. The second antibody is added to the plate and binds to proteins of interest. In the end, substrate is added to the plate, changing the color of the wells when substrate is cleaved by the enzyme attached to the second antibody. The darker the color of each well, the greater the quantity of proteins studied in each well.

R&D ELISA kits (Minneapolis, USA) were used to measure IL-12 and IL-13 due to exclusion of these two cytokines in the human Th1/Th1 cytokine CBA-kit. The assay procedure is similar to that of CBA, however, impossible to be modified except lowering the bottom standard. The highest detectable range for IL-12 is 500 pg/ml and for IL-13 is 4000 pg/ml. For whole blood and plasma/serum IL-12, the coefficients of variation for intra- and inter-assay were 1.1-1.5% and 3.3-7.7%, respectively, while 2.4-6.3% and 3.8-6.5% for IL-13.

The samples used to measure IL-12 and IL-13 were only supernatants of whole blood because the majority of serum samples probably lie under the detectable levels of the kits since most of the attained measures for in-vitro production, particularly those of IL-13, were undetectable. Basically, the steps to detect IL-12 and IL-13 are the same; the only diversity lies in the volumes of various solutions/reagents required. Firstly, the required reagents/solutions such as wash buffer and cytokine standards were prepared. Secondly, 50 μl and 100 μl of assay diluent were added to each well of the IL-12 and IL-13 microplate, respectively. Thirdly, 200 μl and 100 μl of standards or supernatants were added to each well of the IL-12 and IL-13 microplate, correspondingly. Fourthly, the mixtures of the microplate were incubated for 2 hours at room temperature. After 2 hours, the microplate was repeatedly washed and aspirated for three times. Fifthly, the microplate was added with 200 μl of conjugate to each well and incubated for another 2 hours. Two hours later, washing and aspiration were repeated for 3 times. Then, 200 μl of substrate solution was added to each well; the substrate solutions were incubated in darkness for 20 and 30 minutes for IL-12 and IL-13, correspondingly. Finally, 50 μl of stop solution was added to each well. Right after addition of stop solution, the optical density of each well was determined using a microplate reader.

5.4 Hormone assessment

The serum levels of various hormones including prolactin, cortisol, estradiol, testosterone, and the sex hormone binding globuline (SHBG) were measured by using corresponding Elecsys Kits (Roche Diagnostics; Mannheim, Germany). The sensitivities of diverse Kits for the hormones in the order stated above were 0.47 ng/ml, 0.036 µg/dl, 5 pg/ml, 0.02 ng/ml, and 0.35 nmol/l, respectively.

5.5 Statistic analysis

The immunological parameters included Th1 cytokines IFN-γ, IL-2, TNF-α, and IL-12 as well as Th2 cytokines IL-4, IL-10, IL-6, and IL-13. The endocrinological parameters including stress hormones like cortisol, prolactin, sex hormones such as estradiol, testosterone, and the sex hormone binding protein (SHBG) were also assessed since they were reported to have impacts on Th1/Th2 cytokines. The independent variables contained distinct diagnostic groups (e.g. schizophrenia, schizophrenia-related disorders, controls) and various schizophrenic subgroups (e.g. male schizophrenics vs. male controls or paranoid/non-paranoid schizophrenics vs. normal subjects). The dependent variables consisted of different Th1/Th2 cytokines and ratios obtained from serum, whole blood assay, and lymphocyte data. The endocrinological parameters stated above and age were included into the analysis as co-variant(s) if any of them clearly distinguished the corresponding index-groups.

Depending on the questions addressed, diverse statistics were applied to evaluate the data. Cluster-center analysis was used to divide subjects or patients into distinct subgroups. One-way ANOVA was applied to compare the differences in age, illness duration, number of disease episode, and onset age between 2 groups. Cross-table analysis was conducted to compare variables with nominal levels such as case number of nicotine abuse, family psychiatric history, pre-medication (yes vs. no), washout period, drug-free/drug-naïve, first/other-episode, acute/chronic, early/late onset, and clinical diagnostic subgroups. MANOVA was used to compare the diversity in various hormones, SHBG levels, scores on the PANSS scale and the CGI. Additionally, MAN(C)OVA was also applied to compare cytokine productions/levels and Th1/Th2 ratios. Multiple regression analysis was conducted to detect the partial relationships between a criterion (e.g. IFN-γ/IL-4) and distinct predictors (e.g. hormones and cytokines). Furthermore, Pearson correlations were calculated for cytokine levels/productions, Th1/Th2 ratios, and the scores on the PANSS scale.

6   Results

The results contain the following parts: (1) **Demographical data**, (2) **Endocrinological data**, (3) **Clinical data**, (4) **Th1/Th2 cytokines and ratios in schizophrenia**: the whole schizophrenic group & distinct schizophrenic subgroups – results from MAN(C)OVA, (5) **Contributors of Th1/Th2 imbalance in schizophrenia** – outcomes from Multiple Regression, (6) **Influencing factors of Th1/Th2 cytokines in schizophrenia** – findings from Multiple Regression, as well as (7) **Correlations between Th1/Th2 cytokines/ratios and psychopathology in schizophrenics** – results from Pearson correlations.

6.1   Demographical data

Altogether, there were 114 patients with schizophrenia, 36 patients having related disorders (such as schizoaffective disorder, acute psychotic disorder etc.), and 101 healthy controls participated into the study. The total subjects were divided into 3 different groups for various analyses (serum CBA, whole blood CBA, ELISA, and lymphocyte ELISPOT). Therefore, this report contains serum, whole blood, and lymphocyte data. These 3 kinds of data overlapped only partially with one another.

Serum data

Totally, 90 schizophrenic subjects (abbreviated as SCH), 36 patients with schizophrenia-related diseases (SCH-R), and 78 healthy controls (CON) had serum cytokine data. Among them, there were 76 schizophrenics, 75 healthy controls, and 26 patients with schizophrenia-related disorders having complete data for gender, age, hormones, SHBG, and serum Th1/Th2 ratios. Schizophrenics were averagely 36.08 years (SD = 13.31), patients with schizophrenia-related disorders were 32.77 years (SD = 10.54), and controls were 29.59 years old (SD = 8.32). ANOVA post-hoc Schéffe tests revealed that only the diversity between schizophrenics and healthy subjects reached a significance level (SCH vs. CON: F = 12.88, p < .001; SCH-R vs. CON: F = 2.45, p = .12; SCH vs. SCH-R: F = 1.32, p = .25). Furthermore, the female/male ratios among these three diagnostic groups did not significantly differ from one another. There were 42 males and 34 females in the schizophrenic group, 11 men and 15 women in the group having schizophrenia-related disorders as well as 40 males and 35 females in the

control group (SCH vs. CON: χ^2 = .06, p = .81; SCH-R vs. CON: χ^2 = .94, p = .33; SCH vs. SCH-R: χ^2 = 1.30, p = .25).

Whole blood data

Whole blood assay Th1/Th2 cytokine data were collected from 59 schizophrenics, 25 patients having schizophrenia-related disorders, and 78 control subjects. Among those subjects stated above, there were only 44 schizophrenics, 14 patients with schizophrenia-related disorders, and 76 healthy subjects having complete data for whole blood assay Th1/Th2 ratios, age, gender, hormones, and SHBG. The average ages of the schizophrenic and control group were evidently different, but those of patients having schizophrenia-related disorders and normal subjects as well as those of both patient groups were not clearly distinguishable (SCH vs. CON: F = 11.39, p < .001; SCH-R vs. CON: F = 3.41, p = .07; SCH vs. SCH-R: F = 1.32, p = .25). Schizophrenics were the oldest ones having an average age of 36.25 years (SD = 12.68), then patients with schizophrenia-related disorders having a mean age of 34.36 years (SD = 9.61), and the controls were the youngest ones with an average age of 29.75 years (SD = 8.39). Moreover, the male/female ratios were relatively similar among these three diagnostic groups. The schizophrenic group consisted of 26 males and 18 females, the schizophrenia-related patient group contained 9 men and 5 women, while the control group comprised 41 male and 35 female subjects (SCH vs. CON: χ^2 = .30, p = .59; SCH-R vs. CON: χ^2 = .51, p = .47; SCH vs. SCH-R: χ^2 = .12, p = .73).

In addition, there were 25 schizophrenics (12 males + 13 females) and 39 healthy controls (24 males + 15 females) whose IL-12 and IL-13 in PHA-stimulated whole blood were also measured. Nevertheless, only 9 schizophrenics (6 males and 3 females) and 23 controls (12 males and 11 females) had completed demographical, endocrinological data as well as detectable IL-12 in vitro productions. No clear disparity was found between these two diagnostic groups regarding gender distribution and age (SCH – age: M = 33.89 yrs, SD = 13.92; CON – age: M = 29.78, SD = 7.70; F = 1.15, p = .29; gender distribution: χ^2 = .55, p = .46). Totally, there were 10 schizophrenics (4 males and 6 females) and 25controls (18 males and 7 females) having completed data for age, gender, hormones, SHBG, and detectable IL-13. Schizophrenics were apparently older than controls (gender distribution: χ^2 = 3.13, p = .08; SCH – age: M = 37.40 yrs, p = 14.77; CON – age: M = 28.84, SD = 5.48; F = 6.44, p = .02). Moreover, the schizophrenic group tended to have significantly less males than the control group.

Lymphocyte data

Altogether, there were 72 schizophrenics, 30 patients with schizophrenia-related disorders, and 101 controls having lymphocyte-produced cytokine data. However, only 54 schizophrenics, 18 schizophrenia-related patients, and 98 healthy controls had complete data for lymphocyte Th1/Th2 cytokines/ratios, hormones, SHBG, age, and gender. The 54 schizophrenics were averagely 34.43 years old (SD = 12.42), the controls were 29.24 years (SD = 8.90), and the patients with schizophrenia-related disorders were 34.00 years old (SD = 10.50). The disparities between any one patient group and the control group were conspicuously different, however, not that between both patient groups (SCH vs. CON: F = 8.84, p = .003; SCH-R vs. CON: F = 4.10, p = .05; SCH vs. SCH-R: F = .02, p = .90).

Table 6-1(1): A summary of subject numbers (N), ages (means/standard deviations), and gender distributions (male = ♂, female = ♀) in both schizophrenic and control group who had either complete serum or whole blood assay or lymphocyte data.

Diagnostic group	SCH		CON	
Gender	♀	♂	♀	♂
Analysis-materials	N; M of age (SD)	N; M of age (SD)	N; M of age (SD)	N; M of age (SD)
Serum	34; 39.97 (12.14)	42; 32.93 (13.51)	35; 29.34(7.84)	40; 29.80 (8.81)
	Σ = 76; 36.08 (13.31)		Σ = 75; 29.59 (8.32)	
Whole blood assay	18; 39.17 (12.39)	26; 34.23 (12.72)	35; 29.34(7.84)	41; 30.10(8.91)
	Σ = 44; 36.25 (12.68)		Σ = 76; 29.75(8.39)	
Lymphocyte	22; 38.41 (13.14)	32; 31.69 (11.31)	45; 29.80(9.01)	53; 28.77(8.86)
	Σ = 54; 34.42 (12.42)		Σ = 98; 27.24 (8.90)	
Note	M = mean; SD = standard deviation. SCH = schizophrenia; CON = healthy controls; age (years old).			

Summary : **Table 6-1(1)** offers an overview for the demographical data of schizophrenics and healthy controls. Totally, 76 schizophrenics, 26 patients with schizophrenia-related disorders, and 75 controls had complete data for serum Th1/Th2 ratios, hormones, SHBG, ages, and genders. The whole blood data for all the parameters stated above were also available in 44 schizophrenics, 14 patients with schizophrenia-related disorders, and 76 healthy subjects. Furthermore, 54 patients with schizophrenia, 18 psychiatric patients having schizophrenia-related disorders as well as 98 control persons had data regarding lymphocyte Th1/Th2 ratios and the rest of parameters mentioned above. Those subjects were included in the following analyses.

6.2 *Endocrinological data*

Details regarding the means and standard deviations of the hormone-data in schizophrenics and normal controls are summarized in **Table 6-2(1)**. It's noteworthy that the aim to measure diverse hormones was to examine whether there was any significant difference in any of the **stress/sex hormones** between two index-groups because they were believed to have impacts on Th1/Th2 balance. If there were, then they would be included into the corresponding multi-variance analysis as **co-variants**.

Serum data

In general, patients with schizophrenia-related disorders had the lowest cortisol and testosterone levels, nonetheless, the highest prolactin and estradiol levels among these three diagnostic groups. The controls had the highest cortisol, testosterone, SHBG, but the lowest prolactin levels. Nevertheless, schizophrenics had all values in-between but SHBG; they had the lowest SHBG levels among these three groups.

Multi-variant comparisons between the 75 healthy subjects and the 76 schizophrenic patients who had complete data for serum Th1/Th2 ratios, hormones, ages, and gender demonstrated evident disparities between both diagnostic groups in cortisol, prolactin, testosterone, and SHBG (cortisol: F = 7.47, p = .007; prolactin: F = 12.68, p < .001; testosterone: F = 6.29, p = .01; SHBG: F = 10.55, p = .001; estradiol: F = .01, p = .91). Between the 26 patients with schizophrenia-related disorders and control subjects existed also marked diversities in terms of their cortisol and prolactin levels; in addition, the difference in testosterone showed a trend to be significant as well (cortisol: F = 6.21, p = .01; prolactin: F = 15.36, p < .001; estradiol: F = 1.19, p = .28; testosterone: F = 2.86, p = .09; SHBG: F = .71, p = .40). However, between both patient groups no obvious diversity was observed in those regards (cortisol: F = .82, p = .37; prolactin: F = .24, p = .63; estradiol: F = 1.47, p = .23; testosterone: F = .01, p = .93; SHBG: F = 2.76, p = .10).

Whole blood data

Whole blood assay data demonstrated that schizophrenics had the lowest levels of cortisol and testosterone, but the highest estradiol levels. Patients with schizophrenia-related disorders had the highest prolactin, but the lowest SHBG levels. However, the controls had the highest cortisol, testosterone, and SHBG levels, although their prolactin and estradiol levels were the lowest among these three groups.

The findings from multi-variance analysis revealed no distinct discrepancy between both patient groups regarding cortisol, prolactin, estradiol, testosterone, and SHBG levels. Compared to controls, schizophrenics had significantly lower cortisol and SHBG, nevertheless, higher prolactin, while patients with schizophrenia-related diseases had markedly higher prolactin levels (SCH vs. CON – cortisol: F = 4.60, p = .03; prolactin: F = 15.20, p < .001; SHBG: F= 3.84, p = .05; testosterone: F = 2.89, p = .09; estradiol: F = .17, p = .68; SCH-R vs. CON – prolactin: F = 11.91, p = .001; cortisol: F = 1.39, p = .24; SHBG: F = 2.18, p = .14; testosterone: F = .13, p = .72; estradiol: F = .02, p = .88; SCH vs. SCH-R – cortisol: F = .02, p = .89; prolactin: F = .03, p = .87; estradiol: F = .01, p = .91; testosterone: F = .52, p = .48; SHBG: F = .07, p = .79).

Additionally, between the 9 schizophrenics and 23 healthy controls having complete ELISA IL-12 data, the diversities in prolactin and estradiol tended to be significantly different. Schizophrenics tended to have markedly higher prolactin and lower estradiol levels than healthy controls (IL-12 – cortisol: F = 1.30, p = .26; prolactin: F = 3.83, p = .06; estradiol: F = 3.96, p = .06; testosterone: F = .90, p = .35; SHBG: F = 2.10, p = .16). Ten schizophrenic patients and 25 controls had complete ELISA IL-13 data; between these 2 groups, the disparity in testosterone levels was significant as well. Schizophrenics had remarkably lower testosterone than their healthy counterparts (IL-13 – cortisol: F = .81, p = .38; prolactin: F = .10, p = .76; estradiol: F = .25, p = .62; testosterone: F = 6.40, p = .02; SHBG: F = .59, p = .45).

Lymphocyte data

Generally speaking, among the subjects who had complete lymphocyte data, schizophrenics had the highest prolactin, nevertheless, the lowest estradiol, testosterone, and SHBG levels. The controls had the highest cortisol, testosterone, and SHBG, but the lowest prolactin levels. However, the patients with schizophrenia-related disorders had the highest estradiol, nonetheless, the lowest cortisol levels among these three diagnostic groups.

Similar outcomes were obtained from multi-variance analysis of lymphocyte data. Between the 18 patients with schizophrenia-related disorders and the controls or the schizophrenic group, no marked difference was shown in cortisol, prolactin, estradiol, testosterone, and SHBG. Nevertheless, the 54 schizophrenics did have noticeably lower SHBG and tended to have significantly lower cortisol and testosterone levels than the 98 healthy subjects (SCH vs. CON – SHBG: F = 9.21, p = .003; cortisol: F = 3.74, p = .06; testosterone: F = 3.01, p = .09;

prolactin: F = 2.50, p = .12; estradiol: F = .01, p = .91; SCH-R vs. CON – cortisol: F = 2.30, p = .13; prolactin: F = .41, p = .52; estradiol: F = .52, p = .47; testosterone: F = .06, p = .81; SHBG: F = 2.48, p = .12; SCH vs. SCH-R – cortisol: F = .18, p = .68; prolactin: F = .17, p = .68; estradiol: F = .57, p = .45; testosterone: F = .84, p = .36; SHBG: F = .23, p = .64).

Table 6-2(1): A summary of hormone levels including cortisol, prolactin, estradiol, testosterone, and the sex hormone-binding globulin (SHBG) in schizophrenics and healthy controls.

Diagnostic group		**SCH**: M(SD)		**CON**: M (SD)	
Hormone	Material	♀	♂	♀	♂
Cortisol (µg/L)	Serum	160.25 (65.68)	172.18 (66.51)	233.86 (116.24)	176.27 (60.94)
	Σ	166.85 (65.97)		203.14 (94.88)	
	WB	160.00 (70.21)	172.57 (58.25)	233.86 (116.24)	174.52 (61.20)
	Σ	167.43 (62.93)		201.85 (94.92)	
	Lymph	165.17 (70.40)	175.87 (70.05)	233.85 (128.21)	174.41 (63.01)
	Σ	171.51 (69.73)		201.70 (102.33)	
Prolactin (ng/ml)	Serum	36.61 (51.82)	27.78 (23.00)	14.81 (5.80)	15.87 (12.06)
	Σ	31.73 (38.60)		15.37 (9.61)	
	WB	36.65 (47.75)	28.22 (21.43)	14.81 (5.80)	15.74 (11.94)
	Σ	31.67 (34.44)		15.31 (9.56)	
	Lymph	44.63 (24.85)	24.85 (20.03)	17.40 (14.86)	23.58 (62.90)
	Σ	32.91 (41.83)		20.74 (47.23)	
Estradiol (pg/ml)	Serum	63.01 (66.44)	31.57 (8.08)	62.28 (69.01)	32.72 (9.32)
	Σ	45.64 (47.17)		46.51 (49.54)	
	WB	77.23 (86.09)	31.94 (8.84)	62.28 (69.01)	32.65 (9.21)
	Σ	50.47 (59.02)		46.30 (49.24)	
	Lymph	64.08 (69.93)	30.98 (8.50)	61.73 (66.70)	31.57 (9.58)
	Σ	44.46 (47.43)		45.42 (47.91)	
(Total) Testosterone (ng/ml)	Serum	.53 (.27)	4.56 (2.13)	.73 (.93)	6.82 (1.74)
	Σ	2.75 (2.56)		3.97 (3.37)	
	WB	.49 (.26)	4.77 (2.06)	.73 (.93)	6.84 (1.73)
	Σ	3.02 (2.65)		4.03 (3.38)	
	Lymph	.58 (.30)	4.65 (2.03)	.84 (1.43)	6.53 (2.19)
	Σ	3.00 (2.56)		3.92 (3.41)	
SHBG (nmol/L)	Serum	73.49 (45.98)	33.02 (13.41)	116.38 (62.63)	42.41 (17.21)
	Σ	51.12 (37.93)		76.93 (57.78)	
	WB	91.26 (52.67)	32.90 (13.07)	116.38 (62.63)	42.60 (17.04)
	Σ	56.77 (45.15)		76.58 (57.48)	
	Lymph	76.25 (45.15)	32.01 (11.20)	116.09 (65.04)	44.42 (27.46)
	Σ	50.04 (36.91)		77.33 (60.10)	
Note	**Schizophrenia** (SCH) – *serum*: female N = 34, male N = 42; *WB* (whole blood): female N = 18, male N = 26; *lymph* (lymphocyte): female N = 22, male N = 32; **Control** (CON) – *serum*: female N = 35, male N = 40; *whole blood*: female N = 35, male N = 41; *lymphocyte*: female N = 45, male N = 53.				

Summary : Therefore, regardless of analysis material (serum or whole blood or lymphocyte), schizophrenic patients had usually lower cortisol, testosterone, and SHBG, nevertheless, higher prolactin levels. Their estradiol levels were comparable to those of healthy subjects. Those hormones and SHBG may have interacted with the cytokine network dynamically when cytokines were produced in vivo. They were also constantly present in whole blood as it was stimulated with PHA to release cytokines in vitro. They together or alone might, hence, have direct impacts on the balance between the Th1 and Th2 system according to several lines of evidence. Thus, they were included into the analysis of serum and whole blood assay data in order to control their effects on Th1/Th2 ratios if any of them was clearly distinguishable between two index-groups.

6.3   Clinical data

In the following sections, the clinical data are reported. There were missing data in distinct clinical variables including body weight, onset age, pre-medication, number of episode, wash-out-period, nicotine abuse, CGI (Clinical Global Impressions scale) and PANSS (the Positive and Negative Syndrome Scale) scores. The total numbers (Ns) of distinct clinical parameters vary not only with analysis material (serum, whole blood, lymphocyte) but also with clinical variables. The clinical data of schizophrenic patients are summarized in **Table 6-3(1)** as followed.

Table 6-3(1): A summary of clinical data in male, female, and the whole schizophrenic patients.

Clinical data of schizophrenic patients					
Clinical Variable	Material	♀	♂	Total (is)	Missing
Diagnostic subgroup (par:dis:oth)	Serum	29:4:1	28:9:5	57:13:6	0
	WB	15:2:1	20:4:2	35:6:3	0
	Lymph	17:4:1	25:5:2	42:9:3	0
Illness duration (years)	Serum	6.68 (7.39)	4.86 (7.88)	5.79 (7.60)	33
	WB	6.00 (6.25)	6.00 (8.39)	6.00 (7.25)	18
	Lymph	6.00 (5.79)	3.43 (5.44)	4.46 (5.64)	19
Onset age (years old)	Serum	32.23 (10.41)	26.43 (9.73)	29.40 (10.39)	33
	WB	31.56 (10.36)	26.24 (9.98)	28.08 (10.23)	18
	Lymph	30.50 (9.58)	26.00 (9.76)	27.80 (9.81)	19
Number of disease episode	Serum	2.57 (2.14)	3.10 (4.10)	2.83 (3.24)	34
	WB	1.75 (1.04)	3.59 (4.43)	3.00 (3.76)	19
	Lymph	2.15 (1.46)	2.24 (1.90)	2.21 (1.72)	20

1.episode vs. other episode	Serum	8:13	9:12	17:25	34
	WB	4:4	5:12	9:16	19
	Lymph	5:8	10:11	15:19	20
Drug-naïve vs. drug-free	Serum	6:16	7:14	13:30	33
	WB	3:6	3:14	6:20	18
	Lymph	4:10	8:13	12:23	19
Drug free ≤ 1w: >1w&<3m:≥3m	Serum	10:2:8	8:1:9	18:3:17	38
	WB	4:0:5	8:1:5	12:1:10	18
	Lymph	6:1:6	8:1:10	14:2:16	19
Family psychiatric history (yes vs. no)	Serum	4:16	9:9	13:25	38
	WB	2:10	8:7	10:17	17
	Lymph	2:10	9:10	11:20	23
Pre-medication (yes vs. no)	Serum	12 :10	11 :10	23 :20	33
	WB	4 :5	11 :6	15 :11	18
	Lymph	7 :7	10 :11	17 :18	19
Nicotine abuse (yes vs. no)	Serum	10 :11	12 :11	22 :22	32
	WB	4:4	9:8	13:12	19
	Lymph	8:5	13:9	21:14	19
PANSS (global)	Serum	49.09 (11.59)	50.30 (13.70)	49.65 (12.48)	33
	WB	52.40 (9.00)	53.06 (12.76)	52.81 (11.27)	18
	Lymph	50.55 (13.49)	49.73 (11.87)	50.20 (12.64)	19
PANSS (positive)	Serum	24.35 (7.34)	22.00 (5.41)	23.26 (6.55)	33
	WB	25.90 (6.12)	23.00 (5.59)	24.12 (5.85)	18
	Lymph	24.60 (6.06)	22.15 (5.30)	23.20 (5.69)	19
PANSS (negative)	Serum	25.43 (7.06)	27.80 (9.72)	26.53 (8.38)	33
	WB	26.70 (6.11)	30.88 (7.44)	29.27 (7.14)	18
	Lymph	26.73 (6.08)	27.35 (9.68)	27.09 (8.22)	19
CGI: admission	Serum	5.97 (.78)	5.88 (.81)	5.91 (.79)	6
	WB	6.00 (.76)	5.81 (.85)	5.88 (.82)	1
	Lymph	6.00 (.79)	5.88 (.83)	5.92 (.81)	2
CGI: discharge	Serum	3.90 (.98)	4.02 (.91)	3.97 (.93)	6
	WB	4.06 (1.09)	4.04 (.96)	4.05 (1.00)	1
	Lymph	4.00 (1.08)	4.09 (1.03)	4.06 (1.04)	2
Note	(1) WB = whole blood; Lymph = lymphocytes; (2) par = paranoid schizophrenia, dis = disorganized schizophrenia, oth = other diagnostic subgroups (including catatonic, residual, simple, other schizophrenia); (3) ≤ 1w = ≤ 1 week; >1w&<3m = >1 week & < 3 months; ≥3m = ≥ 3 months. (4) PANSS = the Positive and Negative Syndrome Scale; CGI = Clinical Global Impressions.				

Totally, 76 schizophrenics had total data for in vivo serum Th1/Th2 ratios, age, gender, and diverse endocrinological parameters, 44 schizophrenic patients had entire whole blood data, and 54 patients had complete data for in vitro lymphocyte Th1/Th2 ratios and further variables stated above.

Serum data

The 76 schizophrenics included 57 paranoid, 13 disorganized, 2 catatonic, 2 residual, 1 simple, and 1 other schizophrenia. Forty-two schizophrenic patients had data regarding their disease episode (first vs. other); 17 of them were in the first, while 25 of them were in the other episode. Thirty-eight schizophrenics had information about their family psychiatric history; 13 of them had positive family history, while 25 of them had no family psychiatric history. Forty-three of the 76 schizophrenics had data concerning anti-psychotic medication before recruitment; 23 of them had ever been treated with any neuroleptic, whereas 20 of them had never taken any anti-psychotic before admission in the hospital. Equal numbers of smokers (N = 22) and non-smokers (N = 22) were found among the 44 schizophrenics who had data regarding nicotine abuse. Thirty-eight patients had data concerning how long they had been free of neuroleptics as they were recruited; 18 of them had been drug-free for one week or less (but minimum 3 days), 17 of them being free of neuroleptics for at least 3 months, and 3 of them for a period in-between. The distributions of various clinical diagnostic subgroups, disease episode, neuroleptic medication, nicotine abuse, and washout period were relatively similar in male and female schizophrenics (diagnostic subgroups: χ^2 = 5.16, p = .40; disease episode: χ^2 = .10, p = .75; pre-medication: χ^2 = .02, p = .89; nicotine: χ^2 = .21, p = .65; length of wash-out: χ^2 = 1.45, p = .49). The only exception was family psychiatric history; it appeared that there were significantly more male than female schizophrenics having biological relatives who had ever suffered or been suffering under any psychiatric disorder (χ^2 = 3.79, p = .05).

In addition, the CGI scores at admission and discharge of 70 schizophrenics were available. Forty-three schizophrenic patients had data regarding onset age, illness duration, and scores on the PANSS positive, negative, and global scale. They had averagely been ill for 5.79 years (SD = 7.60) and had a mean disease-episode of 2.83 years (SD = 3.24). The average of their onset ages was 29.40 years old (SD = 10.39); male patients had an earlier onset than their female schizophrenic counterparts (♂: M = 26.43 yrs, SD = 9.73; ♀: M = 32.23 yrs, SD = 10.41). The mean scores on the PANSS negative, positive, and global subscale were 26.53 (SD =8.38), 23.26 (SD = 6.55), and 49.65 (SD = 12.48), respectively. Those for the CGI were 5.91 (SD = .79) at admission and 3.97 (SD = .93) as they were discharged. Between male and female schizophrenics, no noteworthy diversity in those regards stated above was observed except onset age. The difference between male and female schizophrenics in onset age tended to reach a statistic significance level (onset age: F = 3.55, p = .07; episode number: F= .27, p = .61; PANSS positive: F = 1.39, p = .25; PANSS negative: F = .85, p = .36; PANSS global:

F = .10, p = .76; CGI at admission: F = .20, p = .65; CGI at discharge: F = .32, p = .58). Female schizophrenics tended to have later onsets than their male schizophrenic counterparts.

Whole blood assay data

Among the 44 schizophrenic patients who had complete data for whole blood assay Th1/Th2 ratios, hormones, SHBG, age, and gender, there were 23 schizophrenics having data for wash-out period, 26 having data for drug-naïve/free, onset age, illness duration, and scores for the PANSS negative, positive, and global scale, 43 having data for the CGI at admission and discharge, 25 having information about disease episode, number of episode, and nicotine abuse, and 27 of the 44 schizophrenics having data for family psychiatric history.

On average, their diseases were manifested at the age of 28.08 years old (SD = 10.23). They had been suffering under schizophrenia averagely for 6 years (SD = 7.58) and had on average 3 disease episodes (SD = 3.76). There were 35 paranoid, 6 disorganized, 1 catatonic, 1 residual, and 1 other schizophrenia among them. Nine were in the first, while 16 were in other episode of disease. Ten of them had positive family psychiatric history, but 17 of them did not. Fifteen of them had ever been under neuroleptic medication, while 11 had never been treated with any anti-psychotic. Thirteen were smokers and 12 were non-smokers. Twelve of them had been neuroleptic-free for one week or less (but ≥ 3 days), 10 had a drug-free period of at least 3 months, and 1 had been free of neuroleptics for a period between 1 week and 3 months. If both pre-medication and disease episode are considered as standards for being drug-naïve/free, then 6 patients were drug-naïve (no pre-medication + 1. episode) and 20 of them were drug-free. At admission, their average scores on the PANSS positive, negative, global, and the CGI were 24.12 (SD = 5.85), 29.27 (SD = 7.14), 52.81 (SD = 11.27), and 5.88 (SD = .82), correspondingly. As they were discharged, the mean score on the CGI was 4.05 (SD = 1.00).

Similar to the serum data, between male and female schizophrenics, no clear diversity was found in any of those variables except family psychiatric history. There were proportionally more schizophrenic men than women who had positive family psychiatric history (family history: $\chi^2 = 3.84$, p = .05; diagnostic subgroup: $\chi^2 = 3.01$, p = .55; episode: $\chi^2 = 1.00$, p = .32; pre-medication: $\chi^2 = .99$, p = .32; nicotine abuse: $\chi^2 = .01$, p = .89; wash-out period: $\chi^2 = 3.07$, p = .22; naïve/free: $\chi^2 = .82$, p = .37; CGI at admission: F = .56, p = .46; CGI at discharge: F = .004, p = .95; PANSS positive: F = 1.54, p = .23; PANSS negative: F = 2.21, p = .15; PANSS

global: F = .02, p = .89; illness duration: F = .00, p = 1.00; episode number: F = 1.32, p = .26; onset age: F = 1.63, p = .21).

Lymphocyte data

Among the 54 schizophrenics, 52 of them had data concerning CGI scores at admission and discharge, 32 of them had data about wash-out period and family history, 34 of them had data concerning number of episode and disease episode (first vs. other), and 35 of them had data regarding pre-medication, nicotine abuse, drug-naïve/free, scores on the PANSS positive, negative, global scale, onset age, and illness duration.

The schizophrenic patients who had complete lymphocyte Th1/Th2 data and other parameters measured in this study started their diseases at an average age of 27.80 years (SD = 9.81). They had been through 2.21 disease episodes (SD = 1.72) and been ill for 4.46 years (SD = 5.64). As they were recruited in this study, the average scores on the CGI, the PANSS positive, negative, and global scale were 5.92 (SD = .81), 23.20 (SD = 5.69), 27.09 (SD = 8.22), and 50.20 (SD = 12.64), respectively. Totally, there were 42 paranoid, 9 disorganized, 1 catatonic, 1 residual, and 1 other schizophrenia having complete lymphocyte data. Fifteen were in their first disease episode, while 19 were in other episode. Eleven patients had positive family psychiatric history, while 20 of them did not. Seventeen schizophrenics had been pre-medicated, whereas 18 patients had never been treated with any neuroleptic. Twenty-one were smokers, while 14 of them were non-smokers. Fourteen schizophrenic patients had been drug-free maximum for one week, but at least for three days, 16 of them had been drug-free for at least 3 months; additionally, 2 of them had been free of neuroleptics for a period in-between.

Nonetheless, no significant diversity was observed between male and female schizophrenics in any of those clinical variables assessed in this study (family history: χ^2 = 3.03, p = .08; diagnostic subgroup: χ^2 = 2.88, p = .58; episode: χ^2 = .27, p = .60; pre-medication: χ^2 = .02, p = .89; nicotine: χ^2 = .02, p = .89; wash-out period: χ^2 = .42, p = .81; CGI at admission: F = .29, p = .60; CGI at discharge: F = .10, p = .76; PANSS positive: F = 1.62, p = .21; PANSS negative: F = .05, p = .83; PANSS global: F = .04, p = .85; illness duration: F = .61, p = .44; episode number: F = .27, p = .61; onset age: F = 3.55, p = .07). Female schizophrenics tended to have clear later onsets than their male schizophrenic counterparts. Moreover, it showed a trend to have more male than female schizophrenics with positive family psychiatric history.

Due to incomplete data regarding nicotine abuse in schizophrenics, it won't be included in the multi-variant comparisons between controls and various schizophrenic subgroups, although it was thought to have impacts on cytokine productions (Hinze-Selch and Pollmacher, 2001). Instead, it were be analyzed separately and reported in the section "6.4.3. Nicotine abuse, Th1/Th2 cytokines and ratios" in order to examine whether or not nicotine use had influences on Th1/Th2 cytokines and ratios.

6.4   Th1/Th2 cytokines and ratios in schizophrenia

In the subsequent sections, various figures regarding corresponding results are presented. Due to great deviations of extreme values from the majority and numerous significance markers on one figure (box-plot) in many cases, the figures are barely visible or differentiable from one another if without excluding the extreme values and outliers. Therefore, no extreme value and no outlier is shown in all the figures presented here.

In addition, because of great diversities in distinct cytokine levels/productions, it's impossible to present the results of different cytokines in one figure. So, all cytokine levels/productions and Th1/Th2 ratios shown in all figures of this report were standardized; that is, all cytokines and Th1/Th2 ratios had a mean of 0 and a standard deviation of 1.

6.4.1   The whole schizophrenic group

6.4.1.1   Schizophrenia vs. normal subjects

Serum data

Figure 6-4-1-1-1(1) to **Figure 6-4-1-1-1(3)** offer an overview of the results from comparing the serum Th1/Th2 cytokine data of all study participants.

Totally, 76 schizophrenic patients and 75 healthy subjects were included into this analysis. The schizophrenics were significantly older and had clearly reduced cortisol, testosterone, and SHBG, nevertheless, highly enhanced prolactin levels if compared to healthy controls. Therefore, those variables would be involved in the following multi-variance analysis as co-variants.

Generally speaking, the control group had greater ranges than the schizophrenic group in all cytokines measured. Schizophrenic patients had lower serum cytokine levels and both serum

Th1/Th2 ratios. Multi-variance analysis demonstrated that although no obvious diversity was found between schizophrenics and controls at single cytokine level, both diagnostic groups remarkably differed from each other in both serum Th1/Th2 ratios if including age, cortisol, testosterone, SHBG, and prolactin as co-variants (IFN-γ: F = .49, p = .48; IL-2: F = .36, p = .55; TNF-α: F = .28, p = .60; IL-4: F = .17, p = .68; IL-10: F = .24, p = .62; IL-6: .15, p = .70). The schizophrenic group had a mean of 14.10 (SD = 12.49) for IFN-γ/IL-4 and 15.50 (SD = 19.66) for IFN-γ/IL-10 ratio. In contrast, the controls obtained an average of 20.34 (SD = 18.12) for IFN-γ/IL-4 and 19.66 (SD = 9.73) for IFN-γ/IL-10 ratio. The diversity in IFN-γ/IL-4 achieved a significance level of .02 (F = 6.02) and that in IFN-γ/IL-10 ratio reached a significance level of .002 (F = 10.41).
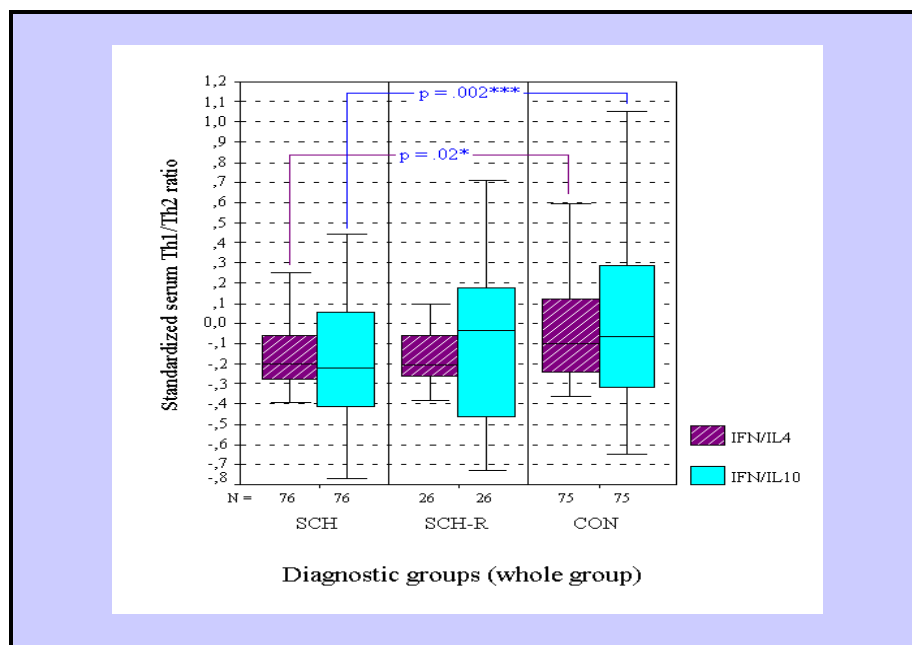
Figure 6-4-1-1-1(1): Standardized serum IFN-γ/IL-4 (IFN/IL4) and IFN-γ/IL-10 (IFN/IL10) ratio in patients with schizophrenia, schizophrenia-related disorders, and healthy subjects (SCH = schizophrenia; SCH-R = schizophrenia-related disorders; CON = controls).

Even if without co-varying with any parameter or if only including age as covariant or if including age, all hormones assessed in this study, and SHBG as co-variants, the findings regarding serum cytokine levels and Th1/Th2 ratios remained relatively constant (no covariant – IFN-γ/IL-4: F = 6.10, p = .02; IFN-γ/IL-10: F = 8.06, p = .005; IFN-γ: F = .88, p = .35; IL-2: F = .65, p = .42; TNF-α: F = .49, p = .49; IL-4: F = .48, p = .49; IL-10: F = .45, p = .50; IL-6: F = .31, p = .58; age as covariant – IFN-γ/IL-4: F = 4.96, p = .03; IFN-γ/ILI-10: F = 6.63, p = .01; IFN-γ: F = .67, p = .41; IL-2: F = .46, p = .50; TNF-α: F = .33, p = .57; IL-4: F

= .37, p = .54; IL-10: F = .31, p = .58; IL-6: F = .24, p = .63; co-vary with all hormones, SHBG, and age – IFN-γ/IL-4: F = 6.08, p = .02; IFN-γ/IL-10: F = 10.51, p = .001; IFN-γ: F = .48, p = .49; IL-2: F = .35, p = .55; TNF-α: F = .28, p = .60; IL-4: F = .16, p = .69; IL-10: F = .24, p = .63; IL-6: F = .15, p = .70). That is, schizophrenic patients had markedly decreased serum IFN-γ/IL-4 and IFN-γ/IL-10 ratios than their healthy counterparts, despite of remarkable disparities in age, hormone, and SHBG between both groups.
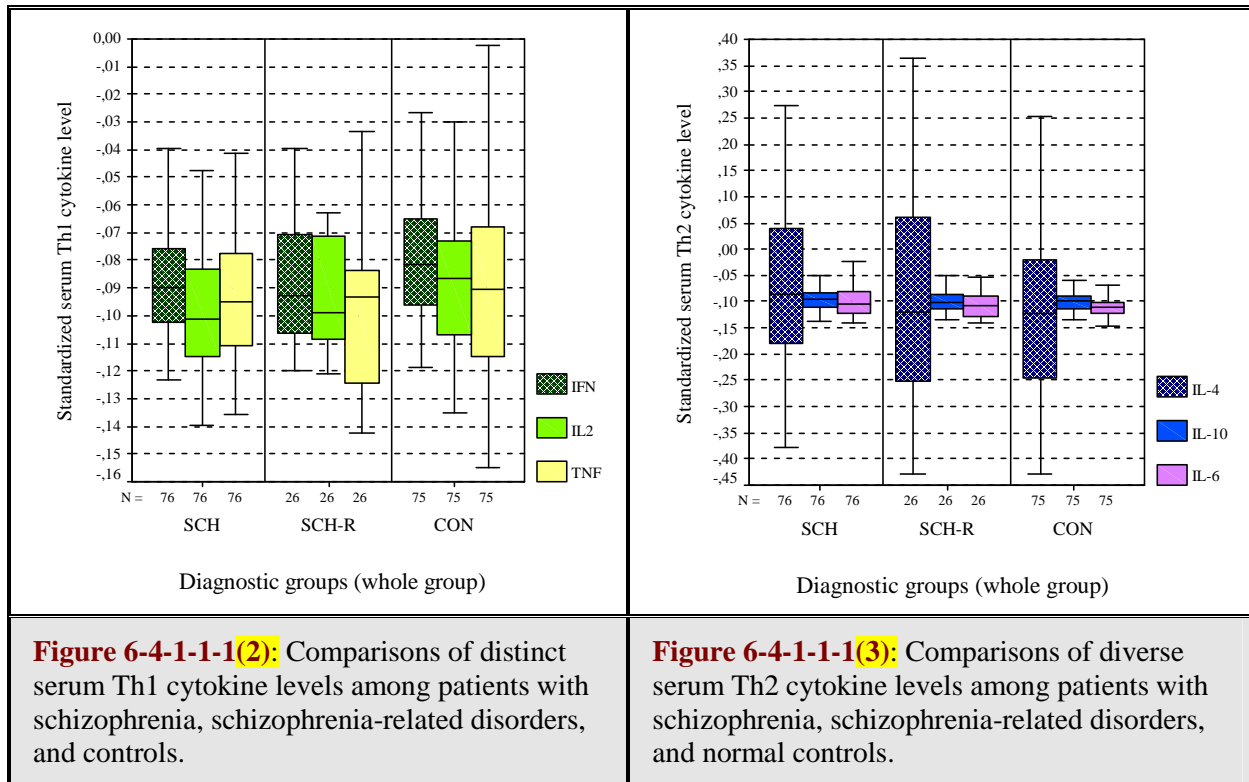
Figure 6-4-1-1-1(2): Comparisons of distinct serum Th1 cytokine levels among patients with schizophrenia, schizophrenia-related disorders, and controls.

Figure 6-4-1-1-1(3): Comparisons of diverse serum Th2 cytokine levels among patients with schizophrenia, schizophrenia-related disorders, and normal controls.

Note: Schizophrenics as a whole group appeared to have similar serum cytokine levels to healthy controls. However, if classifying both schizophrenics and controls together into four serum IFN-γ subgroups through conducting cluster analysis and excluding the 2 extreme cases/subgroups (one control the highest one 13345.6 pg/ml and one schizophrenic patient 1635.3 pg/ml), one way ANCOVA demonstrated that schizophrenics had significantly lower serum IFN-γ than controls if including age, cortisol, prolactin, testosterone, and SHBG as co-variants (F = 6.73, p = .01). Schizophrenics had averagely 39.41 pg/ml IFN-γ (SD = 20.94) and controls obtained 48.89 pg/ml IFN-γ (SD = 28.93). If dividing the subjects into 5 serum IL-2 subgroups and excluding the first two highest subgroups/cases (the highest one control 310.0 pg/ml and the second highest one subject with schizophrenia 83.7 pg/ml), the left over 75 schizophrenics tended to have lower serum IL-2 levels than the left over 74 controls if including age, cortisol, prolactin, testosterone, and SHBG as co-variants (F = 3.21, p = .08).

The mean serum IL-2 levels for schizophrenics and controls were 1.82 pg/ml (SD = .55) and 2.06 pg/ml (SD = .75), correspondingly. If the third highest case also excluded (one schizophrenic patient 11.3 pg/ml), the comparison between the low serum IL-2 schizophrenic patients (N = 72) and the low serum IL-2 normal controls (N = 69) revealed a clear discrepancy between both serum IL-2 subgroups if co-varying with age, cortisol, prolactin, testosterone, and SHBG (F = 5.15, p = .03). The average serum IL-2 level was 1.76 pg/ml (SD = .40) for the low serum IL-2 schizophrenics and 1.94 pg/ml (SD = .47) for controls. That is, one schizophrenic subgroup might have significantly lower serum IL-2 than healthy controls. However, excluding the two extreme serum TNF-α values (one schizophrenic subject 179.2 pg/ml and one control person 56.5 pg/ml), schizophrenics had lower TNF-α than controls; but the reduction failed to reach any significance level (SCH: M = 1.78 pg/ml, SD = .42; CON: M = 1.85, SD = .53; F = .89, p = .35).

If clustering subjects of both diagnostic groups into various serum IL-6 subgroups and excluding the extreme cases/subgroups (one control: the highest one 450 pg/ml; one schizophrenic: 112.1 pg/ml; two schizophrenics: 35.2 pg/ml; 2 subjects of each group: 8.2 pg/ml), the left over 71 schizophrenics did have significantly higher serum IL-6 levels than the remaining 72 controls if including age, cortisol, prolactin, testosterone, and SHBG as co-variants (F = 5.33, p = .02). Schizophrenic patients had a mean of 2.52 pg/ml (SD = .93) and controls had an average of 2.25 pg/ml (SD = .78) serum IL-6. If dividing both schizophrenic and control subjects into three IL-4 subgroups, 3 extreme cases were found; one control had the highest serum IL-4 (104.1 pg/ml), another control case and one schizophrenic subject together obtained a mean serum IL-4 of 15.2 pg/ml. If excluding the 3 extreme values, the 75 schizophrenics tended to have higher serum IL-4 than the remaining 73 controls (F = 3.64, p = .06). The mean of serum IL-4 was 3.40 pg/ml (SD = 1.26) for schizophrenics and 3.04 pg/ml (SD = 1.30) for healthy controls. In addition, cluster analysis divided subjects of both diagnostic groups into 4 serum IL-10 subgroups. Excluding the extreme cases (one control subject 471.6 pg/ml and one schizophrenic patient 143.7 pg/ml), uni-variant comparison revealed that the left over 75 schizophrenics had a higher average of serum IL-10 levels than the remaining 74 controls; however, the enhancement was not statistically significant (F = .11, p = .74). The mean serum IL-10 levels for schizophrenics and controls were 2.71 pg/ml (SD = 1.07) and 2.66 pg/ml (SD = 1.27), respectively.

Summary : Serum data (see **Table 6-4-1(1)**) exhibited no disparity between controls and schizophrenic patients at single serum cytokine level if including extreme cases. Nevertheless, conspicuous differences were observed in both serum IFN-γ/IL-4 and IFN-γ/IL-10 ratio. If excluding extreme values, then schizophrenics had significantly lower serum IFN-γ, however, markedly higher IL-6; additionally, they showed a tendency to have lower serum IL-2, but higher IL-4 levels than normal subjects.

Table 6-4-1(1): Comparisons of serum cytokine levels and Th1/Th2 ratios among schizophrenics, patients with schizophrenia-related disorders, and healthy subjects.

| Serum Th1/Th2 cytokine levels and Th1/Th2 ratios: comparisons among schizophrenia, schizophrenia-related disorders, and healthy controls [M(SD)]			
Group	Schizophrenia (N = 76)	Related disorder (N = 26)	Controls (N = 75)
IFN-γ	60.41 (184.24)/ *39.41(20.94)***	64.44 (136.48)	226.49 (1535.25)/ *48.89 (28.93)*
IL-2	2.91 (9.40)	2.71 (4.03)	6.29 (35.56)
TNF-α	2.50 (6.29)	1.75 (.50)	4.22 (20.48)
IL-4	3.58 (1.97)/*3.40 (1.26)*	3.22 (1.31)	4.53 (11.78)/*3.04 (1.30)*
IL-10	4.56 (16.21)	3.79 (7.14)	8.91 (54.16)
IL-6	4.99 (13.57)/*2.50 (.93)**	3.29 (5.15)	8.39 (51.78)/*2.25(.78)*
IFN/IL4	14.10 (12.49)*	20.09 (33.57)	20.34 (18.12)
IFN/IL10	15.50 (8.26)***	16.48 (7.81)	19.66 (9.73)
Note	(1) Unit of cytokines (IFN-γ, IL-2…IL-6) = pg/ml ; (2) IFN/IL4 = IFN-γ/IL-4; IFN/IL10 = IFN-γ/IL-10; (3) Compared to controls: * $p \le .05$; ** $p \le .01$; *** $p \le .005$; (4) /... (The results from excluding extreme cases)		

Whole blood assay data

Figure 6-4-1-1-2(1) to **Figure 6-4-1-1-2(3)** display the global comparisons and significant results from multi-variance analysis in schizophrenics, patients with schizophrenia-related diseases, and healthy subjects.

Between those 44 schizophrenics and 76 control subjects who had complete data for whole blood assay Th1/Th2 ratios, obvious diversities in ages, cortisol, prolactin, and SHBG levels were found.

Using diagnostic group (schizophrenia vs. control) as independent variable and whole blood assay cytokine productions as well as Th1/Th2 ratios as dependent variables, multi-variant
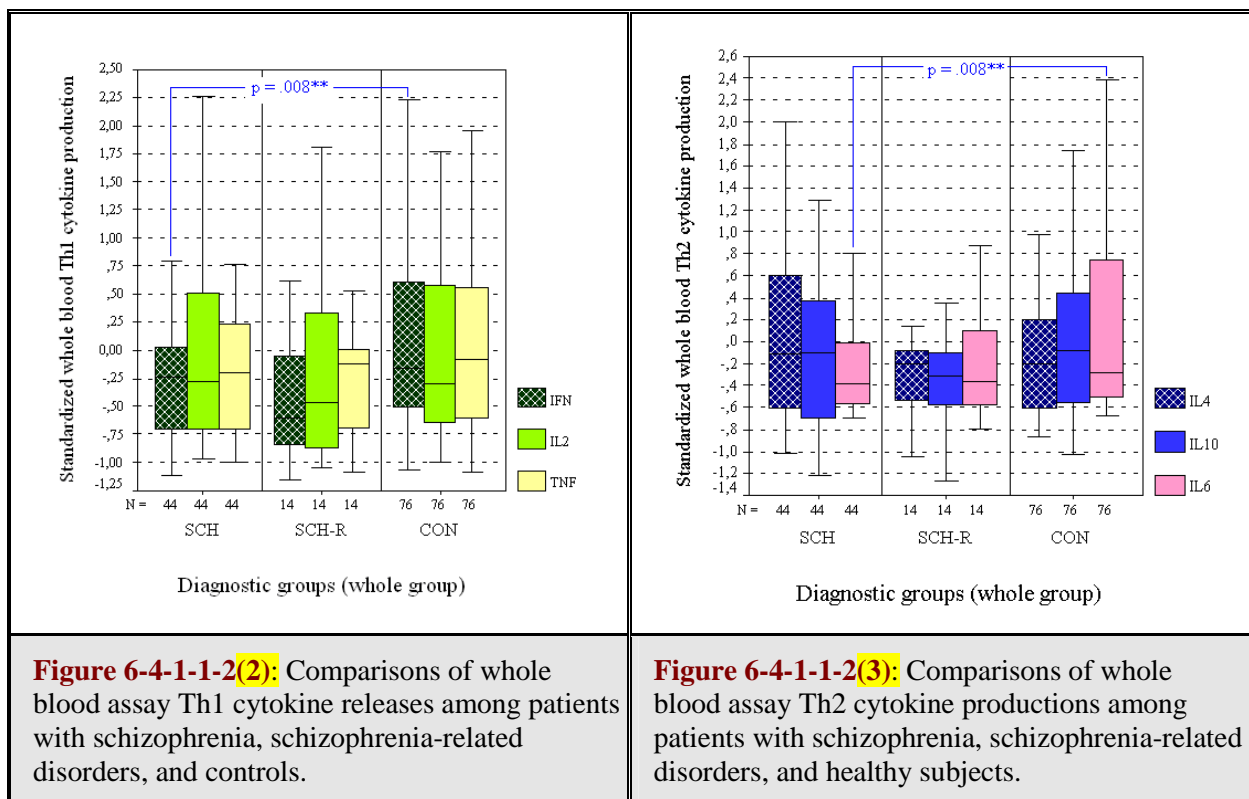
comparisons showed overall reductions in all cytokines and Th1/Th2 ratios except IL-4 if including age, cortisol, prolactin, and SHBG as co-variants into the analysis. Schizophrenics produced less IFN-γ, IL-2, TNF-α, IL-10, and IL-6 in whole blood assay, but secreted more IL-4 than their healthy counterparts. Nevertheless, only the decreases in whole blood assay IFN-γ and IL-6 production reached statistic significance levels. On average, schizophrenic patients released 30469.26 pg/ml of IFN-γ (SD = 20852.69), 1635.61 pg/ml of IL-6 (SD = 1098.39), while controls secreted 45391.47 (SD = 33158.28) and 2913.99 pg/ml (SD = 3031.21) of IFN-γ and IL-6, respectively. The discrepancies in both IFN-γ and IL-6 achieved a significance level of .008 (see **Table 4-1(2)** – IFN-γ: F = 6.74, p = .008; IL-6: F = 5.87, p = .008; IL-2: F = .52, p = .47; TNF-γ: F = 1.81, p = .18; IL-4: F = .03, p = .86; IL-10: F = .58, p = .45). Furthermore, the means of IFN-γ/IL-10 and IFN-γ/IL-4 were 29.80 (SD = 20.14) and 889.75 (SD = 1260.87) for schizophrenics and those for controls' were 39.72 (SD = 26.86) and 1285.00 (SD = 1427.55), correspondingly. However, only the disparity in IFN-γ/IL-10 ratio was unmistakably distinguishable between schizophrenics and healthy controls (IFN-γ/IL-10: F = 4.45, p = .04; IFN-γ/IL-4: F = .74, p = .39).
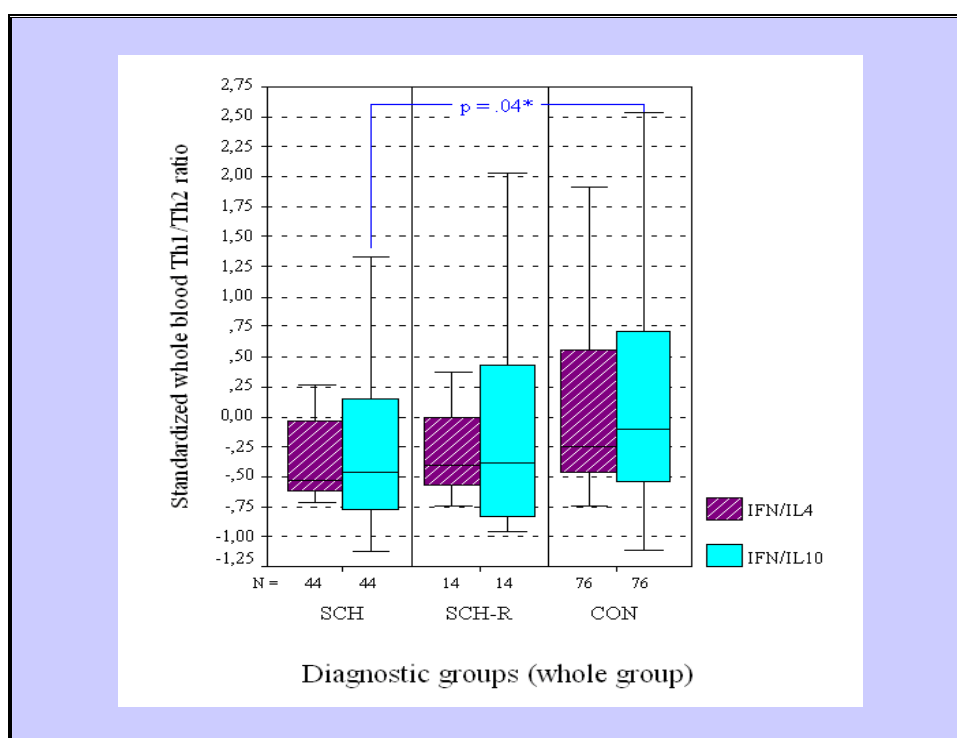
Figure 6-4-1-1-2(1): Standardized whole blood assay IFN-γ/IL-4 (IFN/IL4) and IFN-γ/IL-10 (IFN/IL10) ratio in patients with schizophrenia, schizophrenia-related disorders, and healthy subjects (SCH = schizophrenia; SCH-R = schizophrenia-related disorders; CON = controls).

In contrast to the results from serum data which, despite of co-varying with various parameters, remained relatively consistent, the findings from whole blood assay data varied with covariant(s) included. If without including any variable as covariant, the results demonstrated a similar picture as the outcomes reported above; schizophrenics had noticeably decreased whole blood assay IFN-γ, IL-6 production, and IFN-γ/IL-10 ratios (IFN-γ/IL-10: F = 4.52, p = .04; IFN-γ: F = 7.24, p = .008; IL-6: F = 7.25, p = .008; IL-2: F = .11, p = .74; TNF-α: F = 1.72, p = .19; IL-4: F = .37, p = .55; IL-10: F = .60, p = .44). If only including age as covariant, the clear reductions in whole blood assay IFN-γ and IL-6 remained as they were and that of TNF-α showed a trend to be significant as well (IFN-γ: F = 8.13, p = .005; IL-6: F = 6.41, p = .01; TNF-α: F = 3.43, p = .07; IL-2: F = .55, p = .46; IL-4: F = .01, p = .93; IL-10: F = 1.59, p = .21). Nevertheless, the decrease in IFN-γ/IL-10 ratio became less clear (IFN-γ/IL-10: F = 3.56, p = .06; IFN-γ/IL-4: F = 1.33, p = .25). If all hormones, SHBG, and age taken into account and included into the analysis, then exclusively the diversities in IFN-γ and IL-6 remained significant (whole blood assay IFN-γ/IL-4: F = .24, p = .62; IFN-γ/IL-10: F = 2.49, p = .12; IFN-γ: F = 4.05, p = .05; IL-6: F = 6.19, p = .01; IL-2: F = .14, p = .71; TNF-α: F = .92, p = .34; IL-4: F = .14, p = .71; IL-10: F = .42, p = .52).

Figure 6-4-1-1-2(2): Comparisons of whole blood assay Th1 cytokine releases among patients with schizophrenia, schizophrenia-related disorders, and controls.

Figure 6-4-1-1-2(3): Comparisons of whole blood assay Th2 cytokine productions among patients with schizophrenia, schizophrenia-related disorders, and healthy subjects.

Table 6-4-1(2): Comparisons of whole blood assay cytokine secretions and Th1/Th2 ratios among schizophrenics, patients with schizophrenia-related disorders, and healthy subjects.

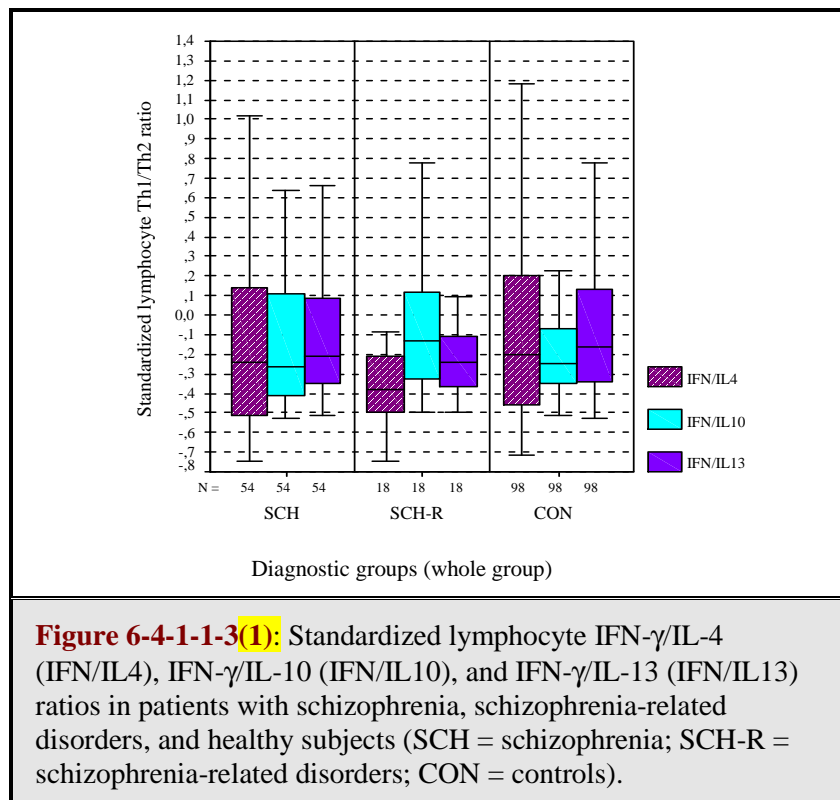| Whole blood assay Th1/Th2 cytokine productions and Th1/Th2 ratios: comparisons among schizophrenia, schizophrenia-related disorders, and healthy controls [M(SD)]			
Group	Schizophrenia (N = 44)	Related disorder (N = 14)	Controls (N = 76)
IFN-γ	30469.26 (20852.26)**	25190.84 (18382.03)*	45391.47 (33158.28)
IL-2	377.11 (318.32)	289.96 (258.55)	398.99 (360.20)
TNF-α	340.73 (221.26)	333.30 (247.61)	412.58 (322.06)
IL-4	60.61 (47.03)	51.56 (47.93)	54.70 (53.90)
IL-10	1172.98 (699.56)	883.86 (403.35)	1299.87 (947.15)
IL-6	1635.61 (1098.39)**	2246.13 (2310.54)	2913.99 (3031.21)
IFN/IL4	889.75 (1260.87)	810.90 (797.61)	1285.00 (1427.55)
IFN/IL10	29.80 (20.14)*	34.35 (27.46)	39.72 (26.86)
Note	(1) Unit of cytokines (IFN-γ, IL-2…IL-6) = pg/ml; (2) IFN/IL4 = IFN-γ/IL-4; IFN/IL10 = IFN-γ/IL-10; (3) Compared to controls: * p ≤ .05; ** p ≤ .01.		

In addition, comparisons between those 9 schizophrenic patients and 23 healthy controls who had whole blood assay IL-12 (ELISA) data showed no clear difference between both groups without including any covariant since both groups were similar in age, hormones, and SHBG (ELISA IL-12: F = 1.18, p = .29). Schizophrenics had a mean of 1.54 pg/ml IL-12 (SD = 1.83), while their healthy counterparts had an average of 2.71 pg/ml IL-12 (SD = 3.00). Similarly, comparing the whole blood assay IL-13 productions (ELISA) of schizophrenics to those of controls demonstrated no noticeable disparity between both groups if including age and testosterone as co-variants due to marked disparities between them in those parameters (SCH: ELISA IL-13 – M = 52.44 pg/ml, SD = 65.21; CON: M = 34.01, SD = 29.50; F = 1.42, p = .24). Because the male/female ratios in both diagnostic groups who had whole blood assay IL-13 data tended to be markedly different, the whole blood assay IL-13 of both genders were analyzed separately. No noticeable disparity in whole blood assay IL-13 was found between both gender subgroups, although both male and female schizophrenics averagely produced more IL-13 than controls of corresponding sex (♂SCH vs. ♂CON – age: F = .98, p = .33; cortisol: F = .27, p = .61; prolactin: F = .33, p = .57; estradiol: F = .02, p = .89; testosterone: F = 4.26, p = .05; SHBG: F = 1.83, p = .19; ELISA IL-13: F = .44, p = .51; ♀SCH vs. ♀CON – age: F = 4.64, p = .05; cortisol: F = 1.30, p = .28; prolactin: F = .01, p = .94; estradiol: F = .53, p = .48; testosterone: F = 2.61, p = .13; SHBG: F = .98, p = .35; ELISA IL-13: F = .004, p = .95). Averagely, male schizophrenics released 69.57 pg/ml of IL-13 (SD = 100.91), male controls produced 38.64 pg/ml (SD = 31.93), female schizophrenic patients

had 41.02 pg/ml (SD = 33.96), and healthy women secreted 22.11 pg/ml IL-13 (SD = 19.05) in PHA-stimulated whole blood.

Summary : Schizophrenics showed reduced whole blood assay IFN-γ/IL-4 and IFN-γ/IL-10 ratios. However, only the decrease in IFN-γ/IL-10 reached a significance level. In addition, schizophrenics also had markedly lower whole blood assay IFN-γ and IL-6 than normal controls.
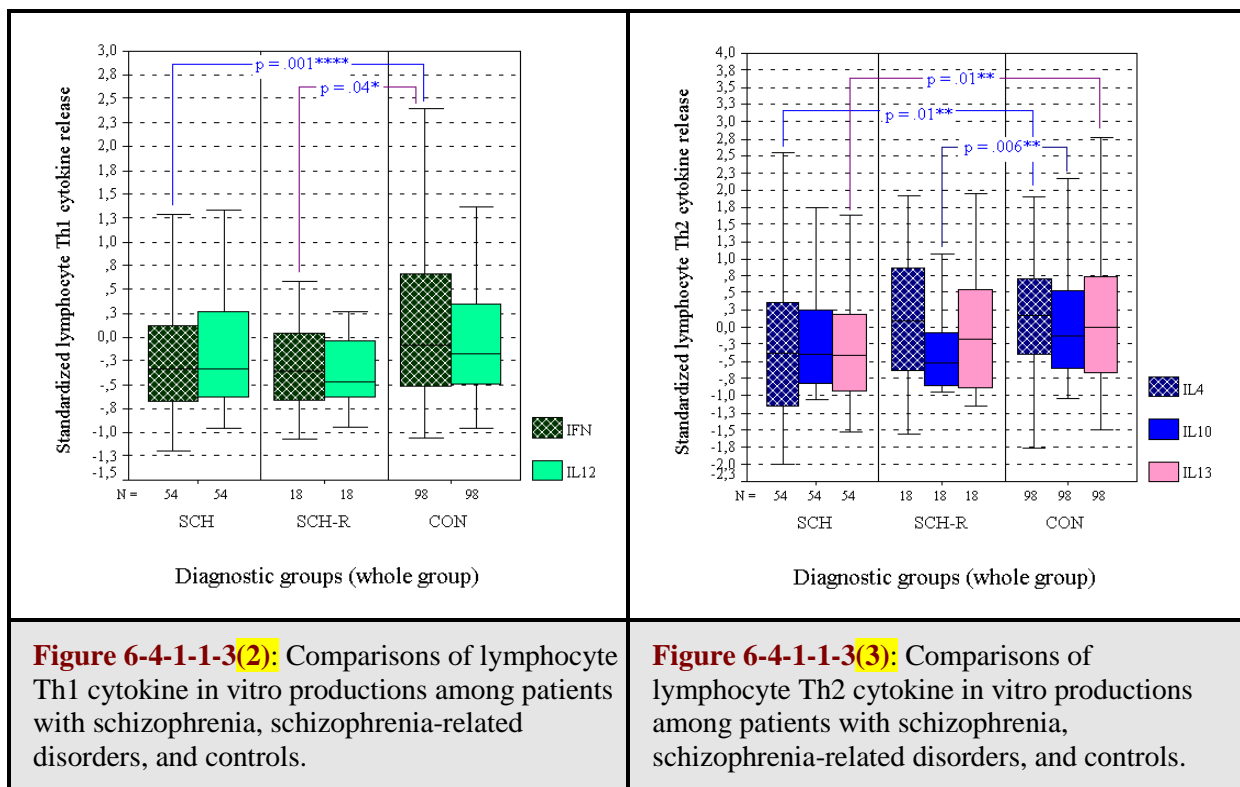
Lymphocyte data

Figure 6-4-1-1-3(1) to **Figure 6-4-1-1-3(3)** give a glance at the findings of comparisons in lymphocyte data among schizophrenics, healthy controls, and patients with schizophrenia-related disorders.

Figure 6-4-1-1-3(1): Standardized lymphocyte IFN-γ/IL-4 (IFN/IL4), IFN-γ/IL-10 (IFN/IL10), and IFN-γ/IL-13 (IFN/IL13) ratios in patients with schizophrenia, schizophrenia-related disorders, and healthy subjects (SCH = schizophrenia; SCH-R = schizophrenia-related disorders; CON = controls).

Nevertheless, MANCOVA of lymphocyte data failed to discover any conspicuous imbalance between the Th1 and Th2 system in schizophrenia, despite of with or without co-varying with age (no covariant – IFN-γ/IL-4: F = .52, p = .47; IFN-γ/IL-10: F = 1.83, p = .18; IFN-γ/IL-13: F = 1.13, p = .29; age as covariant – IFN-γ/IL-4: F = .03, p = .86; IFN-γ/IL-10: F = .75, p = .39; IFN-γ/IL-13: F = .27, p = .60). Generally speaking, schizophrenics produced less lymphocyte IFN-γ, IL-4, IL-13, and IL-10; however, they released more IL-12 and had higher

lymphocyte IFN-γ/IL-4, IFN-γ/IL-10, and IFN-γ/IL-13 ratios. Nevertheless, only the reductions in lymphocyte IFN-γ, IL-4, and IL-13 in schizophrenic patients were remarkable if compared with healthy subjects (see **Table 6-4-1(3)** – IFN-γ: F = 11.76, p = .001; IL-4: F = 6.83, p = .01; IL-13: F = 6.47, p = .01; IL-12: F = .71, p = .40; IL-10: F = 1.12, p = .29). Averagely, schizophrenics produced 716.92 IFN-γ spots pro 40,000 lymphocytes (SD = 443.74), 338.78 IL-4 spots pro 200,000 cells (SD = 202.56), and 588.87 IL-13 spots pro 200,000 lymphocytes (SD = 390.58), while their healthy counterparts released 1045.25 IFN-γ spots (SD = 820.16), 410.47 IL-4 spots (SD = 161.43), and 806.80 IL-13 spots (SD = 514.61) with the same cell concentrations as mentioned in schizophrenics.

Figure 6-4-1-1-3(2): Comparisons of lymphocyte Th1 cytokine in vitro productions among patients with schizophrenia, schizophrenia-related disorders, and controls.

Figure 6-4-1-1-3(3): Comparisons of lymphocyte Th2 cytokine in vitro productions among patients with schizophrenia, schizophrenia-related disorders, and controls.

Summary: No clear reduction in any of the lymphocyte IFN-γ/IL-4, IFN-γ/IL-10, and IFN-γ/IL-13 ratio was found in schizophrenics as a whole group. Nevertheless, schizophrenic patients released tremendously less lymphocyte IFN-γ, IL-4, and IL-13 than healthy subjects.

6.4.1.2 *Schizophrenia vs. schizophrenia-related disorders*

In order to find out which of the above discovered Th1/Th2 abnormalities was specific for schizophrenia, we compared schizophrenics with other psychiatric patients suffering under

schizophrenia-related disorders such as other psychotic and schizoaffective disorders etc. Due to no significant difference in age, hormones, and SHBG between both patient groups, no further variable except cytokines and Th1/Th2 ratios were included into the multi-variance analysis.

Table 6-4-1(3): Comparisons of lymphocyte cytokine releases and Th1/Th2 ratios among schizophrenics, patients with schizophrenia-related disorders, and healthy subjects.

Lymphocyte data: comparisons among schizophrenia, schizophrenia-related disorders, and healthy controls [M(SD)]			
Group	Schizophrenia (N = 54)	Related disorder (N = 18)	Controls (N = 98)
IFN-γ	716.92 (443.74)****	768.38 (648.96)*	1045.25 (820.16)
IL-12	371.74 (487.40)	237.31 (190.15)	301.97 (196.73)
IL-4	338.78 (202.56)**	395.33 (180.15)	410.47 (161.43)
IL-10	480.65 (509.95)	290.13 (225.43)**	535.28 (458.92)
IL-13	588.87 (390.58)**	700.65 (409.66)	806.80 (514.61)
IFN/IL4	3.26 (4.17)	2.23 (2.09)	2.88 (2.37)
IFN/IL10	5.11 (11.71)	3.76 (2.90)	3.34 (4.10)
IFN/IL13	2.37 (4.80)	1.21 (.75)	1.77 (2.21)
Note	(1) Unit of cytokines (IFN-γ, IL-12…IL-13) = spot; cell concentration: IFN-γ = pro 40 000 cells; IL-12 = pro 160 000 cells; IL-4 & IL-13 = pro 200 000 cells; IL-10 = pro 80 000 cells; (2) IFN/IL4 = IFN-γ/IL-4; IFN/IL10 = IFN-γ/IL-10; IFN/IL13 = IFN-γ/IL-13; (3) Compared to controls: * p ≤ .05; ** p ≤ .01, **** p ≤ .001.		

In general, schizophrenics had higher serum IL-2, TNF-α, IL-4, IL-10, and IL-6, nevertheless, lower IFN-γ, IFN-γ/IL-4, and IFN-γ/IL-10 than patients with schizophrenia-related disorders. Schizophrenic patients produced more whole blood assay IFN-γ, IL-2, TNF-α, IL-4, IL-10 and had higher IFN-γ/IL-4. But they released less whole blood assay IL-6 and had lower IFN-γ/IL-10 than other psychiatric patients having schizophrenia-related disorders. In addition, patients with schizophrenia secreted lower IFN-γ, IL-4, and IL-13 in PHA-stimulated lymphocytes, but had higher lymphocyte Th1/Th2 ratios including IFN-γ/IL-4, IFN-γ/IL-10, and IFN-γ/IL-13 than their patient counterparts with schizophrenia-related psychiatric diseases.

However, regardless of which analysis material used (serum or whole blood or lymphocyte), no noteworthy alteration was observed in schizophrenics if compared with their psychiatric counterparts having schizophrenia-related disorders (see **Tables 6-4-1(1), 6-4-1(2) & 6-4-1(3)**: serum – IFN-γ/IL-4: F = 1.75, p = .19; IFN-γ/IL-10: F = .28, p = .60; IFN-γ: F = .01, p =

.92; IL-2: F = .01, p = .92; TNF-α: F = .37, p = .55; IL-4: F = .77, p = .38; IL-10: F = .05, p = .82; IL-6: F = .39, p = .54; whole blood assay – IFN-γ/IL-4: F = .05, p = .83; IFN-γ/IL-10: F = .45, p = .50; IFN-γ: F = .72, p = .40; IL-2: F = .86, p = .36; TNF-α: F = .01, p = .92; IL-4: F = .39, p = .54; IL-10: F = 2.15, p = .15; IL-6: F = 1.83, p = .18; lymphocyte – IFN-γ/IL-4: F = .23, p = .63; IFN-γ/IL-13: F = 1.04, p = .31; IFN-γ/IL-10: F = .23, p = .63; IFN-γ: F = .14, p = .71; IL-12: F = 1.29, p = .26; IL-4: F = 1.11, p = .30; IL-13: F = 1.08, p = .30; IL-10: F = 2.34, p = .13).

Summary : Schizophrenics and patients with related disorders seemed to be undistinguishable in those endocrinological and immunological parameters measured in this study. The next step is to detect whether the immunological alterations found in schizophrenics were also observed in patients with schizophrenia-related disorders if compared to healthy controls.

6.4.1.3   Schizophrenia-related disorders vs. controls

Generally speaking, patients with schizophrenia-related disorders had decreased serum cytokine levels and Th1/Th2 ratios if compared to normal subjects. Similar findings were found in both whole blood assay and lymphocyte data. Controls had higher cytokine productions in PHA-stimulated lymphocytes and whole blood as well as higher Th1/Th2 ratios than patients with schizophrenia-related disorders. The only exception was lymphocyte IFN-γ/IL-10 ratio; patients with schizophrenia-related disorders had higher lymphocyte IFN-γ/IL-10 ratios than controls.

Because normal controls and patients having schizophrenia-related disorders were of similar ages, only cortisol and prolactin were included into the analysis since both groups were clearly distinguishable in these respects. However, multi-variance analysis showed no marked alteration in serum and whole blood assay Th1/Th2 cytokines/ratios of patients with schizophrenia-related disorders if compared to those of normal subjects except the whole blood assay IFN-γ production (see **Tables 6-4-1(1) & 6-4-1(2)**: serum – IFN-γ/IL-4: F = .03, p = .86; IFN-γ/IL-10: F = 2.77, p = .10; IFN-γ: F = .98, p = .32; IL-2: F = .94, p = .34; TNF-α: F = 1.12, p = .29; IL-4: F = 1.20, p = .28; IL-10: F = .86, p = .36; IL-6: F = .88, p = .35; whole blood assay – IFN-γ/IL-4: F = 1.18, p = .28; IFN-γ/IL-10: F = .42, p = .52; IFN-γ: F = 4.09, p = .05; IL-2: F = .62, p = .43; TNF-α: F = .24, p = .62; IL-4: F = .02, p = .89; IL-10: F

= 1.75, p = .19; IL-6: F = .71, p = .40). Nonetheless, lymphocyte data demonstrated distinct changes in IFN-γ and IL-10 productions as well as a tendency to have decreased lymphocyte IFN-γ/IL-4 ratios in psychiatric patients suffering under schizophrenia-related disorders (see **Table 6-4-1(3):** lymphocyte – IFN-γ/IL-4: F = 3.41, p = .07; IFN-γ/IL-10: F = .30, p = .59; IFN-γ/IL-13: F = 2.00, p = .16; IFN-γ: F = 4.53, p = .04; IL-12: F = 2.71, p = .10; IL-4: F = .31, p = .58; IL-13: F = .79, p = .38; IL-10: F = 7.85, p = .006). Patients with schizophrenia-related disorders released markedly less whole blood assay IFN-γ as well as lymphocyte IFN-γ and IL-10 if compared to controls.

Summary: The comparisons between schizophrenics, controls, and patients with schizophrenia-related disorders revealed that significantly reduced serum Th1/Th2 ratios were only found in schizophrenia, but not in patients having related diseases. Additional characteristic Th1/Th2 abnormalities for schizophrenia include lower whole blood assay IFN-γ/IL-10, reduced PHA-stimulated whole blood IL-6, decreased PHA-stimulated lymphocyte IFN-γ, IL-4, and IL-13 production. Schizophrenics and patients having related disorders shared deficits in whole blood assay and lymphocyte IFN-γ secretion. However, patients with schizophrenia-related disorders had noticeably reduced lymphocyte IL-10 secretion.

6.4.2   Distinct schizophrenic subgroups

Since schizophrenia has been considered as a heterogeneous disease, in the following sections, we attempted to divide the schizophrenic patients into various subgroups and to examine whether or not any apparent diversity existed between/among those schizophrenic subgroups. However, due to the **limited space of this report**, many missing data in distinct clinical variables, and the **great diversity in case numbers** between schizophrenic subgroups with 2 polarized clinical features (e.g. early-onset vs. late-onset), the **focus** of this report lies on the comparisons between **gender subgroups**. The outcomes from MANCOVA regarding different clinical subgroups were briefly summarized in two tables in order to offer an overview of Th1/Th2 imbalance in diverse schizophrenic clinical subgroups.

6.4.2.1   Schizophrenic gender subgroups vs. normal controls

Gender is being increasingly recognized as an important factor influencing CNS structure and function (Zubieta et al., 1999). The possible role of gender in the etiology of schizophrenia was likewise lately emphasized (Halbreich and Kahn, 2003; Hafner, 2003). In the following paragraphs, schizophrenic subjects were divided into a male and a female subgroup and compared to the normal subjects of corresponding sex.

6.4.2.1.1 Female schizophrenics vs. female controls
6.4.2.1.1.1 The whole group of schizophrenic and normal females

Figure 6-4-2-1-1(1) to **Figure 6-4-2-1-1(1-2)** give an overview of the differences in serum Th1/Th2 cytokines/ratios among schizophrenic females, normal female subjects, and female patients having schizophrenia-related disorders.
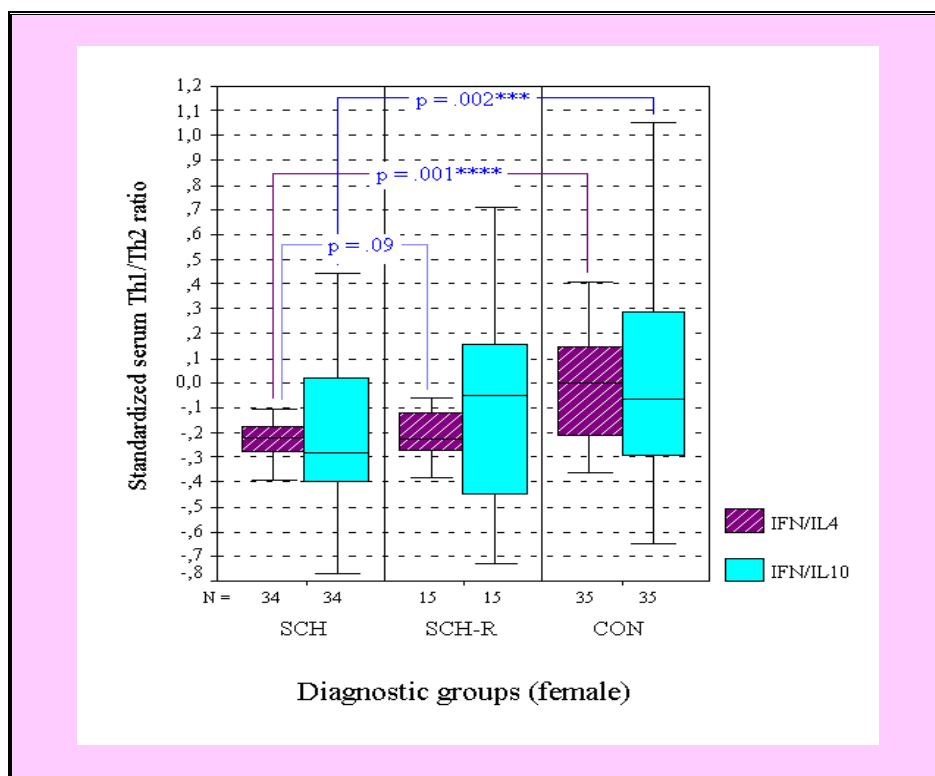

Figure 6-4-2-1-1(1): Standardized serum IFN-γ/IL-4 (IFN/IL4) and IFN-γ/IL-10 (IFN/IL10) ratios in female patients with schizophrenia, schizophrenia-related disorders, and female healthy subjects (SCH = schizophrenia; SCH-R = schizophrenia-related disorders; CON = controls).
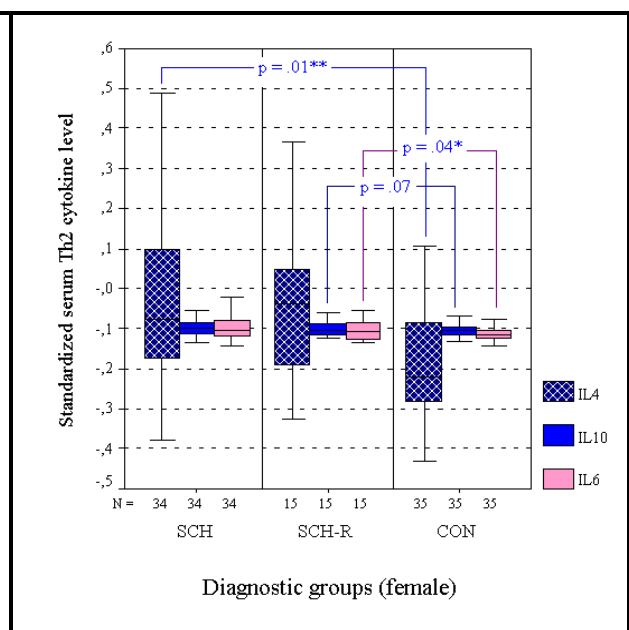
Serum data

Compared to the 35 normal females, the 34 schizophrenic women were tremendously older and had significantly enhanced prolactin, but markedly decreased cortisol and SHBG levels

71

($\female$SCH vs. $\female$CON – age: F = 18.76, p < .001; prolactin: F = 6.12, p = .02; SHBG: F = 10.46, p = .002; cortisol: F = 10.40, p = .002; estradiol: F = .002, p = .97; testosterone: F = 1.44, p = .23). The 15 females with schizophrenia-related diseases and the 35 control women were of relatively similar ages, females patients with schizophrenia-related disorders had tremendously higher prolactin, however, lower cortisol levels than healthy women ($\female$SCH-R vs. $\female$CON – age: F = 2.74, p = .10; cortisol: F = 5.20, p = .03; prolactin: F = 22.43, p < .001; estradiol: F = .45, p = .50; testosterone: F = .05, p = .83; SHBG: F = 3.58, p = .06). Between females of both patient groups, almost no disparity was found except that schizophrenic females had markedly reduced testosterone levels than their female counterparts with schizophrenia-related disorders ($\female$SCH vs. $\female$SCH-R – age: F = 2.48, p = .12; testosterone: F = 4.95, p = .03; cortisol: F = .001, p = .98; prolactin: F = 1.29, p = .26; estradiol: F = .43, p = .52; SHBG: F = .27, p = .60). Those diversities reported here were included into the following multi-variant comparisons.
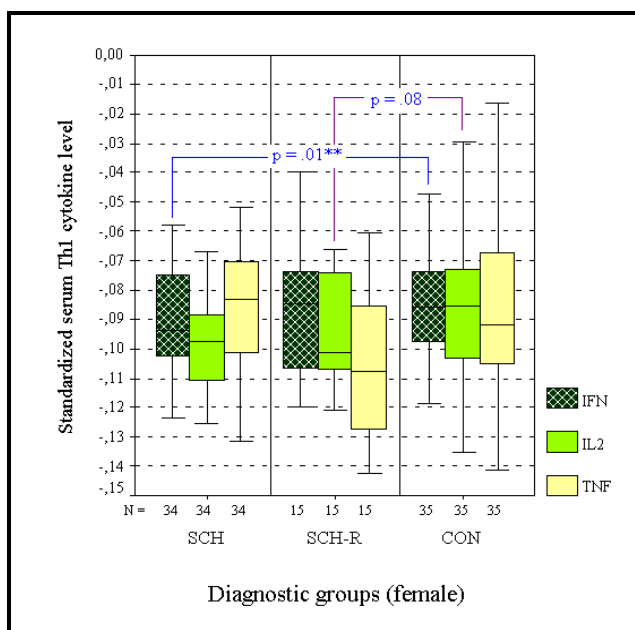
| **Figure 6-4-2-1-1(1-1)**: Comparisons of serum Th1 cytokine levels among female patients with schizophrenia, schizophrenia-related disorders, and female controls. | **Figure 6-4-2-1-1(1-2)**: Comparisons of serum Th2 cytokine levels among female patients with schizophrenia, schizophrenia-related disorders, and female controls. |

Although the data regarding nicotine abuse (the ratio between smoker and non-smoker) were not complete for all subjects, according to those data available, no noteworthy disparity was observed between each pair of the three female subgroups ($\female$SCH vs. $\female$SCH-R – nicotine: χ^2 = .01, p = .93; $\female$SCH vs. $\female$CON – nicotine: χ^2 = .66, p = .42; $\female$SCH-R vs. $\female$CON – nicotine: χ^2 = .11, p = .74).

72

If including age, prolactin, cortisol, and SHBG into the analysis due to clear diversities between schizophrenic and control females, extremely clear alterations in both serum IFN-γ/IL-4 and IFN-γ/IL-10 ratios were found in female schizophrenics (see **Table 6-4-2-1(1)**: IFN-γ/IL-4: F = 12.85, p = .001; IFN-γ/IL-10: F = 10.01, p = .002). The means obtained for both serum IFN-γ/IL-4 and IFN-γ/IL-10 were 10.96 (SD = 5.82) and 14.29 (SD = 6.94) for female schizophrenic subjects as well as 21.31 (SD = 14.77) and 19.29 (SD = 8.98) for control women, correspondingly. Schizophrenic women had clearly reduced serum Th1/Th2 ratios, compared to their healthy female counterparts. Additionally, female schizophrenic patients had remarkably lower serum IFN-γ, nevertheless, clearly higher serum IL-4 levels than healthy women (IFN-γ: F = 6.70, p = .01; IL-4: F = 6.77, p = .01). The schizophrenic females had a mean IFN-γ level of 35.93 pg/ml (SD = 16.26) and an average IL-4 level of 3.62 pg/ml (SD = 1.41), while the normal women had averagely 45.69 pg/ml IFN-γ (SD = 24.22) and 2.67 pg/ml IL-4 (SD = 1.32) in serum. Furthermore, schizophrenic females had lower serum IL-2, however, higher IL-10 and IL-6 levels than control females; nevertheless, those disparities were not clear between both female diagnostic groups ($\female$SCH vs. $\female$CON – IL-2: F = .83, p = .37; TNF-α: F = .68, p = .41; IL-10: F = 1.11, p = .30; IL-6: F = 2.60, p = .11).

However, the comparisons between the 15 female patients with schizophrenia-related disorders and the 35 control females showed another pictures if including cortisol and prolactin into the analysis for the reason that females of both groups were obviously different in both stress hormones. Female patients with schizophrenia-related disorders had apparently enhanced serum IL-6 levels and tended to have increased serum IL-2 and IL-10 levels if compared to healthy female subjects (IL-6: F = 4.41, p = .04; IL-2: F = 3.32, p = .08; IL-10: F = 3.44, p = .07). Female patients with schizophrenia-related disorders obtained averagely 4.02 pg/ml for IL-6 (SD = 6.75), 3.26 pg/ml for IL-2 (SD = 5.30), and 4.78 pg/ml for IL-10 (SD = 9.38). In addition to the disparities stated above, females of both groups were relatively comparable in the remaining cytokines as well as both serum Th1/Th2 ratios ($\female$SCH-R vs. $\female$CON – IFN-γ/IL-4: F = 1.28, p = .26; IFN-γ/IL-10: F = .72, p = .40; IFN-γ: F = 2.75, p = .10; TNF-α: F = .49, p = .49; IL-4: F = 1.14, p = .29).

Compared to female patients having schizophrenia-related disorders, schizophrenic females had generally higher serum levels in Th1 cytokines such as IFN-γ, IL-2, and TNF-α, however, lower Th1/Th2 ratios and Th2 cytokines including IL-4, IL-6, and IL-10. Nevertheless, multi-variance analysis showed no obvious diversity in diverse serum cytokine levels and IFN-γ/IL-10 ratio between both female patient groups (♀SCH vs. ♀SCH-R – IFN-γ: F = 2.42, p = .13; IL-2: F = 2.44, p = .13; IL-4: F = .17, p = .68; IL-6: F = .04, p = .84; TNF-α: F = 1.24, p = .27; IL-10: F = 1.65, p = .21). However, schizophrenic women tended to have lower IFN-γ/IL-4 ratios than female patients having related-diseases (IFN-γ/IL-4: F = 3.09, p = .09; IFN-γ/IL-10: F = 1.01, p = .32).

Table 6-4-2-1(1): Comparisons of serum cytokine levels and Th1/Th2 ratios between schizophrenic and control males/females who had complete data for age, gender, hormones, SHBG, and both Th1/Th2 ratios.

Serum Th1/Th2 ratios and cytokines: comparisons (1) between schizophrenic and control females & (2) between schizophrenic and control males [M(SD)]				
Group	Female		Male	
Gender	SCH (N = 34)	CON (N = 35)	SCH (N = 42)	CON (N = 40)
IFN-γ	35.93 (16.26)**	45.69 (24.22)	80.22 (246.94)	384.69 (2101.60)
IL-2	1.85 (.43)	2.04 (.54)	3.76 (12.64)	10.01 (48.67)
TNF-α	1.87 (.45)	1.81 (.43)	3.02 (8.46)	6.32 (28.04)
IL-4	3.62 (1.41)**	2.67 (1.32)	3.56 (2.35)	6.16 (16.00)
IL-10	2.71 (1.09)	2.39 (.83)	6.06 (21.78)	14.61 (74.12)
IL-6	4.50 (7.84)	2.32 (.94)	5.39 (16.95)	13.70 (70.88)
IFN/IL4	10.96 (5.82)****	21.31 (14.77)	16.64 (15.60)	19.49 (20.76)
IFN/IL10	14.29 (6.93)***	19.65 (8.98)	16.47 (9.16)*	19.67 (10.47)
Note	(1) Unit of cytokines (IFN-γ, IL-2…IL-6) = pg/ml; (2) IFN/IL4 = IFN-γ/IL-4; IFN/IL10 = IFN-γ/IL-10; (3) Compared to corresponding sex: * p ≤ .05; ** p ≤ .01; *** p ≤ .005; **** p ≤ .001.			

Whole blood assay data

Figure 6-4-2-1-1(2) to **Figure 6-4-2-1-1(2-2)** offer a glance at the discrepancies in whole blood assay Th1/Th2 cytokines/ratios among schizophrenic females, normal female subjects, and female patients having schizophrenia-related disorders.

According to the whole blood assay data, the 18 female schizophrenics were extremely older and had significantly higher prolactin, nevertheless, lower cortisol levels than the 35 female controls (♀SCH vs. ♀CON – age: F = 12.44, p = .001; cortisol: F = 6.09, p = .02; prolactin: F

= 7.25, p = .01; estradiol: F = .47, p = .50; testosterone: F = 1.11, p = -30; SHBG: F = 2.12, p = .15). The 5 females with schizophrenia-related disorders were not significantly older, but they did have clearly enhanced prolactin, nonetheless, decreased SHBG levels than normal women ($♀$SCH-R vs. $♀$CON – age: F = 3.77, p = .06; prolactin: F = 50.33, p < .001; SHBG: F = 4.22, p = .05; cortisol: F = .90, p = .35; estradiol: F = .11, p = .75; testosterone: F = .003, p = .95). Nevertheless, women of both patient groups were relatively comparable in all respects stated above ($♀$SCH vs. $♀$SCH-R – age: F = .10, p = .76; cortisol: F = .41, p = .53; prolactin: F = 2.18, p = .16; estradiol: F = .01, p = .93; testosterone: F = 2.05, p = .17; SHBG: F = 1.86, p = .19). Furthermore, between those female patients and control females who had complete whole blood assay Th1/Th2 ratios and endocrinological data, no clear diversity was shown between each pair of them regarding the smoker/no-smoker ratios ($♀$SCH vs. $♀$CON – χ^2 = .21, p = .65; $♀$SCH-R vs. $♀$CON – χ^2 = .73, p = .39; $♀$SCH vs. $♀$SCH-R – χ^2 = .24, p = .62).
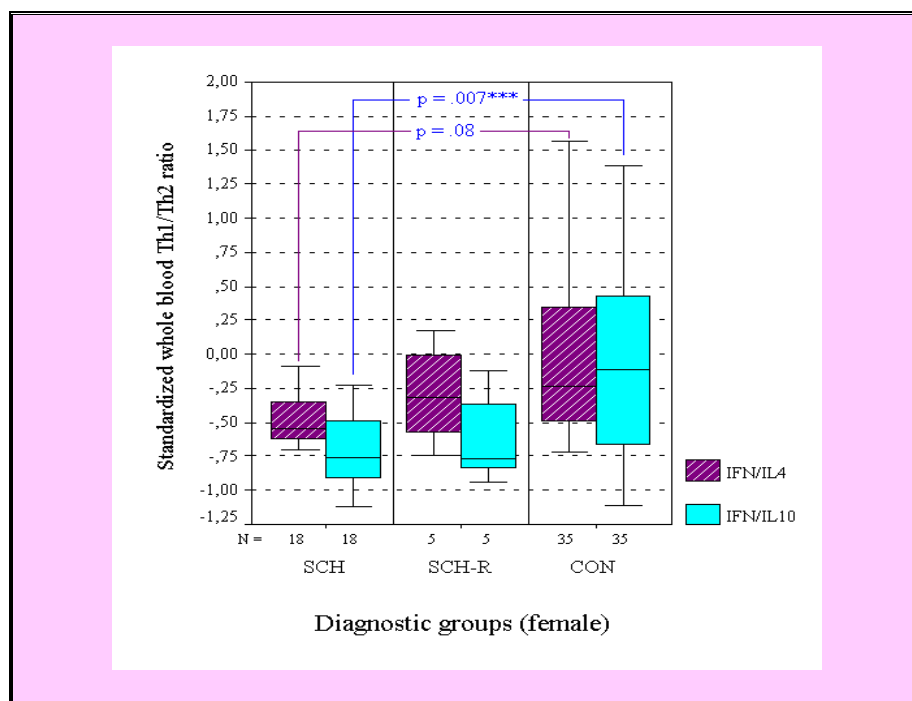
Figure 6-4-2-1-1(2): Standardized whole blood assay IFN-γ/IL-4 (IFN/IL4) and IFN-γ/IL-10 (IFN/IL10) ratios in female patients with schizophrenia, schizophrenia-related disorders, and female healthy subjects (SCH = schizophrenia; SCH-R = schizophrenia-related disorders; CON = controls).

SCH females vs. CON females

Multi-variance analysis revealed that schizophrenic females had highly reduced whole blood assay IFN-γ/IL-10 ratios than healthy women (see **Table 6-4-2-1(2)**: F = 7.86, p = .007).

Female schizophrenics obtained a mean of 19.64 IFN-γ/IL-10 (SD = 12.07), whereas female controls had an average IFN-γ/IL-10 34.77 (SD = 21.52). Schizophrenic women also had reduced whole blood assay IFN-γ/IL-4 ratios, nevertheless, the difference only showed a trend to be significant (F = 3.30, p = .08). The mean whole blood assay IFN-γ/IL-4 ratios for both schizophrenic and control female groups were 462.03 (SD = 310.06) and 1290.69 (SD = 1620.74), respectively. Additionally, female schizophrenics produced markedly less IFN-γ and tended to release less IL-6 than their healthy female counterparts; however, no marked disparity was found in the productions of the remaining cytokines (♀SCH vs. ♀CON – IL-2: F = .19, p = .67; TNF-α: F = .91, p = .34; IL-4: F = .31, p = .58; IL-10: F = .04, p = .85). The means of whole blood IFN-γ and IL-6 were 23410.06 pg/ml (SD = 13379.42) and 1326.20 pg/ml (SD = 751.37) for schizophrenic women as well as 45221.91 pg/ml (SD = 32808.98) and 3300.15 pg/ml (SD = 3259.17) for control females, correspondingly (IFN-γ: F = 5.56, p = .02; IL-6: F = 3.44, p = .07).
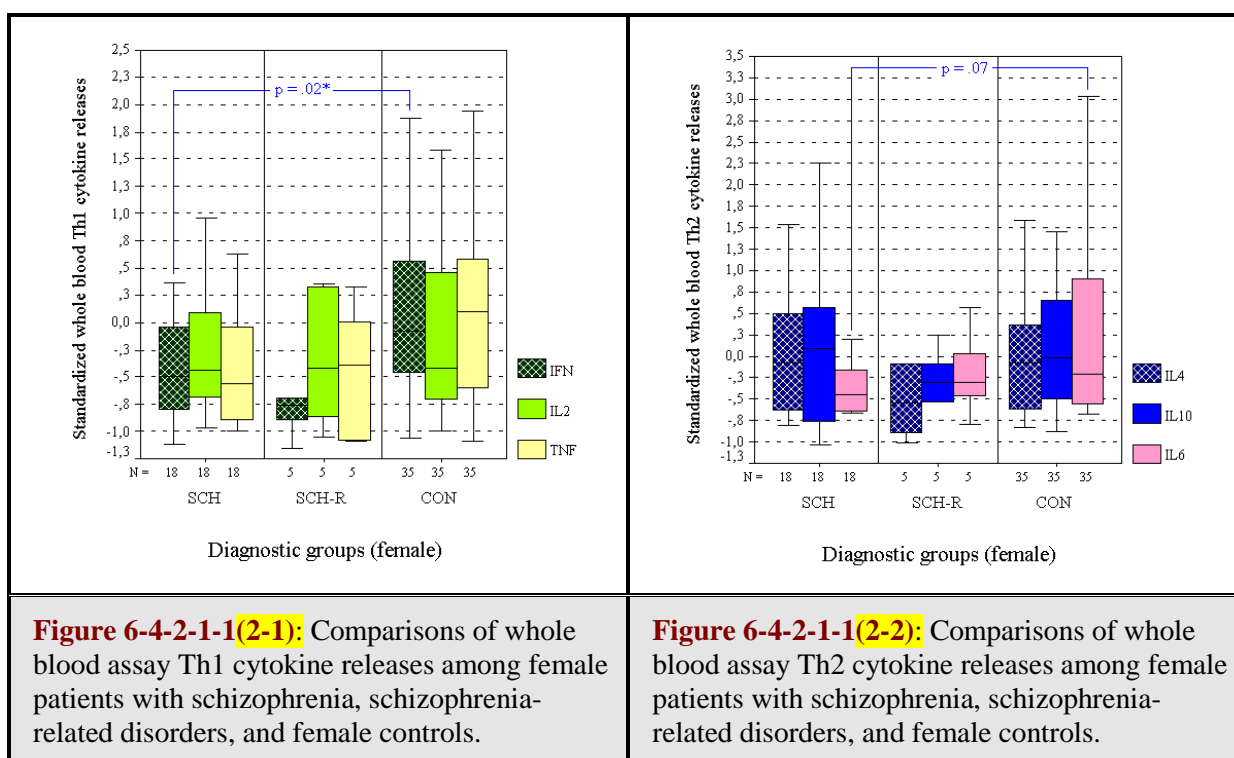
| **Figure 6-4-2-1-1(2-1):** Comparisons of whole blood assay Th1 cytokine releases among female patients with schizophrenia, schizophrenia-related disorders, and female controls. | **Figure 6-4-2-1-1(2-2):** Comparisons of whole blood assay Th2 cytokine releases among female patients with schizophrenia, schizophrenia-related disorders, and female controls. |

SCH-R females vs. CON females & SCH-R females vs. SCH females

Nevertheless, the comparisons between female patients with schizophrenia-related disorders and controls as well as those between both female patient groups (schizophrenia vs. schizophrenia-related disorders) showed similarities in all regards measured (♀SCH-R vs. ♀CON – whole blood assay IFN-γ/IL-4: F = .002, p = .96; IFN-γ/IL-10: F = 2.05, p = .16;

IFN-γ: F = .25, p = .62 ; IL-2: F = .002, p = .97; TNF-α: F = .10, p = .76; IL-4: F = .65, p = .43; IL-10: F = .04, p = .84; IL-6: F = .64, p = .43; ♀SCH-R vs. ♀SCH – IFN-γ/IL-4: F = .1.41, p = .25; IFN-γ/IL-10: F = .002, p = .97; IFN-γ: F = 1.27, p = .27; IL-2: F = .08; p = .78; TNF-α: F = .14, p = .71; IL-4: F = .65, p = .43; IL-10: F = 1.40, p = .25; IL-6: F = 1.17, p = .29).

Table 6-4-2-1(2): Comparisons of whole blood assay cytokine levels and Th1/Th2 ratios among schizophrenic and control males/females who had complete data for age, gender, hormones, SHBG, and both Th1/Th2 ratios.
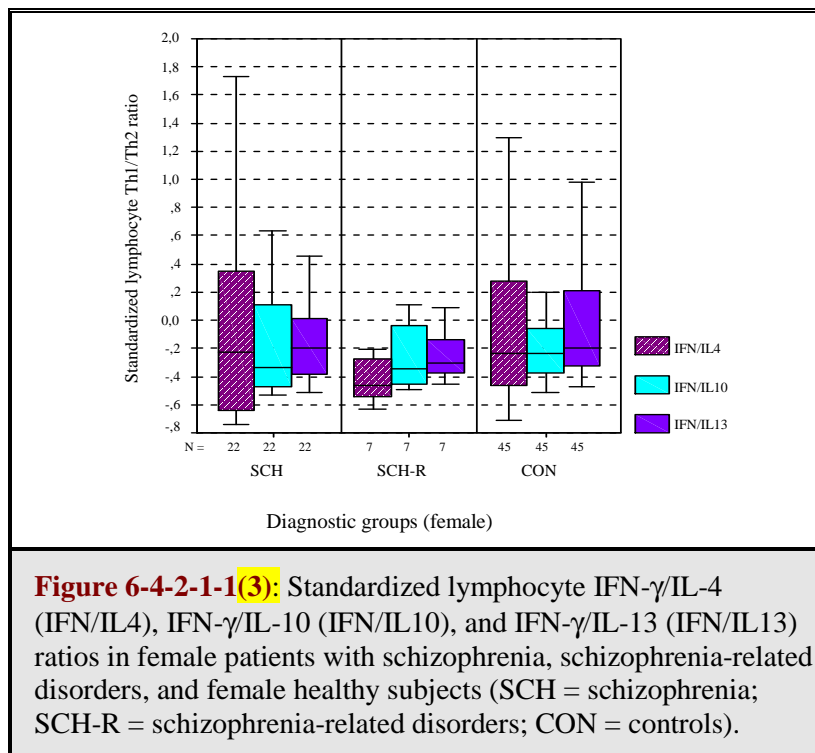
Whole blood assay Th1/Th2 ratios and cytokines: comparisons (1) between schizophrenic and control females & (2) between schizophrenic and control males [M(SD)]				
Group	Female		Male	
Gender	SCH (N = 18)	CON (N = 35)	SCH (N = 26)	CON (N = 41)
IFN-γ	23410.06 (13379.42) **	45221.91 (32808.98)	35356.40 (23779.62)	45536.21 (33859.97)
IL-2	286.66 (194.80)	358.56 (361.86)	439.74 (372.17)	433.50 (359.60)
TNF-α	293.78 (264.20)	413.25 (284.68)	373.24 (184.54)	412.01 (354.39)
IL-4	63.30 (49.37)	54.85 (38.84)	58.75 (46.24)	54.58 (64.55)
IL-10	1368.32 (926.69)	1397.22 (800.39)	1037.74 (459.66)	1216.78 (1059.32)
IL-6	1326.20 (751.37)	3300.15 (3259.17)	1849.35 (1254.81)	2584.35 (2820.86)
IFN/IL4	462.03 (310.06)	1290.69 (1620.74)	1185.87 (1564.02)	1280.13 (1260.25)
IFN/IL10	19.64 (12.07)**	34.77 (21.52)	36.84 (21.74)	43.95 (30.31)
Note	(1) Unit of cytokines (IFN-γ, IL-2…IL-6) = pg/ml; (2) IFN/IL4 = IFN-γ/IL-4; IFN/IL10 = IFN-γ/IL-10; (3) Compared to corresponding sex: ** p ≤ .01.			

Lymphocyte data

Figure 6-4-2-1-1(3) to **Figure 6-4-2-1-1(3-2)** give a glance at the results from comparing the lymphocyte Th1/Th2 data of schizophrenic women, normal females, and female patients having schizophrenia-related diseases.

The diversities in the epidemiological and endocrinological data among these three female subgroups who had complete lymphocyte data, hormones, SHBG, and age are as followed. Apart from being significantly older, the 22 schizophrenic females were also evidently distinguishable from the 45 normal women in cortisol, prolactin, and SHBG levels. Schizophrenic females had remarkably lower cortisol and SHBG, but higher prolactin levels than their healthy female counterparts (♀SCH vs. ♀ CON – age: F = 9.89, p = .003; cortisol: F = 5.48, p = .02; prolactin: F = 8.38, p = .005; SHBG: F = 6.66, p = .01; estradiol: F = .02, p

= .90; testosterone: F = .73, p = .40). Between the 7 female patients having schizophrenia-related disorders and the 45 female controls, only the diversities in prolactin and SHBG reached statistic significance levels, but not age, both sex hormones, and cortisol (♀SCH-R vs. ♀CON – prolactin: F = 17.96, p < .001; SHBG: F = 4.83, p = .03; age: F = 2.48, p = .12; cortisol: F = 1.86, p = .18; estradiol: F = .71, p = .41; testosterone: F = .01, p = .92). Female patients with schizophrenia-related diseases had highly higher prolactin, but lower SBHG levels than the female controls. However, between both patient groups, no clear disparity was observed in those respects (♀SCH vs. ♀SCH-R – age: F = .58, p = .45; cortisol: F = .001, p = .98; prolactin: F = .19, p = .67; estradiol: F = .46, p = .51; testosterone: F = 1.94, p = .18; SHBG: F = .71, p = .41).

Figure 6-4-2-1-1(3): Standardized lymphocyte IFN-γ/IL-4 (IFN/IL4), IFN-γ/IL-10 (IFN/IL10), and IFN-γ/IL-13 (IFN/IL13) ratios in female patients with schizophrenia, schizophrenia-related disorders, and female healthy subjects (SCH = schizophrenia; SCH-R = schizophrenia-related disorders; CON = controls).

In addition, comparing the ratios of smoker/non-smoker demonstrated similarities among these 3 female diagnostic groups (♀SCH vs. ♀CON – χ^2 = .28, p = .60; ♀SCH vs. ♀SCH-R – χ^2 = .17, p = .68; ♀SCH-R vs. ♀CON – χ^2 = .02, p = .90).

SCH females vs. CON females

MANCOVA demonstrated that, in addition to a marked reduction in lymphocyte IFN-γ production and a tendency to release less IL-4 in schizophrenic women, no further discrepancy was found in the other lymphocyte parameters between schizophrenic and control females (see **Table 6-4-2-1(3)**: lymphocyte IFN-γ/IL-4: F = .21, p = .65; IFN-γ/IL-10: F = .03,

p = .87; IFN-γ/IL-13: F = .01, p = .94; IFN-γ: F = 8.33, p = .005; IL-4: F = 3.00, p = .09; IL-12: F = .71, p = .40; IL-13: F = 2.49, p = .12; IL-10: F = .01, p = .94).

| **Figure 6-4-2-1-1(3-1)**: Comparisons of lymphocyte Th1 cytokine productions among female patients with schizophrenia, schizophrenia-related disorders, and female controls. | **Figure 6-4-2-1-1(3-2)**: Comparisons of lymphocyte Th2 cytokine productions among female patients with schizophrenia, schizophrenia-related disorders, and female controls. |
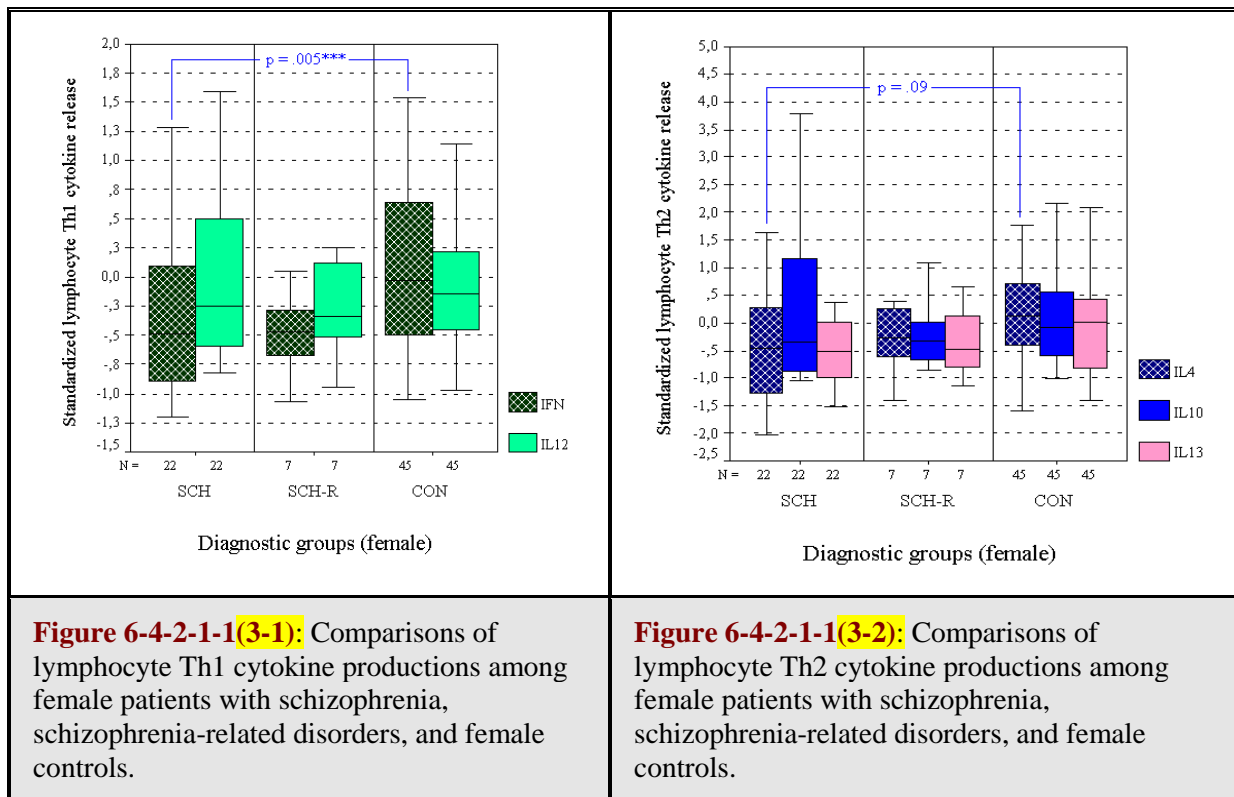
SCH-R females vs. CON females & SCH-R females vs. SCH females

Nevertheless, comparisons between either female patient group and normal females failed to show any marked disparity in lymphocyte cytokine production and Th1/Th2 ratio (♀SCH-R vs. ♀CON – IFN-γ/IL-4: F = 2.29, p = .14; IFN-γ/IL-10: F = .25, p = .62; IFN-γ/IL-13: F = .76, p = .39; IFN-γ: F = 2.62, p = .11; IL-12: F = .002, p = .96; IL-4: F = .42, p = .52; IL-13: F = 1.06, p = .31; IL-10: F = .76, p = .39; ♀SCH-R vs. ♀SCH – IFN-γ/IL-4: F = 1.02, p = .32; IFN-γ/IL-10: F = .28, p = .60; IFN-γ/IL-13: F = .50, p = .49; IFN-γ: F = .30, p = .59; IL-12: F = .37, p = .55; IL-4: F = .46, p = .51; IL-13: F = .03, p = .87; IL-10: F = .59, p = .45).

Summary: Schizophrenic females had significantly lower serum IFN-γ/IL-4, IFN-γ/IL-10, whole blood assay IFN-γ/IL-10, serum, whole blood assay, and lymphocyte IFN-γ, however, higher serum IL-4 than female normal subjects, but not female patients having schizophrenia-related disorders. In addition, schizophrenic women also tended to have markedly decreased whole blood assay IFN-γ/IL-4, IL-6, and lymphocyte IL-4 if compared to female controls. Nevertheless, female patients having schizophrenia-related disorders had significantly

enhanced serum IL-6 and tended to have elevated serum IL-10 levels if compared with normal female controls. Furthermore, schizophrenic women tended to have lower serum IFN-γ/IL-4 than their female patient counterparts having schizophrenia-related diseases.

Table 6-4-2-1(3): Comparisons of lymphocyte cytokine levels and Th1/Th2 ratios between schizophrenic and control males/females who had complete data for age, gender, hormones, SHBG, and both Th1/Th2 ratios.

Lymphocyte Th1/Th2 ratios and cytokines: comparisons (1) between schizophrenic and control females & (2) between schizophrenic and control males [M(SD)]				
Group	Female		Male	
Gender	SCH (N = 22)	CON (N = 45)	SCH (N = 32)	CON (N = 53)
IFN-γ	617.13 (435.92)***	1121.88 (969.10)	785.53 (442.71)	980.19 (671.36)
IL-12	394.95 (459.68)	287.16 (158.10)	355.78 (512.20)	314.54 (225.15)
IL-4	311.48 (211.81)	416.50 (164.66)	357.56 (197.14)	405.35 (160.04)
IL-10	577.52 (634.72)	553.35 (493.19)	414.05 (400.56)	519.91 (431.89)
IL-13	540.22 (413.49)	766.80 (493.92)	622.31 (377.04)*	840.76 (533.87)
IFN/IL4	3.64 (5.64)	3.00 (2.67)	3.00 (2.83)	2.78 (2.09)
IFN/IL10	6.27 (17.58)	3.66 (4.80)	4.31 (4.83)	3.07 (3.43)
IFN/IL13	3.00 (7.19)	2.05 (3.03)	1.94 (1.95)	1.53 (1.12)
Note	(1) Unit of cytokines (IFN-γ, IL-12…IL-13) = spot; cell concentration: IFN-γ = pro 40 000 cells; IL-12 = pro 160 000 cells; IL-4 & IL-13 = pro 200 000 cells; IL-10 = pro 80 000 cells; (2) IFN/IL4 = IFN-γ/IL-4; IFN/IL10 = IFN-γ/IL-10; (3) Compared to corresponding sex: * $p \le .05$; *** $p \le .005$.			

6.4.2.1.1.2 Th1/Th2 subgroups of female schizophrenic patients

Although female schizophrenics as a whole group had significantly reduced Th1/Th2 ratios than normal women, they can be further divided into various heterogeneous subgroups according to their Th1/Th2 ratios. The following sections are the report of such attempts.

Serum Th1/Th2 subgroups of female schizophrenics

If including serum IFN-γ/IL-4 and IFN-γ/IL-10 as clustering standards at the same time to classify the female patients, two extremely heterogeneous subgroups were obtained: one low IFN-γ/IL-4 and IFN-γ/IL-10 (abbreviated as low Th1/Th2) as well as one high Th1/Th2 subgroup (low Th1/Th2 ♀SCH – IFN-γ/IL-4: M = 8.21, SD = 3.78; IFN-γ/IL-10: M = 9.70, SD = 3.73; high Th1/Th2 ♀SCH – IFN-γ/IL-4: M = 14.88, SD = 6.08; IFN-γ/IL-10: M = 20.84, SD = 4.83). There were 20 females in the low Th1/Th2 subgroup and 14 in the high Th1/Th2 female schizophrenic group. These two female schizophrenic subgroups had highly different serum IFN-γ/IL-4 and IFN-γ/IL-10 ratios (IFN-γ/IL-4: F = 15.58, p < .0001; IFN-

γ/IL-10: F = 57.55, p < .00001). The low Th1/Th2 female subgroup tended to have higher scores on the PANSS negative subscale than the high one (low Th1/Th2 group: M = 27.13, SD = 6.43; high: M = 21.57, SD = 7.37; F = 3.34, p = .08). In contrast, the high Th1/Th2 female subgroup had a higher average on the PANSS positive subscale (low Th1/Th2 ♀SCH: M = 23.19, SD = 7.57; high Th1/Th2 ♀SCH: M = 27.00, SD = 6.51). It's worthy to note that although the low Th1/Th2 female schizophrenics tended to have higher scores on the PANSS negative scale, their average score was still smaller than 28; the mean of all 7 PANSS negative subscales is 28.

Serum IFN-γ/IL-4 female schizophrenic subgroups

Further attempts to classify female patients into 3 serum IFN-γ/IL-4 subgroups showed a tendency - lower serum IFN-γ/IL-4 ratio, lower PANSS positive and negative score as well as higher serum IFN-γ/IL-4 ratio, higher PANSS positive and negative score; however, this tendency was statistically not significant. Nevertheless, serum IFN-γ/IL-10 seemed not to have similar associations with the PANSS scores as those described above with IFN-γ/IL-4.

Whole blood assay Th1/Th2 subgroups of females with schizophrenia

If including both whole blood assay IFN-γ/IL-4 and IFN-γ/IL-10 together as classification criteria into the cluster-center analysis, two female schizophrenic subgroups were obtained; they were very different from each other in both whole blood assay IFN-γ/IL-4 and IFN-γ/IL-10 ratios (IFN-γ/IL-4: F = 54.52, p < .001; IFN-γ/IL-10: F = 8.78, p = .009). Four patients were divided into the high whole blood assay Th1/Th2 subgroups, while 14 of them were classified as the low Th1/Th2 group. The high Th1/Th2 female schizophrenics had an average of 957.70 (SD = 249.95) for IFN-γ/IL-4 and 32.70 (SD = 20.35) for IFN-γ/IL-10, while the low female group had a mean of 320.41 (SD = 118.78) for IFN-γ/IL-4 and 15.90 (SD = 5.24) for IFN-γ/IL-10. Nevertheless, none of those demographical, clinical, and endocrinological variables was able to distinguish both female schizophrenic subgroups from each other.

Lymphocyte Th1/Th2 subgroups of female schizophrenia

Similarly, through clustering the female schizophrenics according to their lymphocyte IFN-γ/IL-4 ratios, two extremely heterogeneous subgroups in terms of lymphocyte IFN-γ/IL-4 ratio were obtained, despite that female schizophrenics as a whole group did not have markedly decreased lymphocyte Th1/Th2 ratios (lymphocyte IFN-γ/IL-4: F = 14.60, p = .001; IFN-γ/IL-13: F = 6.20, p = .02; IFN-γ/IL-10: F = .29, p = .60). The high lymphocyte IFN-γ/IL-4 female patient group consisted of 6 persons, while the low one contained 16 patients.

These two lymphocyte IFN-γ/IL-4 female schizophrenic subgroups were, however, similar in all clinical and endocrinological parameters which were available in this study.

6.4.2.1.2 Schizophrenic males vs. healthy men

6.4.2.1.2.1 The whole group of schizophrenic and control males

Serum data

Figure 6-4-2-1-2(1) to **Figure 6-4-2-1-2(1-2)** offer an overview of the results from comparing the serum Th1/Th2 data of schizophrenic men, normal males, and male patients having schizophrenia-related diseases.

The 42 schizophrenic and the 40 control men were of similar ages, however, the schizophrenic males had noticeably lower testosterone and SHBG, but higher prolactin levels than the control men (♂SCH vs. ♂CON – age: $F = 1.53$, $p = .22$; prolactin: $F = 8.50$, $p = .005$; testosterone: $F = 27.64$, $p < .001$; SHBG: $F = 7.64$, $p = .007$; cortisol: $F = .08$, $p = .77$; estradiol: $F = .35$, $p = .55$). No clear disparity was shown regarding the distributions of non-smoker/smoker in both groups ($\chi^2 = .12$, $p = .73$). Comparisons between the 11 male patients with schizophrenia-related disorders and the 40 healthy male subjects revealed that male patients had noticeably reduced testosterone levels compared to male controls. But both schizophrenic and control males had relatively similar cortisol, prolactin, estradiol, SHBG levels, smoker/non-smoker ratios, and ages. (♂SCH-R: ♂CON – testosterone: $F = 4.11$, $p = .05$; cortisol: $F = 2.55$, $p = .12$; prolactin: $F = 1.73$, $p = .20$; estradiol: $F = .68$, $p = .42$; SHBG: $F = .28$, $p = .60$; age: $F = .16$, $p = .69$; nicotine: $\chi^2 = 1.61$, $p = .21$). Between both male patient groups exhibited similarities regarding cortisol, estradiol, testosterone, age, and non-smoker/smoker ratio (♂SCH vs. ♂SCH-R – cortisol: $F = 1.70$, $p = .20$; estradiol: $F = 1.76$, $p = .19$: testosterone: $F = .85$, $p = .36$; age: $F = .20$, $p = .66$; nicotine: $\chi^2 = 1.09$, $p = .30$). Remarkable disparities were found in prolactin and SHBG (prolactin: $F = 5.74$, $p = .02$; SHBG: $F = 5.10$, $p = .03$). Schizophrenic males had highly enhanced prolactin, however, significantly reduced SHBG levels compared with male patients suffering under other related psychiatric disorders.
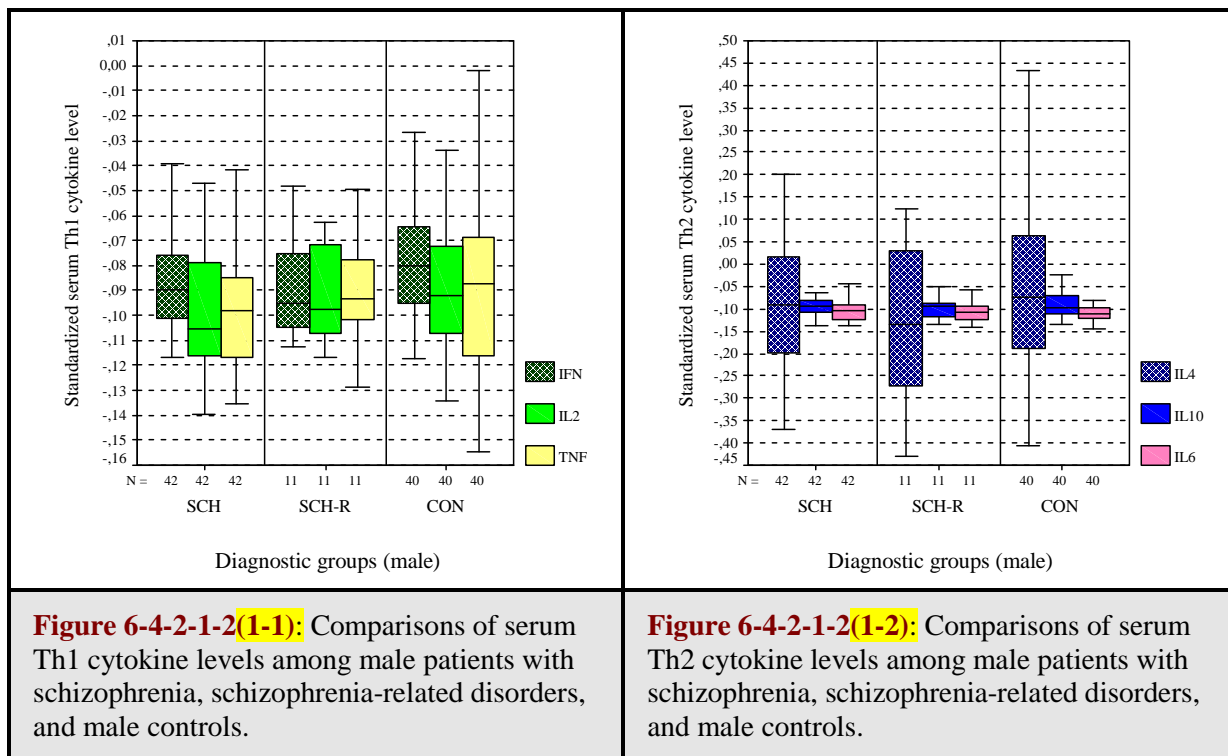
SCH males vs. CON males vs. SCH-R males

On average, both male patient groups had lower serum cytokine levels and Th1/Th2 ratios than the control males. But schizophrenic males had higher in vivo cytokine levels and serum IFN-γ/IL-4 than male patients having schizophrenia-related disorders.
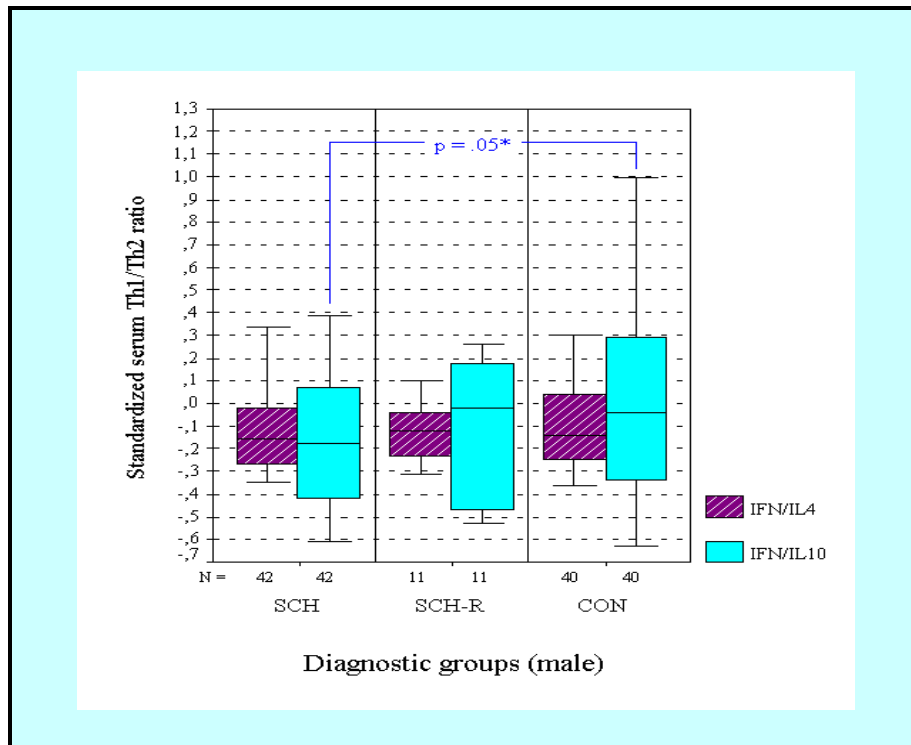
Figure 6-4-2-1-2(1): Standardized serum IFN-γ/IL-4 (IFN/IL4) and IFN-γ/IL-10 (IFN/IL10) ratios in male patients with schizophrenia, schizophrenia-related disorders, and male healthy subjects (SCH = schizophrenia; SCH-R = schizophrenia-related disorders; CON = controls).

MANCOVA comparisons between each pair of these three male groups failed to detect any obvious diversity in terms of serum cytokine levels and Th1/Th2 ratios (♂SCH vs. ♂CON – serum IFN-γ/IL-4: F = 1.90, p = .17; IFN-γ: F = .61, p = .44; IL-2: F = .52, p = .47; TNF-α: F = .47, p = .50; IL-4: F = .64, p = .43; IL-10: F = .45, p = .51; IL-6: F = .43, p = .51; ♂SCH vs. ♂CON – IFN-γ/IL-4: F = .24, p = .63; IFN-γ/IL-10: F = 1.15, p = .29; IFN-γ: F = .08, p = .78; IL-2: F = .08, p = .78; TNF-α: F = .08, p = .79; IL-4: F = .19, p = .67; IL-10: F = .08, p = .78; IL-6: F = .07, p = .79; ♂SCH vs. ♂SCH-R – IFN-γ/IL-4: F = .003, p = .96; IFN-γ/IL-10: F = .45, p = .50; IFN-γ: F = .08, p = .78; IL-2: F = .06, p = .81; TNF-α: F = .06, p = .81; IL-4: F = .62, p = .44; IL-10: F = .12, p = .74; IL-6: F = .20, p = .66). The only exception was the disparity between the male schizophrenics and normal males in serum IFN-γ/IL-10 ratio. Schizophrenic males had significantly reduced IFN-γ/IL-10 ratios compared to control men (F = 4.09, p = .05); the mean IFN-γ/IL-10 ratios for schizophrenic and control men were 16.47 (SD = 9.16) and 19.67 (SD = 10.47). **Table 6-4-2-1(1)** which was presented in the comparisons among various female subject groups summarizes the parameters stated above in both male schizophrenics and controls.

| Figure 6-4-2-1-2(1-1): Comparisons of serum Th1 cytokine levels among male patients with schizophrenia, schizophrenia-related disorders, and male controls. | Figure 6-4-2-1-2(1-2): Comparisons of serum Th2 cytokine levels among male patients with schizophrenia, schizophrenia-related disorders, and male controls. |

Whole blood assay data

Figure 6-4-2-1-2(2) to **Figure 6-4-2-1-2(2-2)** offer a global view of the outcomes from comparing the whole blood assay Th1/Th2 data of schizophrenic men, normal males, and male patients having schizophrenia-related diseases.

Totally, 26 male schizophrenics and 41 normal males had complete whole blood assay data. They had relatively similar ages, cortisol, and estradiol levels (♂SCH vs. ♂CON – age: F = 2.45, p = .12; cortisol: F = .02, p = .90; estradiol: F = .10, p = .75). However, the schizophrenic males had evidently lower SHBG and testosterone, but higher prolactin levels (SHBG: F = 6.13, p = .02; testosterone: F = 19.77, p < .001; prolactin: F = 9.37, p =.003). Concerning the smoker/non-smoker ratio, no apparent disparity was shown between both male groups (χ^2 = .04, p = .84). Nevertheless, (1) between the 9 male patients with schizophrenia-related disorders and healthy males as well as (2) between both male patient groups, no clear diversity was found in age, nicotine abuse, SHBG, and all hormones measured except the SHBG and prolactin levels between both male patient groups (♂SCH-R vs. ♂CON – age: F = .69, p = .41; nicotine: χ^2 = 1.61, p = .21; cortisol: F = .23, p = .64; prolactin: F = .91, p = .35; estradiol: F = .35, p = .56; testosterone: F = 3.67, p= .06; SHBG: F = 1.49, p = .23; ♂SCH-R vs. ♂SCH –age: F = .10, p = .75; nicotine: χ^2 = 1.17, p = .28; cortisol: F = .14, p = .71; estradiol: F = .62, p = .44; testosterone: F = .30, p = .59; prolactin: F

= 5.03, p = .03; SHBG: F = 7.43, p = .01). Schizophrenic patients had significantly higher prolactin, but lower SHBG levels than male patients with schizophrenia-related disorders.
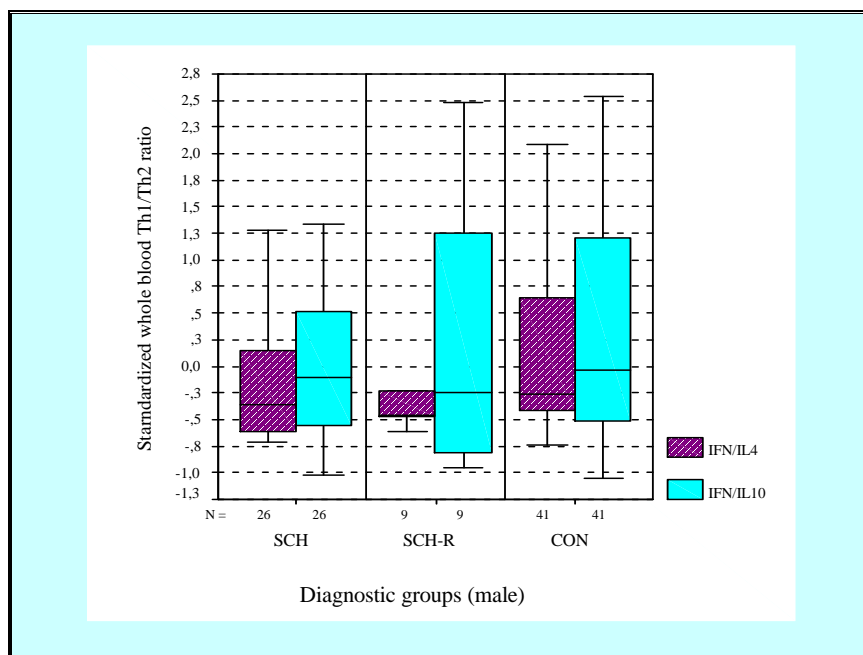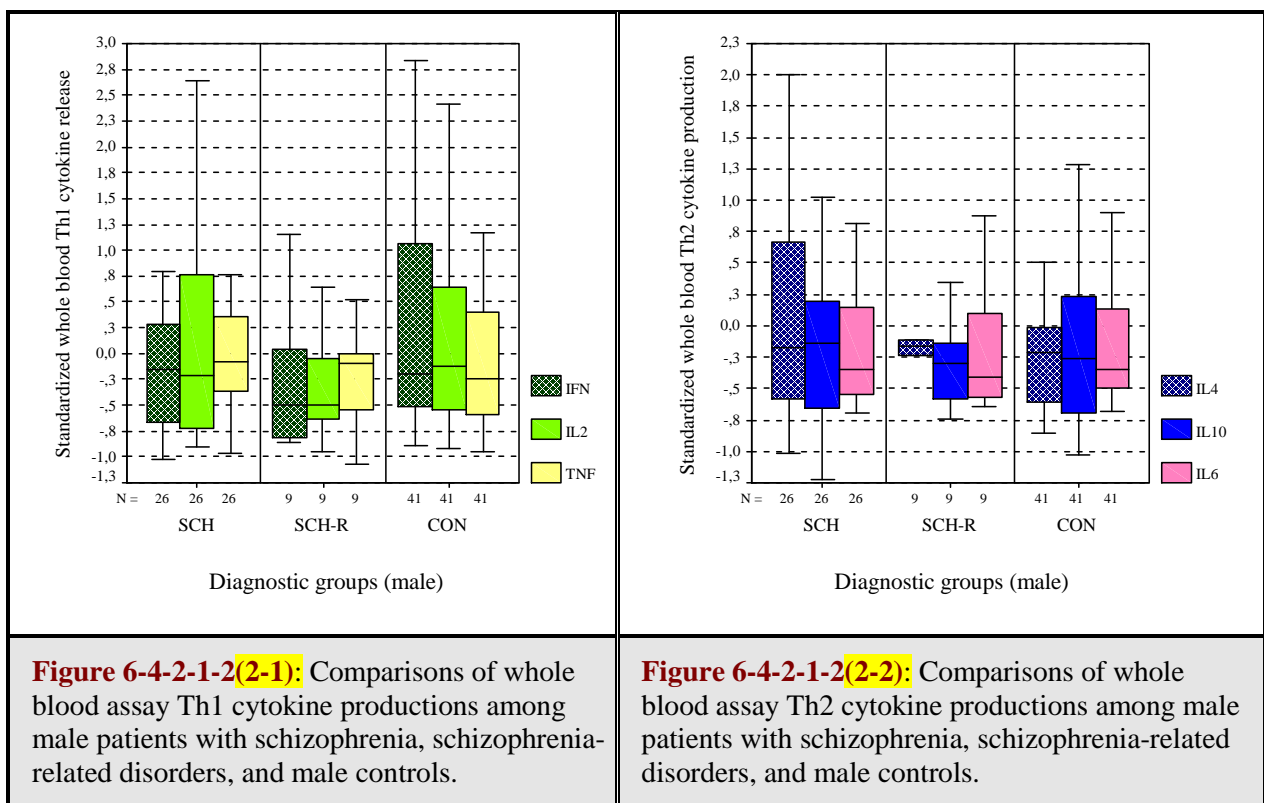

Figure 6-4-2-1-2(2): Standardized whole blood assay IFN-γ/IL-4 (IFN/IL4) and IFN-γ/IL-10 (IFN/IL10) ratios in male patients with schizophrenia, schizophrenia-related disorders, and male healthy subjects (SCH = schizophrenia; SCH-R = schizophrenia-related disorders; CON = controls).

Averagely, schizophrenic men had lower whole blood assay Th1/Th2 ratios and cytokine productions than normal males except IL-2 and IL-4. Male patients with schizophrenia-related disorders had lower whole blood assay Th1/Th2 ratios and cytokine in vitro releases than healthy men except IL-4. Nevertheless, schizophrenic men secreted less TNF-α and IL-6, but more IFN-γ, IL-2, and IL-4 than male schizophrenia-related psychiatric patients. Moreover, schizophrenic male patients had increased whole blood assay IFN-γ/IL-4, but decreased IFN-γ/IL-10 ratio if compared with their male patient counterparts with schizophrenia-related diseases.

Despite of various disparities in age and hormones, no single diversity in terms of whole blood assay cytokine productions and Th1/Th2 ratios was shown (1) between schizophrenic and control males, (2) between male patients with schizophrenia-related diseases and healthy men as well as (3) between both male patient groups (♂SCH vs. ♂CON – whole blood assay IFN-γ/IL-4: F = .26, p = .61; IFN-γ/IL-10: F = .21, p = .65; IFN-γ: F = .09, p = .76; IL-2: F =

.00, p = 1.00; TNF-α: F = .09, p = .77; IL-4: F = .71, p = .40; IL-10: F = .08, p = .77; IL-6: F = 1.30, p = .26; ♂SCH-R vs. ♂CON – IFN-γ/IL-4: F .76, p = .39; IFN-γ/IL-10: F = .02, p = .89; IFN-γ: F = 1.67, p = .20; IL-2: F= .96, p = .33; TNF-α: F = .06, p = .81; IL-4: F = .01, p = .95; IL-10: F = .77, p =.38; IL-6: F = .01, p = .92; ♂SCH vs. ♂SCH-R – IFN-γ/IL-4: F = .84, p = .37; IFN-γ/IL-10: F = .02, p = .89; IFN-γ: F = 2.18, p =.15; IL-2: F = .23, p = .64; TNF-α: F = .25, p = .62; IL-4: F = .05, p = .82; IL-10: F = 1.07, p = .31; IL-6: F = .05, p = .82). The results of both the schizophrenic and control male group were summarized in **Table 6-4-2-1(2)** which is shown in the former section concerning the comparisons among various female subject groups.

| **Figure 6-4-2-1-2(2-1)**: Comparisons of whole blood assay Th1 cytokine productions among male patients with schizophrenia, schizophrenia-related disorders, and male controls. | **Figure 6-4-2-1-2(2-2)**: Comparisons of whole blood assay Th2 cytokine productions among male patients with schizophrenia, schizophrenia-related disorders, and male controls. |

Lymphocyte data

Figure 6-4-2-1-2(3) to **Figure 6-4-2-1-2(3-1)** give a glance at the findings from comparing the lymphocyte Th1/Th2 data of schizophrenic men, normal males, and male patients having schizophrenia-related diseases.

Thirty-two schizophrenic and 53 control males had complete lymphocyte data. They had similar ages, smoker/non-smoker ratios, cortisol, prolactin, and estradiol levels. But the male schizophrenics had lower testosterone and SHBG levels than the healthy men (♂SCH vs. ♂CON – age: F = 1.75, p = .19; nicotine: χ² = .75, p = .39; cortisol: F = .01, p = .92;

prolactin: F = .01, p = .91; estradiol: F = .08, p = .78; testosterone: F = 15.30, p < .001; SHBG: F = 5.92, p = .02). Between the 11 male patients with schizophrenia-related disorders and the control males, no clear diversity in all regards mentioned above was detected (♂SCH-R vs. ♂CON – age: F = 2.92, p = .09; nicotine: χ² = 2.68, p = .10; cortisol: F = .33, p = .57; prolactin: F = .44, p = .51; estradiol: F = 1.16, p = .29; testosterone: F = 1.42, p= .24; SHBG: F = .49, p = .49). However, male patients with schizophrenia-related disorders had significantly lower prolactin, however, higher SHBG than schizophrenic men (♂SCH vs. ♂SCH-R – prolactin: F = 5.11, p = .03; SHBG: F = 12.23, p = .001). Other than these two diversities stated above, no further disparity was observed between both male patient groups (age: F = .33, p = .57; nicotine: F = 1.21, p = .27; cortisol: F = .31, p = .58; estradiol: F = 1.72, p = .20; testosterone: F = 1.10, p = .30).

SCH males vs. CON males vs. SCH-R males

Generally speaking, schizophrenic males had reduced lymphocyte IFN-γ, IL-4, IL-13, and IL-10 productions, however, increased IL-12 releases and all 3 lymphocyte Th1/Th2 ratios if compared with normal males. In contrast, male patients with schizophrenia-related disorders had lower lymphocyte cytokine secretions and Th1/Th2 ratios but IFN-γ/IL-10 if compared to healthy males. However, compared to schizophrenic men, male patients with schizophrenia-related diseases produced less IFN-γ, IL-4, and IL-13, but more IL-12 and IL-10; in addition, they had increased lymphocyte IFN-γ/IL-4 and IFN-γ/IL-13, however, decreased IFN-γ/IL-10 ratios.

Multi-variance analysis showed that (1) schizophrenic males produced remarkably less lymphocyte IL-13 (F = 4.11, p = .05) and that (2) male patients with schizophrenia-related disorders released markedly less lymphocyte IL-10 than control males (F = 4.79, p = .03). Apart from these two noticeable alterations in both male patient groups, no other noteworthy diversity was shown between any pair of these three diagnostic male subgroups (♂SCH vs. ♂CON – lymphocyte IFN-γ/IL-4: F = .17, p = .69; IFN-γ/IL-10: F = 1.91, p = .17; IFN-γ/IL-13: F = 1.58, p = .21; IFN-γ: F = 2.13, p = .15; IL-12: F = .26, p = .61; IL-4: F = 1.49, p = .23; IL-10: F = 1.27, p = .26; ♂SCH-R vs. ♂CON – IFN-γ/IL-4: F = .01, p = .93; IFN-γ/IL-10: F = 1.49, p = .23; IFN-γ/IL-13: F = .36, p = .55; IFN-γ: F = .06, p = .81; IL-12: F = 2.21, p = .14; IL-4: F = .01, p = ,93; IL-13: F = .10, p = .75; ♂SCH vs. ♂SCH-R – IFN-γ/IL-4: F = .09, p = .77; IFN-γ/IL-10: F = .01, p = .95; IFN-γ/IL-13: F = 1.08, p = .31; IFN-γ: F = .54, p = .47; IL-12: F = .88, p = .36; IL-4: F = .60, p = .44; IL-13: F = 1.37, p = .25; IL-10: F = 2.19, p = .15).

The results from both male schizophrenics and controls are presented in **Table 6-4-2-1(3)** which is shown in the previous section regarding the Th1/Th2 cytokines and ratios in females.
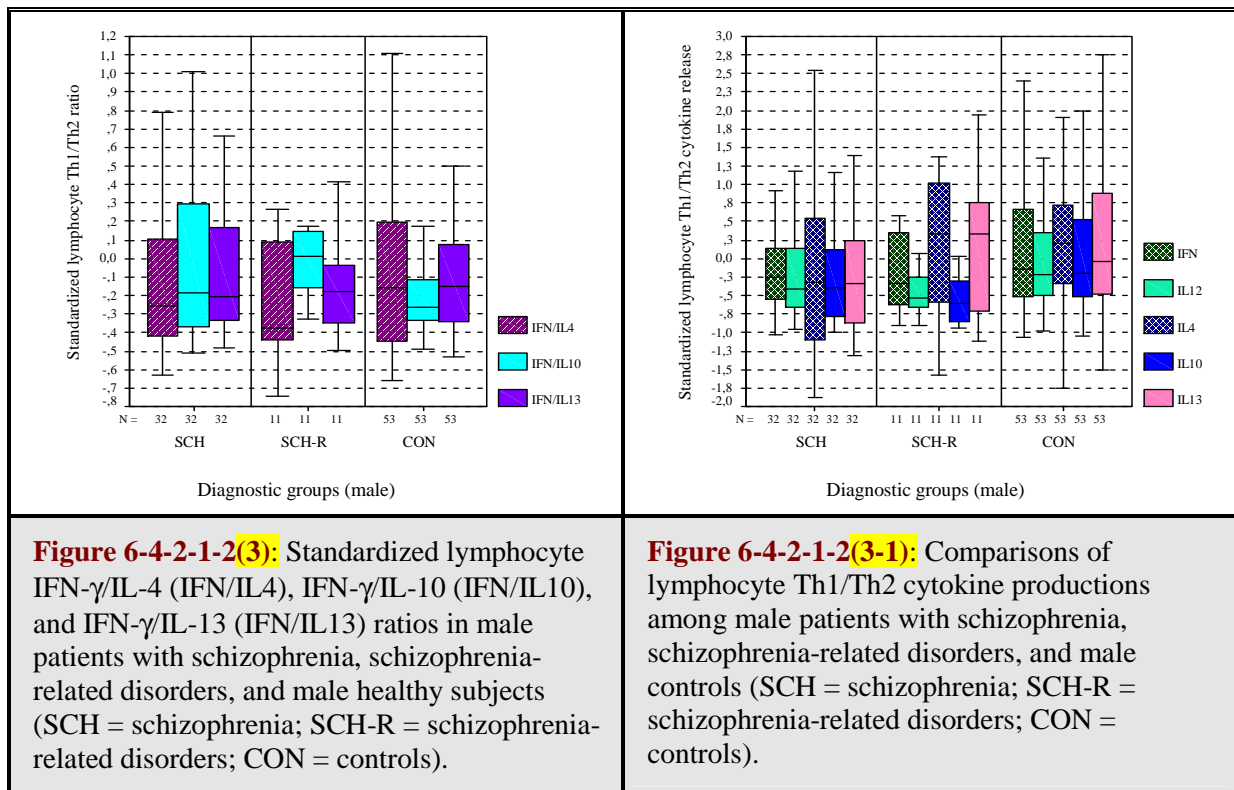
Figure 6-4-2-1-2(3): Standardized lymphocyte IFN-γ/IL-4 (IFN/IL4), IFN-γ/IL-10 (IFN/IL10), and IFN-γ/IL-13 (IFN/IL13) ratios in male patients with schizophrenia, schizophrenia-related disorders, and male healthy subjects (SCH = schizophrenia; SCH-R = schizophrenia-related disorders; CON = controls).	**Figure 6-4-2-1-2(3-1):** Comparisons of lymphocyte Th1/Th2 cytokine productions among male patients with schizophrenia, schizophrenia-related disorders, and male controls (SCH = schizophrenia; SCH-R = schizophrenia-related disorders; CON = controls).

Summary: The only characteristic alteration observed in male schizophrenics as a whole group might be reduced serum IFN-γ/IL-10 ratio and decreased lymphocyte IL-13 production.

6.4.2.1.2.2 Serum IFN-γ/IL-4 subgroups of males with schizophrenia

Serum data

Male schizophrenics as a whole group did not have significantly reduced serum IFN-γ/IL-4 ratios. In this section, we attempted to sub-divide schizophrenic males into various serum IFN-γ/IL-4 subgroups to detect whether or not a subgroup of male schizophrenic patients existed, having significantly reduced serum IFN-γ/IL-4 ratios compared to healthy males. Cluster analysis resulted in two distinct serum IFN-γ/IL-4 male schizophrenic subgroups. The high group contained 20 patients and the low one consisted of 22 male schizophrenics. Both groups were of similar ages as well as had similar levels of hormones, SHBG, and in vivo cytokine levels (high IFN4 ♂SCH vs. low IFN4 ♂SCH – age: F = .90, p = .35; cortisol: F = 1.51, p = .23; prolactin: F = 1.41, p = .24; estradiol: F = .12, p = .74; testosterone: F = 1.60, p

= .21; SHBG: F = .03, p = .87; IFN-γ: F = 1.95, p = .17; IL-2: F = 1.15, p = .29; TNF-α: F = 1.17, p = .29; IL-4: F = .43, p = .52; IL-10: F = 1.16, p = .29; IL-6: F = 1.04, p = .31).

However, both serum IFN-γ/IL-4 male subgroups tremendously differed from each other in both serum Th1/Th2 ratios (high IFN4 ♂SCH vs. low IFN4 ♂SCH – IFN-γ/IL-4: F = 20.64, p < .001; IFN-γ/IL-10: F = 6.27, p = .02). In addition to significantly decreased IFN-γ/IL-4, the low IFN-γ/IL-4 male schizophrenics had markedly reduced IFN-γ/IL-10 ratios compared to the high ones. The means of IFN-γ/IL-4 and IFN-γ/IL-10 ratio were 26.01 (SD = 18.38), 19.97 (SD = 8.95) for the high IFN-γ/IL-4 subgroup and 8.12 (SD = 3.10), 13.30 (SD = 8.31) for the low IFN-γ/IL-4 schizophrenic males. Nevertheless, none of the clinical data measured in this study was sufficient to distinguish both subgroups of male schizophrenics from each other.

Comparisons between the 20 high IFN-γ/IL-4 male schizophrenics and the 40 male controls showed no noticeable diversity in serum cytokine levels and Th1/Th2 ratios, despite of having significantly higher prolactin, however, lower testosterone and SHBG levels in the high IFN-γ/IL-4 male schizophrenics (high IFN4 ♂SCH vs. ♂CON – serum IFN-γ/IL-4: F = .24, p = .63; IFN-γ/IL-10: F = .37, p = .55; IFN-γ: F = .18, p = .67; IL-2: F = .11, p = .74; TNF-α: F = .08, p = .78; IL-4: F = .38, p = .54; IL-10: F = .07, p = .79; IL-6: F = .08, p = .77; age: F = .12, p = .73; cortisol: F = 1.14, p = .29; estradiol: F = .46, p = .50; prolactin: F = 10.92, p = .002; testosterone: F = 27.65, p < .001; SHBG: F = 4.31, p = .04). The high IFN-γ/IL-4 male schizophrenics had lower serum levels in all Th1/Th2 cytokines assessed in this study, but higher IFN-γ/IL-4 and IFN-γ/IL-10 than male controls.

In general, the low IFN-γ/IL-4 schizophrenic males had low serum cytokine levels and Th1/Th2 ratios than male controls. Multi-variance analysis demonstrated that, unlike the female schizophrenics, the low IFN-γ/IL-4 male patient subgroup did not have significantly decreased serum IFN-γ and elevated IL-4 levels if compared to healthy male subjects. However, they did have clearly lower IFN-γ/IL-4 and IFN-γ/IL-10 ratios than the normal men if including prolactin, testosterone, and SHBG into the analysis as co-variants due to remarkable disparities between both male groups in these variables (low IFN4 ♂SCH vs. ♂CON – IFN-γ/IL-4: F = 6.80, p = .01; IFN-γ/IL-10: F = 5.78, p = .02; IFN-γ: F = .61, p = .44; IL-2: F = .60, p = .44; TNF-α: F = .58, p = .45; IL-4: F = .44, p = .51; IL-10: F = .56, p =

.46; IL-6: F = .51, p = .48; age: F = 3.45, p = .07; cortisol: F = .20, p = .66; estradiol: F = .09, p = .77; prolactin: F = 3.96, p = .05; testosterone: F = 14.10, p < .001; SHBG: F = 5.23, p = .03).

Summary : Male schizophrenics as a whole group showed a significantly reduced serum IFN-γ/IL-10. However, there was also a subgroup of male schizophrenics who had significantly decreased serum IFN-γ/IL-4 and IFN-γ/IL-10.

6.4.2.2 Schizophrenic clinical subgroups vs. healthy subjects

The case numbers and the outcomes regarding Th1/Th2 imbalance in diverse schizophrenic clinical subgroups are summarized in **Table 6-4-2-2(1)** and **Table 4-4-2-2(2)**.

As shown in **Table 6-4-2-2(1)**, a number of schizophrenic clinical subgroups only contain a small number of cases, particularly if compared to healthy controls. The results of comparisons between distinct schizophrenic clinical subgroups and healthy subjects are briefly summarized in **Table 6-4-2-2(2)** in order to reduce the complexity of this report as elucidated in a previous section and offer an impression of Th1/Th2 imbalance in different clinical subgroups.

Table 6-4-2-2(1): The case numbers in diverse schizophrenic clinical subgroups.

Case numbers of diverse schizophrenic clinical subgroups						
Analysis materials	Serum		Whole blood		Lymphocyte	
Schizophrenic Subgroup	Female	Male	Female	Male	Female	Male
Paranoid	29	28	15	20	17	25
Non-paranoid	5	14	3	6	5	7
Drug-naive	6	7	3	3	4	8
Drug-free	16	14	6	14	10	13
Premed	12	11	4	11	7	10
No premed	10	10	5	6	7	11
First episode	8	9	4	5	5	10
Other episode	13	12	4	12	8	11
Drug-free ≤ 1 week	10	8	4	8	6	8
Drug-free ≥ 3 months	8	9	5	5	6	10
Positive FH	4	9	2	8	2	9
Negative FH	16	9	10	7	10	10
Acute	7	5	3	5	8	4
Chronic	15	16	6	12	6	17

Early onset	5	11	2	10	3	12
Middle onset	12	8				
Late onset	5	11	7	9	11	9
PANSS positive (>28)	7	3	3	3	3	3
PANSS positive (≤28)	16	17	8	13	12	17
PANSS negative (>28)	9	8	5	8	7	7
PANSS negative (≤ 28)	14	12	6	8	8	13
PANSS global (> 56)	6	5	4	5	4	5
PANSS global (≤ 56)	17	15	7	11	11	15
Low CGI-difference	6	15	5	10	15	20
High CGI-difference	23	26	12	16	5	12
≥Severely ill (High CGI-t1)	20	27	12	16	14	21
≤Markedly ill (Low CGI-t1)	9	14	5	10	6	11
≥Markedly ill (High CGI-t9)	7	13	5	8	14	23
≤ Moderate ill (Low CGI-t9)	22	28	12	18	6	9
Note	Whole blood = PHA-stimulated whole blood; lymphocyte = PHA-stimulated lymphocytes					

Table 6-4-2-2(2): An overview of Th1/Th2 imbalance in various schizophrenic clinical subgroups – results of significance tests from MANCOVA.

Th1/Th2 imbalance in different schizophrenic clinical subgroups							
Schizophrenic Subgroup	Serum		Whole Blood		lymphocyte		
	IFN-γ/IL4	IFN-γ/IL10	IFN-γ/IL4	IFN-γ/IL10	IFN-γ/IL4	IFN-γ/IL10	IFN-γ/IL-13
Paranoid	F = 4.18, p = .04↓	F = 10.52, p = .002↓	n.s.	F = 1.95, p = .17↓	n.s.	n.s.	n.s.
Non-paranoid	F = 3.88, p = .05↓	F = 2.32, p = .13↓	F = 3.19, p = .08↓	F = 6.17, p = .02↓	F = 2.57, p = .11↓	n.s.	n.s.
Drug-naive	F = 2.11, p = .15↓	F = 1.76, p = .19↓	F = 2.34, p = .13↓	F = 3.42. P = .07↓	F = 2.04, p = .16↑	n.s.	n.s.
Drug-free	F = 7.69, P = .007↓	F = 11.65, p = .001↓	n.s.	n.s.	F = 1.88, P = .17↓	n.s.	n.s.
Premed	F = 9.31, P = .003↓	F = 11.65, p = .001↓	n.s.	F = 2.22, P = .14↓	F = 1.72, P = .19↓	n.s.	n.s.
No premed	F = 1.71, p = .19↓	F = 2.83, P = .10↓	n.s.	F = 1.73, p = .19↓	n.s.	n.s.	n.s.
First episode	F = 3.14, p = .08↓	F = 3.66, p = .06↓	n.s.	F = 1.71, p = .19↓	n.s.	n.s.	n.s.
Other episode	F = 6.87, p = .01↓	F = 9.68, p = .002↓	n.s.	n.s.	F = 2.73, P = .10↓	n.s.	F = 2.40, P = .12↓
Drug-free ≤ 1 week	F = 8.83, p = .004↓	F = 12.98, p = .001↓	n.s.	F = 2.05, p = .16↓	F = 1.83, p = .18↓	n.s.	n.s.
Drug-free ≥ 3 months	n.s.	n.s.	n.s.	F = 5.26, p = .02↓	n.s.	n.s.	n.s.
Positive FH	F = 5.44, P = .02↓	F = 5.45, p = .02↓	n.s.	F = 4.24, P = .04↓	n.s.	n.s.	n.s.
Negative FH	F = 7.46, P = .008↓	F =15.48,	n.s.	n.s.	F = 2.41,	n.s.	n.s.

		P < .001↓			*P = .12↓*		
Acute	F = 8.82, P = .004↓	F = 9.33, P = .003↓	n.s.	n.s.	n.s.	n.s.	n.s.
Chronic	F = 3.46, P = .07↓	F = 5.64, P = .02↓	F = 3.29, P = .07↓	F = 4.54, P = .04↓	F = 8.78, P = .004↓	n.s.	n.s.
Early onset	F = 4.72, p = .03↓	F =9.39, p = .003↓	n.s.	n.s.	F = 4.48, p = .04↑	n.s.	*F = 1.84, p = .18↑*
Middle onset	F = 6.07, p = .02↓	F = 8.53, p = .004↓					
Late onset	n.s.	n.s.	n.s.	*F = 2.26, p = .14↓*	F = 6.33, P = .01↓	n.s.	F = 3.94, P = .05↓
PANSS Positive (>28)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
PANSS positive (≤28)	F = 8.88, p = .004↓	F = 16.48, p < .001↓	n.s.	n.s.	n.s.	n.s.	n.s.
PANSS negative (>28)	F = 2.54, p = .12↓	F = 2.93, p = .09↓	n.s.	n.s.	n.s.	n.s.	n.s.
PANSS negative (≤ 28)	F = 5.49, p = .02↓	F = 10.50, p = .002↓	n.s.	F = 3.94, p = .05↓	n.s.	n.s.	n.s.
PANSS global (> 56)	F = 2.87, p = .09↓	F = 7.49, p = .008↓	n.s.	n.s.	n.s.	n.s.	n.s.
PANSS global (≤ 56)	F = 7.88, p = .006↓	F = 9.18, p = .003↓	n.s.	*F = 2.20, p = .14↓*	n.s.	n.s.	n.s.
Low CGI-d	*F = 2.39, p = .13↓*	F = 4.09, p = .05↓	F = 4.25, p = .04↓	F = 7.38, p = .008 ↓	n.s.	n.s.	n.s.
High CGI-d	F = 5.59, p = .02↓	F = 8.71, p = .004↓	n.s.	n.s.	n.s.	n.s.	n.s.
≥Severely ill High CGI-t1	F = 4.25, p = .04↓	F = 6.10, p = .02↓	n.s.	n.s.	n.s.	n.s.	n.s.
≤Markedly ill Low CGI-t1	F = 4.52, p = .04↓	F = 9.26, p = .003↓	F = 4.48, p = .04↓	F = 6.40, p = .01↓	n.s.	n.s.	n.s.
≥Markedly ill High CGI-t9	F = 4.13, p = .05↓	F = 3.59, p = .06↓	F = 4.25, p = .04↓	F = 5.51, p = .02↓	n.s.	n.s.	n.s.
≤Moderate ill Low CGI-t9	F = 4.29, p = .04↓	F = 10.83, p = .001↓	n.s.	n.s.	n.s.	n.s.	n.s.
Note	(1) The p-values which are ≥ .10 and ≤ .19 are also listed in order to offer a better overview of detailed results **SINCE** the case numbers of all the clinical schizophrenic subgroups are so much lower than those of healthy controls (vary between 2/3 and 1/13). (2) n.s. = not significant. (3) CGI-d = score difference in CGI between at admission and discharge; CGI-t1: CGI score at admission; CGI-t9 = CGI score at discharge (CGI = Clinical Global Impressions).						

6.4.3 Nicotine abuse Th1/Th2 cytokines and ratios

Due to nicotine being deemed to have impacts on cytokine productions (Jiao et al., 1998), the effects of nicotine abuse on Th1/Th2 cytokines and ratios were also examined. Both healthy controls and patients were included into the analysis.

Totally, 62 smokers and 55 non-smokers had complete data for serum Th1/Th2 ratios, hormones, SHBG, age, and gender. No remarkable diversity between both groups was shown regarding those respects stated above except cortisol levels (age: F = .23, p = .63; cortisol: F = 3.76, p = .06; prolactin: F = .37, p = .55; estradiol: F = .59, p = .45; testosterone: F = .20, p = .66; SHBG: F = .70, p = .41). The smokers tended to have higher cortisol levels than the non-smokers. Multi-variant analysis exhibited no marked disparity between both nicotine subgroups in terms of their serum cytokine levels and Th1/Th2 ratios without co-varying with any parameter. Smokers had higher serum cytokine levels and IFN-γ/IL-4; however, the increases were not significant (IFN-γ/IL-4: F= .84, p = .36; IFN-γ/IL-10: F = .57, p = .45; IFN-γ: F = .90, p = .35; IL-2: F = .83, p = .37; TNF-α: F = .83, p = .36; IL-4: F = .72, p = .40; IL-10: F = .98, p= .32; IL-6: F = 1.10, p= .30).

Whole blood assay data

Complete data for whole blood assay Th1/Th2 ratios, hormones, age, and gender of 53 smokers and 46 non-smokers were available. No clear discrepancy was detected between both groups regarding their ages, hormones, and SHBG levels except cortisol (age: F = .41, p = .52; cortisol: F = 3.20, p = .08; prolactin: F = .18, p = .67; estradiol: F = .76, p = .38; testosterone: F = .08, p = .78; SHBG: F = .22, p = .64). Smokers tended to have higher cortisol levels than their non-smoker counterparts. On average, smokers produced more cytokines in whole blood assay than non-smokers. However, multi-variance analysis revealed that none of the diversities was significant (IFN-γ: F = .003, p = .96; IL-2: F = 2.14, p = .15; TNF-α: F = .003, p = .96; IL-4: F = .43, p = .52; IL-10: F = .14, p = .71; IL-6: F = .08, p = .77). Similarly, they had higher whole blood assay Th1/Th2 ratios than non-smokers. But the enhancements were not remarkable (IFN-γ/IL-4: F = 1.01, p = .32; IFN-γ/IL-10: F = .16, p = .69).

Lymphocyte data

Seventy smokers and 62 non-smokers had complete data for lymphocyte Th1/Th2 ratios, hormones, age, and gender. Both groups were of similar ages and had similar levels of diverse hormones and SHBG except that smokers tended to have higher cortisol levels (age: F = .18, p = .68; cortisol: F = 3.23, p = .07; prolactin: F = 1.34, p = .25; estradiol: F = 1.42, p = .24; testosterone: F = .05, p= .82; SHBG: F = .00, p = .98). Generally, smokers had lower averages for lymphocyte Th1/Th2 cytokines and ratios than non-smokers except IL-12. Nevertheless, MANOVA demonstrated that smokers had obviously lower lymphocyte IFN-γ and tended to release less IL-4 and IL-13 than non-smokers. But no noteworthy disparity in lymphocyte

Th1/Th2 ratios was found between both groups (IFN-γ: F = 5.04, p = .03; IL-4: F = 3.29, p = .07; IL-13: F = 3.07, p = .08; IL-12: F = .02, p = .90; IL-10: F = .02, p = .89; IFN-γ/IL-4: F = .09, p = .89; IFN-γ/IL-10: F = 1.47, p = .23; IFN-γ/IL-13: F = .01, p = .92).

Summary: MANOVA showed that nicotine abuse could have a substantial impact on lymphocyte IFN-γ productions, but it did not exert any clear influence on serum, whole blood assay cytokines and Th1/Th2 ratios.

Sub-sample (having entire serum, whole blood assay, lymphocyte immunological variables, endocrinological parameters, age, and gender data)

In order to explore the possible sources of Th1/Th2 imbalance within the same schizophrenics in a later section, a sub-sample only including those subjects who had complete data for serum, whole blood assay, lymphocyte cytokines, age, gender, hormones, and SHBG was chosen. This sub-sample contained 40 schizophrenics and 72 healthy subjects; it merely contains about 50% of the original schizophrenic subjects who had complete data for serum Th1/Th2 cytokines, age, gender, and distinct hormones.

(1) Whole SCH vs. whole CON

This sub-sample shared many similarities with the original total samples; they were significantly older, had remarkably lower cortisol and SHBG, but higher prolactin levels than their healthy counterparts. However, no remarkable diversity in estradiol and testosterone was found between both groups (age: F = 7.06, p = .009; cortisol: F = 4.18. p = .04; prolactin: F = 14.83, p < .001; estradiol: F = .01, p = .94; testosterone: F = 1.52, p = .22; SHBG: F = 6.22, p = .01). This schizophrenic sub-sample was averagely 34.93 years old (SD = 12.32). The mean cortisol, prolactin, estradiol, testosterone, and SHBG level for schizophrenic patients were 169.25 µg/l (SD = 65.34), 32.55 ng/ml (SD = 35.68), 47.90 pg/ml (SD = 54.04), 3.18 ng/ml (SD = 2.67), and 51.63 nmol/l (SD = 40.57), correspondingly. Nonetheless, the control sub-sample was 29.68 years old (SD = 8.47) and had averagely 204.27 µg/l cortisol (SD = 96.68), 15.35 ng/ml prolactin (SD = 9.79), 47.11 pg/ml estradiol (SD = 50.44), 3.94 ng/ml testosterone (SD = 3.38), and 77.62 nmol/l SHBG (SD = 58.50). The significant differences in those parameters stated above were included into the following multi-variance analysis of serum and whole blood assay data. For the lymphocyte data, only the age was considered as covariant since isolated lymphocytes were not directly exposed to those influencing factors such as hormones when they were in vitro stimulated to produce cytokines.

94

Multi-variant comparisons between both schizophrenic and control sub-sample showed comparable results to those of the original total serum sample. As a whole group, schizophrenics had clearly reduced serum IFN-γ/IL-4 and IFN-γ/IL-10 ratios compared with healthy controls. The schizophrenics had a mean of 11.42 (SD = 5.84) for IFN-γ/IL-4 and an average of 13.48 (SD = 8.09) for IFN-γ/IL-10, while the controls obtained an average of 20.49 (SD = 18.42) for IFN-γ/IL-4 and 19.75 (SD = 9.75) for IFN-γ/IL-10. The discrepancies in both serum Th1/Th2 ratios achieved very significant levels (serum IFN-γ/IL-4: F = 8.94, p = .003; IFN-γ/IL-10: F = 14.08, p < .001). At single cytokine level, no marked difference was observed between both diagnostic groups if including all extreme values and outliers (IFN-γ: F = 1.47, p = .23; IL-2: F = 1.48, p = .23; TNF-α: F = 1.38, p = .24; IL-4: F = 1.40, p = .24; IL-10: F = 1.30, p = .26; IL-6: F = .81, p = .37).

Whole blood assay data

Besides, the schizophrenic sub-sample tended to have decreased whole blood assay IFN-γ/IL-10 if compared to controls (whole blood assay IFN-γ/IL-10: F = 3.55, p = .06). Schizophrenics had an average of 29.82 (SD = 20.66) for whole blood assay IFN-γ/IL-10, while the average for the control group was 38.59 (SD = 26.41). Additionally, schizophrenics had markedly decreased whole blood assay IFN-γ and IL-6. The mean IFN-γ and IL-6 production for schizophrenic patients were 28989.78 (SD = 17477.00) and 1592.50 pg/ml (SD = 1011.81), correspondingly. In contrast, those of control subjects were 44745.33 (SD = 32904.80) and 2830.09 pg/ml (SD = 2820.63) in the same order as mentioned in schizophrenia (IFN-γ: F = 5.66, p = .02; IL-6: F = 5.15, p = .03). Concerning the remaining cytokine productions, no noteworthy outcome was found (IL-2: F = .40, p = .53; TNF-α: F = 2.55, p = .11; IL-4: F = .03, p = .87; IL-10: F= .39, p = .53).

Lymphocyte data

Generally, lymphocyte data also confirmed the findings from the original total lymphocyte sample. In addition to IFN-γ, IL-4, and IL-13, no further remarkable alteration was shown in schizophrenia as a whole group (lymphocyte IFN-γ: F = 11.98, p = .001; IL-4: F = 6.34, p = .01; IL-13: F = 6.27, p = .01; IL-12: F = .33, p = .57; IL-10: F = 2.72, p = .10; IFN-γ/IL-4: F = .60, p = .44; IFN-γ/IL-10: F = .30, p = .59; IFN-γ/IL-13: F = .18, p = .67). Schizophrenics released noticeably less lymphocyte IFN-γ, IL-4, and IL-13 than controls. The average productions of IFN-γ, IL-4, and IL-13 were 714.61 (SD = 433.90), 345.03 (SD = 200.94), and

556.35 spots (SD = 333.42) for schizophrenics, while those for normal controls were 1094.75 (SD = 874.98), 426.22 (SD = 152.45), and 792.87 spots (SD = 505.88).

(2) Female SCH vs. female CON

Totally, 15 female schizophrenics and 34 female controls had serum, whole blood assay, and lymphocyte Th1/Th2 data. Schizophrenic females were averagely 37.80 years old (SD = 13.01), while control females were 29.29 years old (SD = 7.96). Female patients were obviously older and had apparently higher prolactin, but lower cortisol levels than healthy females (age: F = 7.94, p = .007; cortisol: F = 4.70, p = .04; prolactin: F = 9.10, p = .004). The average cortisol and prolactin level for schizophrenic women were 163.37 µg/ml (SD = 76.00) and 41.23 ng/ml (SD = 41.23), whereas those for female controls were 235.24 µg/ml (SD = 117.70) and 14.79 ng/ml (SD = 5.81), respectively. In addition, female patients tended to have lower SHBG concentrations than control women (F = 2.97, p = .09). However, both female groups have relatively comparable testosterone and estradiol concentrations (testosterone: F = .72, p = .40; estradiol: F = .26, p = .61).

Serum data

On average, female schizophrenics had lower typical Th1 cytokine levels including IFN-γ and IL-2, however, higher Th2-released cytokine serum levels such as IL-4, IL-6, IL-10, TNF-α as well as lower serum Th1/Th2 ratios. Nevertheless, only the diversities in IFN-γ, IL-6, IFN-γ/IL-4, and IFN-γ/IL-10 reached significance levels (serum IFN-γ/IL-10: F = 9.04, p = .004; IFN-γ/IL-4: F = 5.52, p = .02; IL-6: F = 5.50, p = .02; IFN-γ: F = 5.54, p = .02; IL-2: F = .53, p = .47; TNF-α: F = .36, p = .55; IL-4: F = 1.23, p = .27; IL-10: F = 2.61, p = .11). The average IFN-γ and IL-6 level for the schizophrenic females were 31.67 pg/ml (SD = 13.51) and 6.59 pg/ml (SD = 11.63), whereas those for the control women were 45.77 pg/ml (SD = 24.58) and 2.33 pg/ml (SD = .96), respectively. The schizophrenic females obtained averagely 10.78 (SD = 4.47) for serum IFN-γ/IL-4 and 11.85 (SD = 5.61) for IFN-γ/IL-10, while their healthy female counterparts had a mean of 21.19 (SD = 14.98) for IFN-γ/IL-4 and 19.71 (SD = 9.10) for IFN-γ/IL-10.

Whole blood assay data

On the whole, schizophrenic females produced less cytokines in PHA-stimulated whole blood and had lower Th1/Th2 ratios than healthy females except IL-4. However, only the differences in IFN-γ and IFN-γ/IL-10 achieved significance levels; additionally, the disparity in IL-6 also tended to be significant (whole blood assay IFN-γ/IL-10: F = 6.56, p = .01; IFN-γ/IL-4: F = 2.38, p = .13; IFN-γ: F = 4.36, p = .04; IL-6: F = 3.13, p = .08; IL-2: F = .27, p =

.61; TNF-α: F = 1.90, p = .18; IL-4: F = .38, p = .54; IL-10: F = .00, p = 1.00). The mean of IFN-γ/IL-10 for female schizophrenic patients was 19.21 (SD = 13.13), whereas that for healthy women was 34.52 (SD = 21.79). Schizophrenic females produced averagely 23162.80 pg/ml IFN-γ (SD = 12862.94) and 1354.95 pg/ml IL-6 (SD = 745.11), whereas control females secreted averagely 45134.33 pg/ml IFN-γ (SD = 33298.22) and 3365.67 pg/ml IL-6 (SD = 3284.69).

Lymphocyte data

Although the original total female sample did not show any diversity in any of the lymphocyte Th1/Th2 ratios, the sub-sample did reveal a significant discrepancy between both female groups. In general, female schizophrenic patients released less lymphocyte cytokines and had lower lymphocyte Th1/Th2 ratios including IFN-γ/IL-4 and IFN-γ/IL-13, but not IFN-γ/IL-10. However, both female groups were only remarkably distinguishable in IFN-γ/IL-4 ratio and IFN-γ production (lymphocyte IFN-γ/IL-4: F = 4.58, p = .04; IFN-γ/IL-10: F = .05, p = .82; IFN-γ/IL-13: F = 1.33, p = .25; IFN-γ: F = 6.28, p = .02; IL-12: F = .36, p = .55; IL-4: F = .67, p = .42; IL-13: F = 1.15, p = .29; IL-10: F = .47, p = .50). Female schizophrenics obtained an average of 2.61 (SD = 2.25) and control women had a mean of 2.99 (SD = 2.90) for IFN-γ/IL-4 ratio. The female patients produced averagely 625.21 IFN-γ spots (SD = 439.99); in contrast, the female controls released averagely 1083.05 IFN-γ spots (SD = 1032.02) pro 40000 lymphocytes.

(3) Male SCH vs. male CON

Comparisons between male schizophrenics (N = 25) and controls males (N = 38) showed that schizophrenic males had significantly higher prolactin, nevertheless, lower testosterone and SHBG than male normal subjects, although they were of relatively similar ages and had comparable estradiol and cortisol levels (prolactin: F = 7.15, p = .01; testosterone: F = 17.17, p < .001; SHBG: F = 7.58, p = .008; age: F = 1.46, p = .23; cortisol: F = .06, p = .81; estradiol: F = .24, p = .63). Schizophrenic males were averagely 33.20 (SD = 11.83), while their healthy male counterparts were 29.80 years old (SD = 8.82). Furthermore, the mean prolactin, testosterone, and SHBG level were 27.35 ng/ml (SD = 21.40), 4.77 ng/ml (SD = 2.10), and 31.68 nmol/l (SD = 11.75) for schizophrenic men as well as 15.97 ng/ml (SD = 12.37), 6.81 ng/ml (SD = 1.78), and 42.61 nmol/l (SD = 17.75) for control males, correspondingly.

Serum data

In contrast to the results from both female groups, multi-variance analysis showed that male schizophrenics had not only lower serum Th1/Th2 ratios but also cytokine levels than normal

males. Nevertheless, these two male diagnostic groups could be evidently differentiated from each other exclusively in both serum Th1/Th2 ratios (serum IFN-γ/IL-4: F = 4.46, p = .04; IFN-γ/IL-10: F = 8.69, p = .005; IFN-γ: F = .70, p = .41; IL-2: F = .70, p = .41; TNF-α: F = .67, p = .42; IL-4: F = .75, p = .39; IL-10: F = .65, p = .42; IL-6: F = .59, p = .45). The averages of IFN-γ/IL-4 and IFN-γ/IL-10 were 11.81 (SD = 6.59), 14.46 (SD = 9.23) for schizophrenic males and 19.86 (SD = 21.21), 19.78 (SD = 10.42) for male controls.

Whole blood assay data

Male schizophrenics generally produced more IL-2, IL-4 and had higher IFN-γ/IL-10 ratios than male controls. However, they released less IFN-γ, TNF-α, IL-6, IL-10 and had lower IFN-γ/IL-4 ratios than control men. Nevertheless, contrary to the whole blood assay data of female subjects, no single apparent diversity was found between both male groups in terms of their whole blood assay Th1/Th2 ratios and cytokine secretions (whole blood assay IFN-γ/IL-4: F = .71, p = .40; IFN-γ/IL-10: F = .04, p = .84; IFN-γ: F = .14, p = .71; IL-2: F = .01, p = .91; TNF-α: F = .001, p = .98; IL-4: F = .46, p = .50; IL-10: F = .20, p = .65; IL-6: F = 1.09, p = .30).

Lymphocyte data

By and large, male schizophrenics had lower lymphocyte cytokine productions, nevertheless, higher lymphocyte Th1/Th2 ratios than control males. Multi-variance analysis failed to reveal any conspicuous disparity between both male subgroups in lymphocyte Th1/Th2 ratios. However, male schizophrenics released significantly less IFN-γ, IL-4, and IL-13 than their healthy male counterparts (lymphocyte IFN-γ/IL-4: F = .05, p = .82; IFN-γ/IIL-10: F = .61, p = .44; IFN-γ/IL-13: F = 1.30, p = .26; IFN-γ: F = 4.42, p = .04; IL-4: F = 4.16, p = .05; IFN-γ/IL-13: F = 6.92, p = .01). The mean IFN-γ, IL-4, and IL-13 were 768.27 spots pro 40000 lymphocytes (SD = 430.14), 352.47 spots pro 200000 lymphocytes (SD = 188.79), and 569.77 spots pro 200000 cells (SD = 317.21) for schizophrenic men, while those for male controls were 1105.22 (SD = 720.39), 436.62 (SD = 138.70), and 873.62 spots (SD = 515.94) with the same cell concentrations as stated in schizophrenics.

The main Th2-shift findings in the sub-sample are summarized in **Table 6-4**.

Table 6-4: A summary of Th2-shifts in the sub-sample which included the subjects who had complete data for serum, whole blood assay, lymphocyte cytokines, endocrinological parameters, gender, and age.

Comparisons between schizophrenic and control subjects who had complete serum, whole blood assay, and lymphocyte data							
	Serum		**Whole Blood**		**lymphocyte**		
Th1/Th2	**IFN-γ/IL4**	**IFN-γ/IL10**	**IFN-γ/IL4**	**IFN-γ/IL10**	**IFN-γ/IL4**	**IFN-γ/IL10**	**IFNγ/IL13**
SCH vs. CON	F = 8.94, p = .003↓	F = 14.08, p < .001↓	n.s.	F = 3.55, P = .06↓	n.s.	n.s.	n.s.
♀ SCH vs. ♀ CON	F = 5.52, p = .02↓	F = 9.04, P = .004↓	n.s.	F = 6.56, P = .01↓	F = 4.58, p = .04↓	n.s.	n.s.
♂ SCH vs. ♂ CON	F = 4.46, p = .04↓	F = 8.69, P = .005↓	n.s.	n.s.	n.s.	n.s.	n.s.
Note	Total SCH N = 40; ♀SCH N = 15; ♂SCH N = 25; total CON N = 72; ♀CON N = 34; ♂CON N = 38.						

6.5 Contributors of Th1/Th2 imbalance in schizophrenia

In order to detect the individual contribution of each parameter measured in this study to the imbalance of Th1/Th2 systems in schizophrenic patients, multiple regression were used to explore the relationships between various Th1/Th2 ratios and other possible influencing factors measured in this study, using IFN-γ/IL-4 or IFN-γ/IL-10 or IFN-γ/IL-13 as criterion and age, hormones, SHBG, and Th1/Th2 cytokines as predictors. Although the isolated lymphocytes applied in ELISPOT were not directly exposed to other possible influencing factors in serum and whole blood, for the reason that the cytokine producing ability of lymphocytes could reflect (partially) biological processes such as aging or degeneration, a complete model including all Th1/Th2 cytokines assessed in this study, age, hormones, and SHBG were firstly utilized to predict all serum, whole blood assay, and lymphocyte Th1/Th2 ratios. Then depending upon the fitness of the complete model, predictor(s) could be excluded in order to improve the envisaging power of the model and to enable a reliable prediction of a Th1/Th2 ratio.

Due to the small case number in schizophrenic subjects and the great number of predictors involved, the aim to conduct multiple regression analysis to the data of schizophrenic patients was not to generate a multiple regression formula in order to predict further unknown schizophrenic cases. The goal was to detect which of those predictors made a significant contribution to the variance of a Th1/Th2 ratio within the same subject group. The t-test of β-

coefficient (regression coefficient) for each predictor was presented as the individual predictive power or as the magnitude of importance of each predictor in forecasting Th1/Th2 ratios. Because the case numbers of the control and schizophrenic group were somewhat different, the β-coefficients of various predictors in distinct groups won't be directly comparable. The case numbers in various groups of this study lied between 15 (♀SCH) and 72 (whole CON). The t-values for case numbers within the range of our subjects are relatively comparable. For example, the t-value of $p = .0005$ for $N = 15$ is about 4.07, for $N = 25$ (♂SCH) is about 3.72, for $N = 40$ (whole SCH) is about 3.55, while that for $N = 72$ is around 3.46 etc. Therefore, the **t**- and p-value of each predictor (see **Table 6-5-1(1)** to **Table 6-5-3(3)**) were presented in the following sections in order to compare their magnitudes of importance in envisaging Th1/Th2 ratios between different groups. Furthermore, the **F**-value represents the cumulative predictive power of predictor assembly included in the corresponding model. **R²**-value is the portion of a criterion that can be explicated through the predictors included in the model of multiple regression analysis.

6.5.1 Serum data

The complete model using age, cortisol, prolactin, estradiol, testosterone, SHBG, IFN-γ, IL-2, TNF-α, IL-4, IL-10, and IL-6 as predictors appeared to be sufficient to envisage both serum IFN-γ/IL-4 and IFN-γ/IL-10 ratios in the whole group of schizophrenics, the whole control group, the male schizophrenics, the male controls, and the female control group, but not the female schizophrenic group (whole SCH – IFN-γ/IL-4: $F = 22.10$, $p < .001$; IFN-γ/IL-10: $F = 35.49$, $p < .0001$; whole CON – IFN-γ/IL-4: $F = 13.30$, $p < .001$; IFN-γ/IL-10: $F = 11.64$, $p < .001$; ♂SCH – IFN-γ/IL-4: $F = 23.59$, $p < .0001$; IFN-γ/IL-10: $F = 105.12$, $p < .0001$; ♂CON – IFN-γ/IL-4: $F = 13.44$, $p < .001$; IFN-γ/IL-10: $F = 5.27$, $p < .001$; ♀CON – IFN-γ/IL-4: $F = 6.36$, $p < .001$; IFN-γ/IL-10: $F = 17.31$, $p < .001$; ♀SCH – IFN-γ/IL-4: $F = 6.21$, $p = .15$; IFN-γ/IL-10: $F = 8.58$, $p = .11$).

Whole SCH vs. whole CON

Comparisons of the predictive power of individual cytokine in serum (see **Table 6-5-1(1)**) showed that the principal abnormalities of schizophrenics were (1) much too strong influence from IFN-γ (+) as well as (2) IL-4 (-) and IL-10 (-) if the whole schizophrenic group was regarded as a homogenous entity and compared with the whole controls. Within the schizophrenic group, IFN-γ and IL-4 significantly correlated with serum IFN-γ/IL-4 (IFN-γ: t

= 10.38, p < .001; IL-4: t = -8.93, p < .001). Similar outcomes were found between IFN-γ, IL-10 and serum IFN-γ/IL-10 in schizophrenia (whole SCH – serum IFN-γ: t = 14.85, p < .001; IFN-γ/IL-10: t = -9.12, p < .001). In controls, IFN-γ, IL-4, and IL-10 likewise played important roles in predicting serum IFN-γ/IL-4 and IFN-γ/IL-10 variances, the importance magnitudes of these three cytokines were not as overwhelming as those in schizophrenics (whole CON – serum IFN-γ/IL-4 – IFN-γ: t = 4.23, p < .001; IL-4: t = -5.11, p < .001; serum IFN-γ/IL-10: IFN-γ: t = 10.05, p < .001; IL-10: t = -6.40, p < .001). For serum IFN-γ/IL-4 ratio, no other important factor existed among those parameters measured except IFN-γ and IL-4, while for serum IFN-γ/IL-10 ratio, estradiol and IL-6 additionally tended to play a role in the balancing between IFN-γ and IL-10 in schizophrenics and controls, respectively (whole SCH – estradiol: t =1.97, p = .06; whole CON – IL-6: t = -1.89, p = .06).

Table 6-5-1(1): A summary of the individual contributions of predictors in serum IFN-γ/IL-4 and IFN-γ/IL-10 variance in the whole schizophrenic and control group (results from multiple regression).

The individual contributions of predictors in forecasting serum Th1/Th2 ratios (Whole SCH vs. Whole CON)							
Criteria	Serum IFN-γ/IL-4				Serum IFN-γ/IL-10		
	SCH (N = 40)		**CON** (N = 72)		**SCH** (N = 40)		**CON** (N = 72)
Predictor	T	p	T	p	T	p	T	p
IFN-γ	10.38	.000*****	4.12	.000*****	14.85	.000*****	9.83	.000*****
IL-2	-.16	.87	.72	.47	.85	.41	-.30	.76
TNF-α	.91	.37	-1.65	.11	-.67	.51	-.69	.49
IL-4	-8.93	.000*****	-5.19	.000*****	.08	.94	-.94	.35
IL-10	.23	.82	-.93	.36	-9.12	.000*****	-6.28	.000*****
IL-6	-.68	.50	-.97	.34	1.19	.25	-1.80	.08
Cortisol	-1.14	.26	1.42	.16	-.22	.83	.54	.59
Prolactin	.47	.65	-.25	.80	-.91	.37	.16	.87
Estradiol	-.92	.37	.09	.93	1.97	.06	-.62	.54
Testosterone	.91	.37	-.06	.95	-.78	.44	.14	.89
SHBG	1.21	.24	-.31	.76	-.36	.72	-.91	.37
Age	-.50	.62	.21	.84	-1.27	.22	-.13	.90
Adjusted	R² = .87		R² = .68		R² = .91		R² = .64	
Signif. test	F = 22.10, p < .0001		F = 13.30, p < .0001		F = 35.49, p < .0001		F = 11.64, p < .0001	

Besides, the complete model explained higher percentages of serum IFN-γ/IL-4 and IFN-γ/IL-10 variance in schizophrenics, compared to healthy subjects (whole SCH – serum IFN-γ/IL-4: adjusted R² = .87, F = 22.10, p < .0001; IFN-γ/IL-10: adjusted R² = .91, F = 35.49, p < .0001;

whole CON – IFN-γ/IL-4: adjusted R^2 = .67, F = 35.49, p < .0001; IFN-γ/IL-10: adjusted R^2 = .65, F = 12.07, p < .0001).

Table 6-5-1(2): The individual contributions of predictors in envisaging the variances of serum IFN-γ/IL-4 and IFN-γ/IL-10 in schizophrenic and control females (results from multiple regression analysis: underlined = complete model; shaded = new model)

The individual contributions of predictors in forecasting serum Th1/Th2 ratios (♀SCH vs. ♀CON)								
Criteria	Serum IFN-γ/IL-4				Serum IFN-γ/IL-10			
	SCH (N = 15)		CON (N = 34)		SCH (N = 15)		CON (N = 34)	
Predictor	T	p	T	p	T	p	T	p
IFN-γ	2.77	.11	5.24	.000*****	.91	.46	11.68	.000*****
	3.27	.05*	5.32	.000*****	1.25	.30	11.95	.000*****
IL-2	-.77	.52	-.01	1.00	2.81	.11	.78	.44
	-.68	.55	-.06	.95	3.74	.03*	.83	.42
TNF-α	1.24	.34	-.68	.50	-.65	.58	-.04	.97
	1.29	.29	-.70	.49	-.84	.46	-.03	.97
IL-4	-1.36	.31	-4.13	.000*****	-3.23	.08	-.63	.53
	-1.79	.17	-4.27	.000*****	-4.312	.03*	-.63	.54
IL-10	.37	.75	.03	.97	-5.19	.04*	-6.48	.000*****
	.20	.86	.10	.92	-6.83	.006***	-6.70	.000*****
IL-6	-.57	.63	.02	.99	2.62	.12	-.86	.40
	-.40	.72	-.07	.95	3.64	.04*	-.85	.41
Cortisol	-.96	.44	.57	.58	2.82	.11	-1.66	.11
	-.98	.40	.56	.58	3.53	.04*	-1.69	.11
Prolactin	-.37	.75	-.28	.78	1.45	.28	.09	.93
	.04	.97	-.38	.71	2.49	.09	.13	.90
Estradiol	-.93	.45	.32	.75	3.52	.07	-1.31	.21
	-.87	.45	.27	.79	4.68	.02**	-1.32	.20
Testosterone	.78	.52	.09	.93	-1.89	.20	-.71	.49
	.60	.59	-.001	1.00	-3.49	.04*	-.69	.50
SHBG	1.20	.36	.65	.53	-1.63	.24	1.06	.30
	1.25	.30	.57	.58	-2.05	.13	1.15	.26
Age	.57	.63	.50	.62	.06	.96	-.23	.82
Adjusted	R^2 = ~~.82~~ (.86)		R^2 = .66 (.67)		R^2 = ~~.87~~ (.91)		R^2 = .86 (.86)	
Signif.test	~~F = 6.21, p = .15~~		F = 6.36, p < .001		~~F = 8.58, p = .11~~		F = 17.31, p <.0001	
	F = 8.70, p = .05		F = 9.37, p < .001		F = 14.01, p = .03		F = 19.73, p < .0001	

Female SCH vs. female CON

In contrast to female controls whose serum IFN-γ/IL-4 and IFN-γ/IL-10 could be significantly and only explained by IFN-γ, IL-4, and IL-10, none of the predictors included in this study was able to reliably envisage serum IFN-γ/IL-4 and IFN-γ/IL-10 in schizophrenic women if using the complete model. The only exception was IL-10 for IFN-γ/IL-10; however, the extent of importance dropped if compared to that in healthy females (see **Table 6-5-1(2)** – (in

black) ♀CON serum IFN-γ/IL-4 –IFN-γ: t = 5.24, p < .001; IL-4: t = -4.13, p < .001; IFN-γ/IL-10 – IFN-γ: t = 11.68, p < .001; IL-10: t = -6.48, p < .001; ♀SCH serum IFN-γ/IL-4 – IFN-γ: t = 2.77, p = .11; IL-4: t = -1.36, p = .31; IFN-γ/IL-10 – IFN-γ: t = .91, p = .46).

Table 6-5-1(2-1): The inter-correlations among the predictors which were included in the model to forecast serum IFN-γ/IL-4 and IFN-γ in female schizophrenic patients and normal female controls.

	Age	SHBG	IL-4	IL-6	IFN-γ	E2	IL-10	Cort	Testo	TNF	Prl	IL-2
Serum Data: female schizophrenics versus female controls (inter-correlations among the predictors of serum IFN-γ/IL-4 and IFN-γ/IL-10).												
Age		.20	.09	.16	.02	.12	.12	.04	.19	.01	.17	.11
SHBG	.20		-.11	.10	.27	-.05	-.49	-.61	.29	.12	.11	.07
IL-4	.28	.44		.02	.14	.19	.16	.18	.09	-.40	-.25	.19
IL-6	-.45	-.45	-.69		-.11	.08	-.36	-.32	.03	-.22	.20	.08
IFN-γ	.51	.20	.61	-.65		.09	-.34	-.01	.21	-.23	-.16	-.18
E2	-.38	-.69	-.84	.81	-.67		.03	.37	.10	-.10	-.15	-.04
IL-10	.36	.60	.41	-.52	.09	-.48		.39	-.16	-.04	-.10	.01
Cort	-.20	-.83	-.74	.64	-.38	.89	-.53		-.04	-.10	-.36	-.17
Testo	.74	.54	.70	-.77	.77	-.86	.49	-.67		.19	.14	-.16
TNF	.24	.74	.06	-.24	.11	-.45	.35	-.55	.40		.17	-.38
Prl	-.68	-.19	-.65	.73	-.91	.71	-.24	.41	-.88	-.12		-.09
IL-2	-.38	-.66	-.87	.82	-.65	.96	-.58	.86	-.84	-.40	.70	
Note	The left bottom corner = schizophrenic females (N = 15) ; the right above corner = control females (N = 34). E2 = estradiol; Cort = cortisol; Testo = testosterone; Prl = prolactin; TNF = TNF-α; SHBG = sex hormone-binding globulin											

After various attempts, a new model dropping age resulted in similar outcomes in female controls (IFN-γ: t = 5.32, p < .001; IL-4: t = -4.27, p < .001). Nevertheless, those of schizophrenic females had changed a lot; the predictive power of IFN-γ was significant, but not that of IL-4 (IFN-γ: t = 3.27, p = .05; IL-4: t = -1.79, p = .17). The new model could only predict 67% serum IFN-γ/IL-4 variance in control females, but 86% in schizophrenic women (♀CON: F = 7.16, p < .001; ♀SCH: F = 8.70, p = .05). The new model also revealed that the imbalance in serum IFN-γ/IL-4 in schizophrenic females could be a consequence of interactive effects of cytokines, hormones, and age. That is, among the predictors included in the model existed close inter-correlations as shown in **Table 6-5-1(2-1)** which were obtained simultaneously with the outcomes from multiple regression stated above; however, they were totally different in normal females.

Male SCH vs. male CON

Table 6-5-1(3): An overlook of the importance indexes of predictors for serum IFN-γ/IL-4 and IFN-γ/IL-10 variance in schizophrenic and control males (results from multiple regression)

The individual contributions of predictors in forecasting serum Th1/Th2 ratios (♂SCH vs. ♂CON)								
Criteria	Serum IFN-γ/IL-4				Serum IFN-γ/IL-10			
	SCH (N = 25)		CON (N = 38)		SCH (N = 25)		CON (N = 38)	
Predictor	T	p	T	p	T	p	T	p
IFN-γ	8.62	.000*****	2.08	.05*	20.15	.000*****	5.75	.000*****
IL-2	-.15	.89	.44	.67	1.71	.11	-.26	.80
TNF-α	.96	.36	-1.29	.21	-.37	.72	-1.17	.25
IL-4	-8.68	.000*****	-3.37	.002***	-.04	.97	-.76	.46
IL-10	2.29	.04*	-1.28	.21	-6.87	.000*****	-3.51	.002***
IL-6	-2.03	.07	-.15	.88	-.15	.89	-.99	.33
Cortisol	-.20	.85	1.35	.19	-.03	.98	.80	.43
Prolactin	1.18	.26	.07	.95	2.20	.05*	.10	.92
Estradiol	1.96	.07	-.63	.54	2.07	.06	-.30	.77
Testosterone	-1.38	.19	.96	.35	-.88	.40	.81	.43
SHBG	2.68	.02*	-1.32	.20	-1.23	.24	-1.13	.27
Age	-.77	.46	.08	.94	-2.25	.04*	.19	.85
Adjusted	R^2 = .92		R^2 = .79		R^2 = .98		R^2 = .56	
Signif. test	F = 23.59, p < .0001		F = 13.44, p < .001		F = 105.12, p < .0001		F = 5.27, p < .001	

The same new model stated above also improved the power of predictor assembly for serum IFN-γ/IL-10 in schizophrenic women. Nevertheless, it caused almost no change in female controls (♀SCH – the complete model: F = 8.58, p = .11; new model: F = 14.01, p = .03; ♀CON – the complete model: F = 17.31, p < .0001; new model: F = 19.73, p < .001). The new model could interpret 91% and 86% variance of serum IFN-γ/IL-10 ratio in female schizophrenics and controls, respectively. In control females, the most essential factors which made the greatest contributions to IFN-γ/IL-10 variance were IFN-γ and IL-10 (IFN-γ: t = 11.95, p < .001; IL-10: t = -6.70, p < .001). However, it was not that case in schizophrenic women. IL-10, with a slightly weakened effect, still played the most important role. Nonetheless, a variety of other factors except IFN-γ seemed to be involved in the balancing between IFN-γ and IL-10 in female schizophrenic patients (IL-10: t = -6.83, p = .006; IFN-γ: t = 1.25, p = .30). They included estradiol, IL-4, testosterone, IL-2, IL-6, prolactin, and cortisol (estradiol: t = 4.68, p = .02; IL-4: t = -4.13, p = .03; testosterone: t = -3.48, p = .04; IL-2: t = 3.74, p = .03; IL-6: t = 3.64, p = .04; prolactin: t = 2.49, p = .09; cortisol: t = 3.53, p = .04). The significant contributors of serum IFN-γ/IL-10 ratio for schizophrenic females were much

more widespread than those for healthy females. However, the importance of IFN-γ in predicting IFN-γ/IL-4 remained unnoticeable in female schizophrenic subjects (t = 1.25, p = .30).

If compared male schizophrenics to male controls, in addition to enhanced influences from IFN-γ, IL-4, and IL-10 on IFN-γ/IL-4 as well as IFN-γ/IL-10 in schizophrenic males, there were further influencing factors which remarkably contributed to the imbalance of serum IFN-γ/IL-4 and IFN-γ/IL-10 in male schizophrenics (see **Table 6-5-1(3)** – ♂CON: serum IFN-γ/IL-4 – IFN-γ: t = 2.08, p = .05; IL-4: t = -3.37, p = .002; IFN-γ/IL-10 – IFN-γ: t = 5.75, t < .001; IL-10: t = -3.51, p = .002; ♂SCH: serum IFN-γ/IL-4 – IFN-γ: t = 8.62, p < .001; IL-4: t = -8.68, p < .001; IFN-γ/IL-10 – IFN-γ: t = 20.15, p < .001; IL-10: t = -6.87, p < .001). They include serum IL-10 and SHBG for IFN-γ/IL-4 as well as prolactin and age for IFN-γ/IL-10. Nevertheless, those factors did not play any role in serum IFN-γ/IL-4 and IFN-γ/IL-10 in controls (♂SCH serum IFN-γ/IL-4 – IL-10: t = 2.29, p = .04; SHBG: t = 2.68, p = .02; IFN-γ/IL-10 – prolactin: t = 2.20, p = .05; age: t = -2.25, p = .04; ♂CON serum IFN-γ/IL-4 – IL-10: t = -1.29, p = .21; SHBG: t = -1.32, p = .20; IFN-γ/IL-10 – prolactin: t = .10, p = .92; age: t = .19, p = .85).

Similar to the findings concerning female schizophrenics, the complete model could elucidate more Th1/Th2 ratio variance in schizophrenic males than in control men (♂SCH – serum IFN-γ/IL-4: adjusted R² = .92, F = 23.59, p < .0001; IFN-γ/IL-10: adjusted R² = .98, F = 105.12, p < .0001; ♂CON – serum IFN-γ/IL-4: adjusted R² = .79, F = 13.44, p < .0001; IFN-γ/IL-10: adjusted R² = .56, F = 5.26, p < .001).

6.5.2 Whole blood assay data

The complete model was also able to significantly predict whole blood assay IFN-γ/IL-4 and IFN-γ/IL-10 in the whole schizophrenic patients, the whole control group, the male and female control group (whole CON – whole blood assay IFN-γ/IL-4: F = 15.38, p < .001; IFN-γ/IL-10: F = 15.26, p < .001; whole SCH – IFN-γ/IL-4: F = 3.62, p = .003; IL-4: F = 9.94, p < .001; ♂CON – IFN-γ/IL-4: F = 6.77, p < .001; IFN-γ/IL-10: F = 9.28, p < .001; ♀CON – IFN-γ/IL-4: F = 9.64, p < .001; IFN-γ/IL-10: F = 23.72, p < .001). For male schizophrenics, the model was only capable of envisaging the variance of whole blood assay IFN-γ/IL-10, but not

that of IFN-γ/IL-4. However, for schizophrenic females, it failed to predict whole blood assay IFN-γ/IL-4 and IFN-γ/IL-10 sufficiently (♂SCH – whole blood assay IFN-γ/IL-4: F = 2.07, p = .11; IFN-γ/IL-10: F = 9.00, p < .001; ♀SCH – IFN-γ/IL-4: F = 6.26, p = .15; IFN-γ/IL-10: F = 9.83, p = .10).

Whole SCH vs. whole CON

In controls, apart from both key cytokines IFN-γ and IL-4, TNF-α seemed to have an essential contribution to whole blood assay IFN-γ/IL-4 ratio (see **Table 6-5-2(1)** – whole CON – whole blood assay IFN-γ: t = 7.70, p < .001; IL-4: t = -4.79, p < .001; TNF-α: t = -2.45, p = .02). On the contrary, schizophrenics had reduced influences from both IFN-γ and IL-4 on IFN-γ/IL-4, nevertheless, an enhanced impact from IL-6 (whole SCH – whole blood IFN-γ: t = 2.24, p = .03; IL-4: t = -3.40, p =.002; IL-6: t = 2.06, p = .05).

Table 6-5-2(1): A summary of the individual contributions of predictors for whole blood assay IFN-γ/IL-4 and IFN-γ/IL-10 variance in the whole schizophrenic and control group (results from multiple regression).

Criteria	Whole blood IFN-γ/IL-4				Whole blood IFN-γ/IL-10			
	SCH (N = 40)		CON (N = 72)		SCH (N = 40)		CON (N = 72)	
Predictor	T	p	T	p	T	p	T	p
IFN-γ	2.24	.03*	7.70	.000*****	5.43	.000*****	10.39	.000*****
IL-2	-1.60	.12	.20	.84	-1.64	.11	.82	.41
TNF-α	.68	.50	-2.45	.02***	1.632	.11	-.81	.42
IL-4	-3.40	.002***	-4.79	.000******	-1.46	.16	3.02	.004***
IL-10	-.38	.71	1.67	.10	-4.93	.000*****	-9.16	.000*****
IL-6	2.06	.05*	.40	.69	1.23	.23	-1.12	.27
Cortisol	.49	.63	.12	.91	.52	.61	.13	.90
Prolactin	-.35	.73	-1.00	.32	.16	.87	1.12	.27
Estradiol	-.33	.74	.15	.88	-.92	.37	-.42	.67
Testosterone	.38	.71	.13	.90	.47	.64	-1.17	.25
SHBG	.47	.64	-.10	.92	1.50	.15	-1.64	.11
Age	.15	.89	.75	.45	2.02	.05*	-1.15	.26
Adjusted	R^2 = .45		R^2 = .71		R^2 = .73		R^2 = .71	
Signif. test	F = 3.62, p = .003		F = 15.83, p < .0001		F = 9.94, p < .0001		F = 15.26, p < .001	

For the variance of whole blood assay IFN-γ/IL-10, in addition to IFN-γ and IL-10, the ages of schizophrenic men also exerted marked impacts on IFN-γ/IL-10 ratios. However, instead of age in schizophrenia, IL-4 appeared to have considerable influences on IFN-γ/IL-10 ratios in healthy subjects, in addition to IFN-γ and IL-10 (whole SCH – whole blood assay IFN-γ: t =

5.43, p < .001; IL-10: t = -4.93, p < .001; age: t = 2.02, p = .05; whole CON – IFN-γ: t = 10.39, p < .001; IL-10: t = -9.16, p < .001; IL-4: t = 3.02, p = .004). Whole blood assay data suggested that the major resources of Th1/Th2 imbalance in schizophrenia were rather decreased, but not increased IFN-γ, IL-4, and IL-10 impacts. Additionally, other factors could have been involved; they include TNF-α, IL-4, IL-6, and age. TNF-α made a markedly negative contribution to whole blood assay IFN-γ/IL-4 variance in normal controls, while IL-6 made a noticeably positive one to IFN-γ/IL-4 in schizophrenics. IL-4 and age also markedly contributed to IFN-γ/IL-10 variance in healthy controls and schizophrenics, respectively. In addition, the explainable portions of whole blood assay IFN-γ/IL-4 and IFN-γ/IL-10 variance through the complete model were 45% and 73% for schizophrenics as well as 71% and 71% for controls.

<mark>Female schizophrenics vs. control females</mark>

Although the <u>complete model</u> was able to predict 76% variance of <u>whole blood assay IFN-γ/IL-4</u> ratio in female controls, it failed to make a reliable prediction of whole blood assay IFN-γ/IL-4 in female schizophrenics (see **Table 6-5-2(2)** – ♀CON: F = 9.64, p < .001; ♀SCH: F = 6.26, p = .15). For healthy female subjects, IFN-γ and IL-4 played the major roles in the balance between IFN-γ and IL-4 (whole blood assay IFN-γ: t = 5.20, p < .001; IL-4: t = -3.06, p = .006). Besides, TNF-α might play a crucial role and IL-10 seemed to be effectively involved in the balancing process between IFN-γ and IL-4 (♀CON – whole blood assay TNF-α: t = -2.46, p = .02; IL-10: t = 1.76, p = .09).

<u>New model</u> keeping all parameters but SHBG was able to efficiently predict <u>whole blood assay IFN-γ/IL-4</u> ratio in both schizophrenic and control females (♀SCH: R² = 88, F = 10.09, p = .04; ♀CON: R² = 76, F = 10.58, p < .001). Compared to the complete model, no obvious change was observed in control females regarding the importance magnitudes of various predictors (whole blood assay IFN-γ: t = 5.16, p < .001; IL-4: t = -2.98, p = .007; TNF-α: t = -2.56, p = .02). However, the alterations in female schizophrenics were manifold regarding the forecasting powers of various predictors. Almost every predictor significantly contributed to the imbalance between IFN-γ and IL-4 except SHBG (age: t = 5.45, p = .01; prolactin: t = -4.93, p = .02; IL-4: t = -4.52, p = .02; testosterone: t = 4.01, p = .03; IL-6: t = -3.23, p =.05; IFN-γ: t = 3.04, p = .06; estradiol: t = -2.81, p = .07; cortisol: t = 2.58, p = .08; IL-10: t = 2.39, p = .10; TNF-α: t = 2.36, p = .10). Interestingly, the first two most important contributors of

IFN-γ/IL-4 variance were neither IFN-γ nor IL-4, but age and prolactin. IFN-γ was on the sixth and IL-4 on the third place.

Table 6-5-2(2): The individual contributions of predictors in envisaging whole blood assay IFN-γ/IL-4 and IFN-γ/IL-10 in female schizophrenics and controls (results of multiple regression: <u>underlined</u> = complete model; shaded = new model).

	The individual contributions of predictors in forecasting whole blood assay Th1/Th2 ratios (♀SCH vs. ♀CON)							
Criteria	**Whole blood IFN-γ/IL-4**				**Whole blood IFN-γ/IL-10**			
	SCH (N = 15)		**CON (N = 34)**		**SCH (N = 15)**		**CON (N = 34)**	
Predictor	T	p	T	p	T	p	T	p
IFN-γ	2.49	.13	5.20	.000*****	3.57	.07	10.18	.000*****
	3.04	.06	5.16	.000*****	4.31	.02*	10.11	.000*****
IL-2	-.61	.61	-.06	.95	1.25	.34	2.07	.05*
	-.89	.44	.02	.98	1.78	.17	2.17	.04*
TNF-α	1.95	.19	-2.46	.02*	2.63	.12	-.78	.45
	2.36	.10	-2.56	.02*	3.26	.05*	-.86	.40
IL-4	-3.68	.07	-3.06	.006**	-1.08	.39	1.24	.23
	-4.52	.02*	-2.98	.007**	-1.39	.26	1.40	.18
IL-10	1.82	.21	1.76	.09	-4.30	.05*	-10.82	.000*****
	2.39	.10	1.66	.11	-5.73	.01**	-11.07	.000*****
IL-6	-2.63	.12	-.79	.44	-3.79	.06	1.74	.10
	-3.23	.05*	-.59	.56	-4.75	.02*	2.08	.05*
Cortisol	1.49	.28	.24	.81	.35	.76	.28	.78
	2.58	.08	-.07	.95	1.00	.39	-.10	.92
Prolactin	-2.80	.11	-.04	.97	-1.58	.26	.82	.42
	-4.93	.02*	-.04	.97	-3.21	.05*	.82	.42
Estradiol	-2.31	.15	.39	.70	-4.37	.05*	-.44	.67
	-2.81	.07	.40	.70	-5.35	.01**	-.43	.67
Testosterone	2.58	.12	-.54	.60	.74	.54	-.17	.87
	4.01	.03*	-.29	.77	1.45	.24	.17	.87
SHBG	-.17	.88	-.86	.40	.23	.84	-1.05	.31
Age	3.98	.06	-.68	.51	2.22	.16	2.39	.03*
	5.45	.01*	-.49	.63	3.25	.05*	2.73	.01**
Adjusted	R² = ~~.82~~ (.88)		R² = .76 (.76)		R² = ~~.88~~ (.92)		R² = .89 (.89)	
Signif.test	~~F = 6.26, p = .15~~		F = 9.64, p < .001		~~F = 9.83, p = .10~~		F = 23.72, p < .0001	
	F = 10.09, p = .04		F = 10.58, p < .001		F = 15.65, p = .02		F = 25.65, p < .0001	

The complete model also failed to reliably envisage <u>whole blood assay IFN-γ/IL-10</u> in schizophrenic females (F = 9.83, p = .10). A <u>new model</u> dropping SHBG improved the collective envisaging power of all predictors in envisaging IFN-γ/IL-10 in female patients (R² = .92, F = 15.65, p = .02). The exclusion of SHBG led to solely a minor alteration in IL-6 in female controls; IL-10 and IFN-γ kept their dominant roles as usual, IL-2, IL-6, and age still

played crucial roles in the IFN-γ/IL-10 balancing process (♀CON – whole blood assay IL-10: t = -11.07, p < .001; IFN-γ: t = 10.11, p < .001; age: t = 2.73, p = .01; IL-2: t = 2.17, p = .04; IL-6: t = 2.08, p = .05).

Table 6-5-2(2-1): A summary of the inter-correlations among the predictors which were included in the model to envisage whole blood assay IFN-γ/IL-4 and IFN-γ in female schizophrenic patients and normal women.

	Age	SHBG	E2	Prl	IL-6	IL-4	Cort	IL-2	Testo	IFN-γ	IL-10	TNF
Whole blood assay data: female schizophrenics versus female controls (inter-correlations among the predictors of whole blood assay IFN-γ/IL-4 and IFN-γ/IL-10).												
Age		.24	.25	-.13	.46	-.18	.34	.18	.27	-.18	-.16	-.14
SHBG	-.50		.00	.00	.26	.14	-.36	.10	.31	-.12	-.15	-.08
E2	-.46	.12		-.32	.28	-.18	.57	-.02	.17	.19	-.24	-.22
Prl	-.73	.75	.46		-.29	.27	-.53	.07	.01	-.06	.36	.34
IL-6	-.61	.22	.67	.47		-.11	.33	.23	.29	-.39	-.06	-.32
IL-4	-.58	.20	.63	.46	.77		-.28	.07	.09	.35	-.26	-.27
Cort	.56	-.77	-.15	-.70	-.38	-.50		.14	.10	.06	-.25	-.26
IL-2	.05	-.40	.12	-.11	.09	.39	.13		.16	.08	-.34	-.65
Testo	.81	-.66	-.50	-.89	-.43	-.44	.60	.19		.04	-.16	-.22
IFN-γ	-.06	.04	-.27	-.28	.02	-.12	.01	-.60	.14		-.59	-.34
IL-10	.48	-.47	.00	-.40	-.46	-.51	.57	.23	.42	-.47		.29
TNF	.57	-.16	-.77	-.41	-.78	-.91	.38	-.38	.40	.14	.24	
Note	The left bottom corner = schizophrenic females (N = 15) ; the right above corner = control females (N = 34). E2 = estradiol; Cort = cortisol; Testo = testosterone; Prl = prolactin; TNF = TNF-α; SHBG = sex hormone-binding globulin											

The new model could predict as much variance of whole blood assay IFN-γ/IL-10 as the complete one in control females (R^2 = .89, F = 25.65, p < .001). In contrast, distinct changes occurred in female schizophrenics after excluding SHBG regarding the predictive power of individual predictor. The new model significantly predicted 92% variance of IFN-γ/IL-10 in female patients (F = 15.65, p = .02), in contrast to 89% in control females. A variety of additional parameters made significant contributions in envisaging whole blood assay IFN-γ/IL-10; estradiol, IL-6, TNF-α, age and prolactin seemed to be effectively involved in the balancing process between IFN-γ and IL-10 (♀SCH – whole blood assay IFN-γ: t = 4.31, p = .02; IL-10: -5.73, p = .01; estradiol: t = -5.35, p = .01; IL-6: t = -4.75, p = .02; TNF-α: t = 3.26, p = .05; age: t = 3.25, p = .05; prolactin: t = -3.21, p = .05). IL-10 played the most important role in the balancing process, but not IFN-γ; instead, estradiol took over the role of IFN-γ. The predictive powers of IFN-γ and IL-10 were also much weakened in schizophrenic females if

compared with those in control women. Furthermore, the results from whole blood assay data likewise demonstrated that instead of a few dominant contributors, there were various primary influencing factors being able to predict whole blood assay Th1/Th2 ratios in female schizophrenics. Those essential influencing factors correlated closely with one another in schizophrenic women, whereas those in normal females did not (see **Table 6-5-2(2-1)**).

Male schizophrenics vs. male controls (whole blood)

The complete model enabled the predictor assembly to make a significant prediction of both whole blood assay Th1/Th2 ratios in control men, however, only IFN-γ/IL-10 in schizophrenic males (see **Table 6-5-2(3)** – ♂SCH – IFN-γ/IL-4: F = 2.07, p = .11; IFN-γ/IL-10: F = 9.00, p < .001; ♂CON – IFN-γ/IL-4: F = 6.77, p < .001; IFN-γ/IL-10: F = 9.28, p < .001).

Comparisons showed that schizophrenic males had tremendously decreased impacts rather from IFN-γ than from IL-10 on whole blood assay IFN-γ/IL-10 ratio. Healthy subjects had additional remarkable contributions from testosterone and SHBG, whereas schizophrenics had an extra marked input from IL-6, in addition to dominant influences from both IFN-γ and IL-10 (♂SCH – whole blood assay IFN-γ: t = 4.40, p = .001; IL-10: t = -4.47, p = .001; IL-6: t = 2.38, p = .04; ♂CON – IFN-γ: t = 7.26, p < .001; IL-10: t = -5.47, p < .001; testosterone: t = -3.03, p = .006; SHBG: t = 2.30, p = .02). Moreover, IL-4 and estradiol also tended to exert evident effects on whole blood assay IFN-γ/IL-10 ratio in male controls (IL-4: T = 1.77, p = .09; estradiol: F = 1.85, p = .08). The explainable portions of IFN-γ/IL-10 ratios through the complete model were relatively comparable in both groups (♂SCH: adjusted R² = .80, F = 9.00, p < .001; ♂CON: adjusted R² = .73, F = 9.28, p < .001).

The explicable part of whole blood assay IFN-γ/IL-4 variance through the alternative model excluding age and prolactin was similar to that via the complete model in controls. Nevertheless, in contrast to the complete model, the new one was able to predict the variance of IFN-γ/IL-4 sufficiently in the schizophrenic group (♂CON: R² = .65, F = 7.91, p < .001; ♂SCH: R² = .52, F = 4.69, p = .004). The new model exposed a significant shortage in whole blood assay IFN-γ production in male schizophrenics, compared to healthy men (♂SCH: t = 1.42, p = .18; ♂CON: t = 6.02, p < .001). However, the predictive powers of whole blood assay IL-4 in envisaging IFN-γ/IL-4 variances were relatively comparable in both male groups (♂SCH: t = -2.61, p = .02; ♂CON: t = -2.69, p = .01). IL-6 also showed a tendency to

predict whole blood assay IFN-γ/IL-4 significantly in male patients, but not in male controls (♂SCH: t = 2.10, p = .06; ♂CON: t = -.57, p = .57). Besides, only 42% and 65% of the IFN-γ/IL-4 variance could be clarified by the model in schizophrenic and control males, correspondingly.

Table 6-5-2(3): The individual contributions of predictors in envisaging whole blood assay IFN-γ/IL-4 and IFN-γ/IL-10 in male schizophrenics and controls (results of multiple regression: underlined = complete model; shaded = new model).

Criteria	Whole blood IFN-γ/IL-4				Whole blood IFN-γ/IL-10			
	SCH (N = 25)		CON (N = 38)		SCH (N = 25)		CON (N = 38)	
Predictor	T	p	T	p	T	p	T	p
IFN-γ	1.20	.25	6.06	.000*****	4.40	.001****	7.26	.000*****
	1.42	.18	6.02	.000*****				
IL-2	-.67	.52	-.96	.35	-1.39	.19	-.28	.78
	-1.18	.26	-.59	.56				
TNF-α	-.194	.85	-.08	.94	.61	.55	-.27	.79
	-.01	.99	-.15	.89				
IL-4	-2.35	.04*	-2.60	.02*	-2.00	.07	1.77	.09
	-2.61	.02*	-2.69	.01**				
IL-10	-.77	.46	-.42	.68	-4.47	.001****	-5.47	.000*****
	-.75	.46	-.30	.77				
IL-6	1.93	.08	-.67	.51	2.38	.04*	-1.43	.16
	2.10	.06	-.57	.57				
Cortisol	.70	.50	-.72	.48	1.01	.33	.14	.89
	.83	.42	-.77	.45				
Prolactin	-.70	.50	-1.14	.27	.47	.65	.14	.89
Estradiol	.03	.98	1.23	.23	.06	.95	1.85	.08
	.24	.81	.88	.39				
Testosterone	-.36	.73	-1.04	.31	.18	.86	-3.03	.006*
	-.40	.69	-.73	.47				
SHBG	.71	.49	-.09	.93	.21	.84	2.30	.03*
	.69	.51	.19	.85				
Age	-.26	.80	.87	.39	1.22	.25	-1.30	.21
Adjusted	R² = .35 (.42)		R² = .65 (.65)		R² = .80		R² = .73	
Signif.test	F = 2.07, p = .11		F = 6.77, p < .001		F = 9.00, p < .001		F = 9.28, p < .001	
	F = 2.72, p = .04		F = 7.91, p < .0001					

6.5.3 Lymphocyte data

Whole schizophrenic group vs. whole control group

The complete model enabled reliable predictions of lymphocyte IFN-γ/IL-4, IFN-γ/IL-10 as well as IFN-γ/IL-13 ratio in both controls and schizophrenics (whole CON – IFN-γ/IL-4: F =

55.30, p < .001; IFN-γ/IL-10: F = 4.50, p < .001; IFN-γ/IL-13: F = 4.10, p < .001; whole SCH – IFN-γ/IL-4: F = 4.26, p = .001; IFN-γ/IL-10: F = 8.04, p < .001; IFN-γ/IL-13: F = 4.21, p = .01). It was able to explicate 89%, 35%, and 32% variance of lymphocyte IFN-γ/IL-4, IFN-γ/IL-10, and IFN-γ/IL-13 in controls, respectively. On the contrary, it elucidated 48%, 67%, and 48% Th1/Th2 variance in schizophrenics in the same order as stated in controls (see **Table 6-5-3(1)**).

Apart from the predominant factors IFN-γ and IL-4, other parameters such as testosterone, SHBG, and age also played crucial roles in envisaging <u>lymphocyte IFN-γ/IL-4</u> in controls. Instead, IL-13 and estradiol additionally exerted noticeable powers in forecasting IFN-γ/IL-4 ratio in schizophrenics (whole CON – lymphocyte IFN-γ: t = 15.36, p < .0001; IL-4: t = -9.58, p < .001; age: t = 3.11, p = .003; SHBG: t = -2.88, p = .006; testosterone: t = -2.58, p = .01; whole SCH – IL-13: t = 2.20, p = .04; estradiol: t = -1.85, p = .08).

Table 6-5-3(1): A summary of the individual contributions of predictors in lymphocyte IFN-γ/IL-4, IFN-γ/IL-10, and IFN-γ/IL-13 variance in the whole schizophrenic and control group (results from multiple regression).

	The individual contributions of predictors in forecasting lymphocyte Th1/Th2 ratio (whole SCH vs. whole CON)											
Criteria	**Lymphocyte IFN-γ/IL-4**				**Lymphocyte IFN-γ/IL-10**				**Lymphocyte IFN-γ/IL-13**			
	SCH (N=40)		**CON (N=72)**		**SCH (N=40)**		**CON (N=72)**		**SCH (N=40)**		**CON (N=72)**	
Predictor	T	p	T	p	T	p	T	p	T	p	T	p
IFN-γ	4.46	.000*****	15.36	.000******	.41	.68	3.42	.001****	3.39	.002***	4.01	.000*****
IL-12	-.29	.78	-1.22	.23	3.58	.001****	-1.23	.22	-.41	.68	-.30	.76
IL-4	-5.55	.000*****	-9.58	.000******	-1.11	.28	1.74	.09	1.40	.17	-2.07	.04*
IL-13	2.20	.04*	-1.59	.12	.09	.93	-.83	.41	-5.15	.000*****	-3.33	.001****
IL-10	.10	.92	-.29	.77	-3.42	.002***	-4.41	.000*****	-.40	.69	-1.30	.20
Cortisol	-1.66	.11	-.07	.94	1.02	.32	-.76	.45	-.08	.94	-.30	.76
Prolactin	.73	.47	-.35	.73	3.73	.001****	.86	.39	.23	.82	-.39	.70
Estradiol	-1.85	.08	-.92	.36	.61	.55	-1.44	.16	.23	.82	-.88	.38
Testosterone	.23	.82	-2.58	.01**	-.65	.52	-1.34	.19	.52	.61	-1.71	.09
SHBG	.76	.45	-2.88	.006**	-.43	.67	.27	.79	.003	1.00	-1.35	.18
Age	-.12	.91	3.11	.003***	3.04	.005***	.38	.71	.13	.90	.36	.72
Adjusted	R² = .48		R² = .89		R² = .67		R² = .35		R² = .48		R² = .32	
Signif.test	F = 4.26, p = .001		F = 55.30, p < .0001		F = 8.04, p < .001		F = 4.50, p < .001		F = 4.21, p = .001		F = 4.10, p < .001	

However, prolactin, IL-12, and age were further essential predictors for lymphocyte IFN-γ/IL-10 ratio in schizophrenic patients, in addition to IL-10 (whole SCH – prolactin: t = 3.73, p = .001; IL-12: t = 3.58, p = .001; IL-10: t = -3.42, p = .002; age: t = 3.04, p = .005). Interestingly, lymphocyte IFN-γ production was unable to significantly predict IFN-γ/IL-10 ratio in schizophrenia (t = .41, p = .68). IL-10 was not even on the first or the second important place, either. Instead, prolactin and IL-12 took over the first two most essential roles. However, in controls, IL-10 and IFN-γ took the dominant roles in predicting IFN-γ/IL-10 ratio; besides, IL-4 also tended to make a marked contribution (whole CON: lymphocyte IL-10: t = -4.41, p < .001; IFN-γ: t = 3.42, p = .001; IL-4: t = 1.74, p = .09).

For lymphocyte IFN-γ/IL-13 ratio, the diversity between both groups was not clear. The complete model could only enlighten 32% of IFN-γ/IL-13 variance in controls, however, 48% in schizophrenics (whole CON: F = 4.10, p < .001; whole SCH: F = 4.21, p = .001). In both controls and schizophrenics, IFN-γ and IL-13 took over the first and the second central role in envisaging IFN-γ/IL-13 (whole CON – lymphocyte IFN-γ: t = 4.01, p < .001; IL-13: t = -3.33, p = .001; whole SCH – IFN-γ: t = 3.39, p = .002; IL-13: t = -5.15, p < .001). The predicting power of IFN-γ was somewhat reduced and that of IL-13 slightly increased in schizophrenics, compared to those in normal controls. In addition, IL-4 could significantly and testosterone showed a tendency to predict IFN-γ/IL-13 ratio in control subjects, but not in schizophrenic patients (whole CON – IL-4: t = -2.07, p = .04; testosterone: t = -1.71, p = .09).

Female schizophrenic patients vs. control females

The complete model could reliably predict lymphocyte IFN-γ/IL-4, IFN-γ/IL-13, and IFN-γ/IL-10 in female controls, but not those in schizophrenic women (see **Table 6-5-3(2)**: ♀SCH – lymphocyte IFN-γ/IL-4: F = 2.45, p = .25; IFN-γ/IL-13: F = 4.57, p = .12; IFN-γ/IL-10: F = 1.98, p = .31; ♀CON – IFN-γ/IL-4: F = 28.16, p < .001; IFN-γ/IL-13: F = 2.29, p = .05; IFN-γ/IL-10: F = 2.42, p = .04).

An alternative model dropping IL-10 and prolactin led to amendment of the collective forecasting power of the remaining predictors in schizophrenic females. But it did not cause any remarkable alteration in control females (♀SCH: R² = .72, F = 4.94, p = .05; ♀CON: R² = .90, F = 32.69, p < .001). Comparing the individual predictive power of each predictor in both groups showed that in control women, IFN-γ and IL-4 took over the most dominant two

places in envisaging lymphocyte IFN-γ/IL-4 ratio. Additionally, age, IL-12, and testosterone also made remarkable contributions in this regard (♀CON – lymphocyte IFN-γ: t =10.43, p < .001; IL-4: t = -5.07, p < .001; age: t = 2.53, p = .02; IL-12: t = -2.27, p = .03; testosterone: t = -2.06, p = .05; IL-13: t = -1.45, p = .16; cortisol: t = -1.20, p = .24; estradiol: t = -1.15, p = .26; SHBG: t = -1.51, p = .15). Nonetheless, it was not that case in female schizophrenics; testosterone was the most crucial one within the predictors included, then age and IFN-γ (♀SCH – testosterone: t = 3.50, p = .02; age: t = 3.01, p = .03; cortisol: t = -3.00, p = .06; SHBG: t = 2.39, p = .06; estradiol: t = -1.80, p = .13; IFN-γ: t = 2.97, p = .03; IL-13: F = -2.49, p = .06; IL-4: t = .10, p = .92; IL-12: t = -1.76, p = .14). In addition, SHBG, cortisol, and IL-13 tended to have significant predicting powers to envisage IFN-γ/IL-4 in schizophrenics. Surprisingly, IL-4 did not even play a minor role in the balance of lymphocyte IFN-γ/IL-4 in female patients.

Table 6-5-3(2): The individual contributions of predictors in envisaging lymphocyte IFN-γ/IL-4, IFN-γ/IL-10, and IFN-γ/IL-13 in female schizophrenics and controls (results of multiple regression: underlined = complete model; shaded = new model)

	The individual contributions of predictors in forecasting lymphocyte Th1/Th2 ratios (♀SCH vs. ♀CON)											
Criteria	Lymphocyte IFN-γ/IL-4				Lymphocyte IFN-γ/IL-10				Lymphocyte IFN-γ/IL-13			
	SCH (N=15)		CON (N=34)		SCH (N=15)		CON (N=34)		SCH (N=15)		CON (N=34)	
Predictor	T	p	T	p	T	p	T	p	T	p	T	p
IFN-γ	2.14	.12	10.28	.000*****	-.89	.44	1.38	.18	1.52	.23	2.87	.009**
	2.97	.03*	10.34	.000*****	-1.02	.37	1.28	.22	2.18	.07	2.76	.01**
IL-12	-.65	.56	-1.04	.31	2.40	.10	-1.63	.12	-.63	.58	-.10	.92
	-1.76	.14	-2.27	.03*	2.84	.05*	-1.80	.07	-.34	.75	-.94	.36
IL-4	.14	.90	-4.81	.000*****	.45	.68	1.80	.09	1.30	.29	-.88	.39
	.10	.92	-5.07	.000*****	.92	.41	1.77	.09	2.56	.04*	-1.04	.31
IL-13	-1.63	.20	-2.02	.06	-.12	.91	-.61	.55	-2.64	.08	-2.25	.04*
	-2.49	.06	-1.45	.16					-3.83	.009**	-2.35	.03*
IL-10	-.12	.92	-1.71	.10	-.74	.51	-2.14	.04*	-.39	.72	-1.42	.17
					-.87	.43	-2.16	.04*				
Cortisol	-1.81	.17	-.96	.35	1.21	.31	-.21	.84	-.94	.42	-.37	.72
	-3.00	.06	-1.20	.24	1.40	.23	-.14	.89	-1.29	.25	-.86	.40
Prolactin	-.08	.94	-.24	.82	-.89	.44	-1.53	.14	-.32	.77	-1.03	.32
					-1.16	.31	-1.56	.13	-1.40	.21	-1.10	.28
Estradiol	-1.14	.34	-1.26	.22	.68	.55	-1.00	.33	-.16	.88	-.66	.52
	-1.80	.13	-1.15	.26	.77	.48	-1.09	.29				
Testosterone	1.60	.21	-2.48	.02*	.93	.42	-1.04	.31	1.56	.22	-1.38	.18
	3.50	.02*	-2.06	.05*	1.38	.24	-1.03	.31	2.58	.04*	-.89	.38
SHBG	1.37	.27	-1.64	.12	-1.30	.29	-.39	.70	.72	.52	-.75	.46
	2.39	.06	-1.51	.15	-.67	.53	-.60	.55				
Age	1.65	.20	2.87	.009**	1.50	.23	.28	.78	1.52	.23	-.23	.82
	3.01	.03*	2.53	.02*	-1.50	.21	.24	.82	3.23	.02*	-.49	.63
Adjusted	R² = .53 (.72)		R² = .90 (.90)		R² = .74 (.80)		R² = .32 (.34)		R² = .44 (.66)		R² = .30 (.31)	
Signif.test	F = 2.45, p = .25		F = 28.16, p < .0001		F = 4.57, p = .12		F = 2.42, p = .04		F = 1.98, p = .31		F = 2.29, p = .05	
	F= 4.94, p = .05		F = 32.69, p < .0001		F = 6.66, p = .04		F =3.13, p = .01		F = 4.47, p = .04		F = 2.82, p = .02	

According to the underlined complete model, IFN-γ and IL-13 had made the most crucial contributions to lymphocyte IFN-γ/IL-13 in healthy women, however, not in schizophrenic females (♀CON – lymphocyte IFN-γ: t = 2.87, p = .009; IL-13: t = -2.25, p = .04; ♀SCH – lymphocyte IFN-γ: t = 1.52, p = .23; IL-13: t = -2.64, p = .08). An alternative model dropping IL-10, estradiol, and SHBG enabled the remaining predictors to reliably forecast lymphocyte IFN-γ/IL-13 in both schizophrenic and control females (♀SCH: adjusted R² = .66, F = 4.47, p = .04; ♀CON: adjusted R² = .31, F = 2.82, p = .02). The important "donors" of IFN-γ/IL-13 balance in female schizophrenics were IL-13, age, testosterone, and IL-4 (♀SCH – lymphocyte IL-13: t = -3.83, p = .009; age: t = 3.23, p = .02; testosterone: t = 2.58, p = .04; IL-4: t = 2.56, p = .04; IFN-γ: t = 2.18, p = .07). IFN-γ only showed a tendency to be able to predict IFN-γ/IL-13 variance. The power of IFN-γ in envisaging IFN-γ/IL-13 in schizophrenic females was also weaker compared to that in healthy women. Nevertheless, the predicting power of IL-13 was stronger than that in female controls. Furthermore, the alternative model explicated 31% of IFN-γ/IL-13 variance in female controls, however, 66% in schizophrenic women (♀CON: F = 3.70, p = .007; ♀SCH: F = 4.53, p = .03).

A alternative model dropping IL-13 could predict lymphocyte IFN-γ/IL-10 significantly and explain about 80% of its variance in schizophrenic women (F = 6.66, p = .04), in contrast to 34% in female controls (F = 3.13, p = .01). However, no single predictor except IL-12 showing an obvious contribution to the variance of IFN-γ/IL-10 in female schizophrenics (♀SCH – lymphocyte IFN-γ: t = -1.02, p = .37; IL-12: t = 2.84, p = .05; IL-4: t = .92, p = .41; IL-10: t = -.87, p = .43; prolactin: t = -1.16, p = .31; estradiol: t = .77, p = .48; testosterone: t = 1.38, p = .24; age: t = -1.50, p = .21; SHBG: t = -.67, p = .53). Similar findings were found in normal female subjects; no other parameter except IL-10 significantly predicted lymphocyte IFN-γ/IL-10 variance (♀CON – lymphocyte IL-10: t = -2.09, p = .05; IL-4: t = 1.77, p = .09; IL-12: t = -1.80, p = .08; IFN-γ: t = 1.32, p = .20; age: t = .24, p = .82; cortisol: t = -.14, p = .89; prolactin: t = -1.56, p = .13; estradiol: t = -1.09, p = .29; testosterone: t = -1.03, p = .31; SHBG: t = -.60, p = .55). In addition, IL-4 and IL-12 tended to have marked impacts on lymphocyte IFN-γ/IL-10 balance in control women.

Male schizophrenics vs. male controls

The complete model could reliably predict all 3 lymphocyte Th1/Th2 ratios in control males, although it failed to predict any lymphocyte Th1/Th2 ratio significantly except IFN-γ/IL-13 in male schizophrenics (see **Table 6-5-3(3)** – ♂CON – lymphocyte IFN-γ/IL-4: R² = .95, F =

71.10, p < .001; IFN-γ/IL-10: R² = .62, F = 6.53, p < .001; IFN-γ/IL-13: R² = .81, F = 15.02, p < .001; ♂SCH – IFN-γ/IL-4: F = 2.55, p = .06; IFN-γ/IL-10: F = 1.85, p = .15; IFN-γ/IL-13: R² = .70, F = 2.78, p = .04).

Multiple regression analysis showed that the predictive power of lymphocyte IFN-γ to envisage IFN-γ/IL-13 in schizophrenic males was much weaker than that in control males, while those of IL-13 were relatively comparable between both male groups (♂SCH – lymphocyte IFN-γ: t = 1.80, p = .10; IL-13: t = -4.16, p = .001; ♂CON – IFN-γ: t =4.34, p < .001; IL-13: t = -5.13, p < .001). Besides, SHBG seemed to play a role in this regard in healthy males (F = 1.83, p = .08).

Table 6-5-3(3): The importance indexes of predictors for lymphocyte IFN-γ/IL-4, IFN-γ/IL-10, and IFN-γ/IL-13 variance in schizophrenic and control males (results from multiple regression: underlined = complete model; shaded = new model)

The individual contributions of predictors in forecasting lymphocyte Th1/Th2 ratios (♂SCH vs. ♂CON)												
Criteria	Lymphocyte IFN-γ/IL-4				Lymphocyte IFN-γ/IL-10				Lymphocyte IFN-γ/IL-13			
	SCH (N=25)		CON (N=38)		SCH (N=25)		CON (N=38)		SCH (N=25)		CON (N=38)	
Predictor	T	p	T	p	T	p	T	p	T	p	T	p
IFN-γ	2.88 / 2.99	.01** / .01**	13.61 / 13.76	.000***** / .000*****	1.83 / 1.99	.09 / .07	3.34 / 3.47	.003*** / .002***	1.80	.10	4.34	.000*****
IL-12	.13 / .14	.90 / .89	-.81 / -.72	.43 / .48	1.02 / 1.15	.33 / .27	-.04 / .19	.97 / .86	.44	.67	.06	.96
IL-4	-3.45 / -3.70	.004*** / .002***	-9.78 / -9.94	.000***** / .000*****	-.93 / -1.06	.37 / .30	1.08 / 1.18	.29 / .25	.89	.39	-1.13	.27
IL-13	2.13 / 2.23	.05* / .04*	-.51 / -.57	.62 / .58	-1.02 / -1.21	.33 / .24	.32 / .02	.75 / .99	-4.16	.001****	-5.13	.000*****
IL-10	-.002 / -.02	1.00 / .99	1.45 / 1.41	.16 / .17	-2.84 / -3.15	.01** / .007**	-4.80 / -4.92	.000***** / .000*****	-.42	.68	1.22	.23
Cortisol	-.95 / -1.05	.36 / .31	1.05 / 1.03	.30 / .31	-.22	.83	-.65	.52	.71	.49	-.31	.76
Prolactin	-.39 / -.42	.70 / .68	-1.56 / -1.54	.13 / .14	-.45 / -.49	.66 / .63	2.70 / 2.71	.01** / .01**	.64	.53	-.30	.77
Estradiol	-.95 / -.97	.36 / .35	1.73 / 1.71	.10 / .10	.13 / .19	.90 / .85	.44 / .06	.67 / .95	1.07	.30	-.86	.40
Testosterone	.61 / .63	.55 / .54	-1.45 / -1.33	.16 / .19	-.67 / -1.02	.51 / .33	-1.23 / -.48	.23 / .64	-.60	.56	.13	.90
SHBG	-.13	.90	.61	.55	-.20	.85	1.42	.17	-.05	.96	1.83	.08
Age	.42 / .46	.68 / .65	.94 / 1.16	.35 / .26	1.86 / 2.04	.09 / .06	-.86 / -.57	.40 / .57	-.58	.57	.03	.98
Adjusted	R² = .42 (.46)		R² = .95 (.96)		R² = .28 (.37)		R² = .62 (.61)		R² = .45		R² = .81	
Signif.test	F = 2.55, p = .06 / F= 3.02, p= .03		F = 71.10, p < .0001 / F = 80.05, p < .0001		F = 1.85, p = .15 / F = 2.59, p = .05		F = 6.53, p < .001 / F = 7.55, p < .001		F = 2.78, p = .04		F = 15.02, p < .0001	

A new model dropping SHBG enhanced the cumulative envisaging power of the included predictors to forecast the variance of IFN-γ/IL-4 in schizophrenics (R² = .46, F = 3.02, p = .03). Comparisons showed that the predictive powers of both IFN-γ and IL-4 were much

weaker in schizophrenic males than control men ($\male$CON – lymphocyte IFN-γ: t = 13.76, p < .001; IL-4: t = -9.94, p < .001; $\male$SCH – IFN-γ: t = 2.99, p = .01; IL-4: t = -3.70, p = .002). Moreover, IL-13 also played an essential role in predicting lymphocyte IFN-γ/IL-4 in male schizophrenics (t = 2.23, p = .04). This model enlightened 96% IFN-γ/IL-4 variance in healthy males, in contrast to 46% in schizophrenic men.

A model excluding cortisol and SHBG improved the predictive power of the model to forecast <u>IFN-γ/IL-10</u> in schizophrenic males, nevertheless, almost did not change that in control men ($\male$SCH: from R^2 = .28, F = 1.85, p = .15 to R^2 = .37, F = 2.59, p = .05; $\male$CON: from R^2 = .62, F = 7.71, p < .001 to R^2 = .61, F = 7.55, p < .001). In male subject with schizophrenia, both IFN-γ and IL-10, particularly IFN-γ, had markedly weakened predictive powers to forecast IFN-γ/IL-10 if compared to those in control males ($\male$CON – lymphocyte IFN-γ: t = 3.47, p = .002; IL-10: t = -4.92, p < .001; $\male$SCH – IFN-γ: t = 1.99, p = .07; IL-10: t = -3.15, p = .007). Additionally, prolactin appeared to play an essential role in healthy males, while age seemed to have a marked impact on IFN-γ/IL-10 in schizophrenics (prolactin: t = 2.71, p = .01; age: t = 2.04, p = .06). Moreover, this reduced model explicated exclusively 37% IFN-γ/IL-10 variance in schizophrenic men, in contrast to 61% in male controls.

6.6   Influencing factors of Th1/Th2 cytokines in schizophrenia

In the subsequent sections, the inter-correlations among various cytokines, hormones, and SHBG were examined in order to discover possible sources of Th1/Th2 cytokines deficits in schizophrenia from those parameters measured in this study. Such comparisons might offer an overview for the possible consequences of changing a certain cytokine in order to restore Th1/Th2 balance, if re-balance of Th1/Th2 regarded as a co-target of treatment in schizophrenics. The outcomes from the complete model are summarized in **Appendix 6-6(1)** to **6-6(8)**.

6.6.1   IFN-γ: cortisol, prolactin (serum), IL-10, IL-6, IL-4 (whole blood)

Serum data

The complete model, including serum IL-2, TNF-α, IL-4, IL-10, IL-6, hormones, SHBG, and age as predictors, was able to predict 100% variance of serum IFN-γ level in the whole control group and male controls (whole CON: F = 18575.53, p < .001; male CON: F =

13914.45, p < .001). However, it could not sufficiently predict the variance of serum IFN-γ in the whole schizophrenic group, males with schizophrenia, female schizophrenics and control females (whole SCH: F = 1.62, p = .15; male SCH: F = 1.85, p = .15; female SCH: F = 2.01, p = .31; female CON: F = 1.45, p = .22).

Whole SCH vs. whole CON

Dropping testosterone, age, and SHBG, the predictive power of the remaining predictors was improved; it could explain 21% variance of serum IFN-γ in the whole schizophrenic group, while it only caused minor changes in controls (whole SCH: R^2 = .21, F = 2.28, p = .05; whole CON: R^2 = 1.00, F = 25558.02, p < .001). In schizophrenia, cortisol played a significant role and prolactin tended to make a marked contribution to the variance of serum IFN-γ (whole SCH – cortisol: t = -2.42, p = .02; prolactin: t = 1.99, p = .06). In contrast, both prolactin and cortisol played only secondary roles in envisaging serum IFN-γ level in controls; instead, serum IL-6 was the most important contributor. In addition, IL-10 also tended to make a remarkable contribution to IFN-γ variance (whole CON – IL-6: t = 3.15, p = .003; IL-10: t = 1.93, p = .06). The results revealed that barely 80% IFN-γ variance in schizophrenics were unexplainable through the parameters assessed in this study.

Male SCH vs. male CON

An alterative model dropping prolactin and SHBG out of the multiple regression analysis improved the forecasting power of the remaining predictors in the male schizophrenic group. It could explain 38% variance of serum IFN-γ (F = 2.60, p = .05). Nevertheless, none of the predictors alone was able to make a significant prediction for serum IFN-γ in schizophrenic men (IL-2: t = .82, p = .42; TNF-α: p = 1.07, p = .30; IL-4: t = 1.34, p = .20; IL-10: t = .80, p = .44; IL-6: t = -.06, p = .95; cortisol: t = -1.57, p = .14; estradiol: t = -1.07, p = .30; testosterone: t = -1.28, p = .22). The new model did not cause too much alteration and explained, as the complete model, 100% variance of serum IFN-γ in control males (F = 17771.93, p < .001). In contrast to male schizophrenics, serum IL-2 and IL-6 played essential roles in envisaging IFN-γ variance in control males (IL-2: t = 3.46, p = .002; IL-6: t = 2.28, p = .03).

Female SCH vs. Female CON

For both female groups, the only alternative model being able to make a sufficient prediction for serum IFN-γ was to keep IL-2, IL-10, prolactin, and testosterone. The new model explained 46% serum IFN-γ variance in female schizophrenics, while it explicated solely 18% variance of IFN-γ in female controls (♀SCH: F = 3.92, p = .04; ♀CON: F = 2.78, p = .05).

For schizophrenic women, prolactin seemed to play the most dominant role. Instead, both IL-2 and IL-10 made significant contributions to serum IFN-γ variance in healthy females (♀SCH – prolactin: t = 3.42, p = .007; ♀CON – IL-10: t = 2.25, p = .03; IL-2: t = 2.02, p = .05).

Summary : Serum data exhibited,

(1) IL-10, IL-6 were the primary predictors and IL-2 was the secondary contributor for serum IFN-γ in the whole control group, while cortisol was the dominant and prolactin was the secondary influencing factor for serum IFN-γ in the whole schizophrenic group.

(2) Healthy males had clear contributions from IL-2 and IL-6, while no single dominant factor was found in females.

(3) No single primary "donor" of serum IFN-γ variance was found among the parameters measured in this study in male schizophrenics, whereas prolactin played an essential role in predicting serum IFN-γ in female schizophrenics.

Whole blood assay data

Whole SCH vs. whole CON

Comparisons of whole blood assay data between the whole schizophrenic and control group showed that IL-10, IL-4, and IL-6 were the most important contributors of whole blood assay IFN-γ variance, although the magnitudes of importance of these three parameters were much weaker in schizophrenics than those in controls (whole SCH – whole blood assay IL-10: t = 5.70, p < .001; IL-4: t = -3.05, p = .003; IL-6: t = 3.09, p = .003; whole CON – IL-10: t = 2.90, p = .007; IL-4: t = -2.12, p = .04; IL-6: t = 2.02, p = .05). In addition, prolactin and testosterone also played essential roles in predicting whole blood IFN-γ in vitro production in schizophrenics and controls, respectively (prolactin: t = 2.07, p = .04; testosterone: t = 2.18, p = .04). Apparently, all the parameters together could envisage 61% and 63% variance of IFN-γ in the whole schizophrenic and control group, correspondingly (SCH: F = 10.98, p < .001; CON: F = 4.37, p = .001).

Male SCH vs. male CON

Although the complete model could predict 58% variance of whole blood IFN-γ in vitro production in control males, it failed to achieve that in schizophrenic men (♂CON: F = 5.65, p < .001; male SCH: F = 2.35, p = .07). A new model dropping cortisol enhanced the predicting power of the remaining parameters in envisaging whole blood assay IFN-γ in male

schizophrenics. It enabled the predictors included in the model to explicate 42% variance of IFN-γ (F = 2.76, p = .04) in male schizophrenic patients. Nonetheless, none of the predictors dominated over the others and made a noticeable contribution to IFN-γ variance. The only exception was IL-10; it tended to make a significant prediction for whole blood IFN-γ in vitro production in schizophrenic men (♂SCH – whole blood IL-10: t = 1.85, p = .09). The new model only raised minor alterations in control males (R² = .58, F = 6.27, p < .001). IL-10, prolactin, IL-6, and IL-2 all made significant contributions to whole blood assay IFN-γ variance in male controls (♂CON – whole blood assay IL-10: t = 3.47, p = .002; prolactin: t = 2.20, p = .04; IL-6: t = 2.20, p = .04; IL-2: t = 2.17, p = .04).

Female SCH vs. female CON

The complete model could significantly predict the variance of whole blood assay IFN-γ production in female healthy subjects and explain 62% of IFN-γ variance. Nevertheless, it did not succeed in making a reliable prediction for IFN-γ secretion in schizophrenic females (♀CON: F = 5.86, p < .001; ♀SCH: F = 2.99, p = .20). The complete model revealed that IL-10 played a dominant role in predicting whole blood IFN-γ in vitro production and IL-6 also tended to do so in control women (♀CON – IL-10: t = 3.45, p = .002; IL-6: t = 1.99, p = .06).

An alternative model dropping SHBG and cortisol led to improvement of the cumulative forecasting power of the remaining predictors in schizophrenic females. It explained 76% variance of whole blood IFN-γ in vitro production (F = 6.05, p = .03). Nevertheless, none of the parameters kept in the model alone was able to make a marked contribution to the variance of whole blood assay IFN-γ in schizophrenic females (IL-2: t = 1.92, p = .11; TNF-α: t = -.29, p = .78; IL-4: t = .25, p = .81; IL-10: t = 1.58, p = .18; IL-6: t = -.08, p = .94; prolactin: t = 1.16, p = .30; estradiol: t = .64, p = .55; testosterone: t = -.48, p = .65; age: t = .16, p = .88). The new model explicated 65% variance of whole blood assay IFN-γ in control women (F = 7.66, p < .001); IL-10, IL-6, and IL-4 were essential in envisaging IFN-γ. Besides, TNF-α tended to make a marked contribution as well (♀CON – IL-10: t = 4.19, p < .001; IL-6: t = 2.38, p = .03; IL-4: t = -2.07, p = .05; TNF-α: t = 1.93, p = .07).

Summary : Whole blood assay data showed:

(1) IL-10, IL-6, and IL-4 were the 3 most important predictors for whole blood assay IFN-γ in both schizophrenic and control group.

(2) Both IL-10 and IL-6 were crucial contributors for whole blood assay IFN-γ variance in normal males. Nevertheless, IL-10 was the only remarkable and IL-6 was rather a secondary influencing factor in healthy females.

(3) In addition, no single factor among those variables measured in this study was found to be able to predict whole blood assay IFN-γ significantly in both male and female schizophrenics.

Lymphocyte data

Despite that the complete model explained 44%, 61%, and 42% variance of lymphocyte IFN-γ release in the whole control group, male normal subjects, and control females, the model failed to make a reliable prediction for lymphocyte IFN-γ secretion in the whole schizophrenic group and both schizophrenic gender subgroups (whole CON: F = 6.49, p < .001; ♂CON: F = 6.71, p < .001; ♀CON: F = 3.39, p = .007; whole SCH: F = .79, p = .64; ♂SCH: F = 1.53, p = .23; ♀SCH: F = .60, p = .77). For the whole control group, IL-13, IL-10, and age dominated over the other parameters in envisaging lymphocyte IFN-γ production (whole CON – IL-13: t = 3.51, p = .001; IL-10: t = 3.14, p = .003; age: t = 2.07, p = .04). Similarly, IL-13 and IL-10 also made remarkable contributions to the variance of lymphocyte IFN-γ production in female control subjects (♀CON – IL-13: t = 3.34, p = .003; IL-10: t = 2.09, p = .05). For the male controls, prolactin, IL-10, and IL-12 played essential roles in predicting lymphocyte IFN-γ release (♂SCH – prolactin: t = 2.53, p = .02; IL-10: t = 2.55, p = .02; IL-12: t = 2.52, p = .02).

Attempts to find an alternative model within the parameters measured in this study for the whole schizophrenic group and female schizophrenics failed. However, a model dropping IL-13, testosterone, SHBG, and age improved the reliability of the remaining predictors in forecasting IFN-γ in schizophrenic men. It explicated 31% variance of lymphocyte IFN-γ (F = 2.76, p = .04). IL-4 took over the dominant role among those parameters assessed in contributing to lymphocyte IFN-γ variance in male patients (t = 2.54, p = .02). Nevertheless, the new model could clarify 64% of lymphocyte IFN-γ variance in control males (F = 11.77, p < .001). Among the predictors included, IL-12, estradiol, prolactin, and IL-10 made the substantial contributions to lymphocyte IFN-γ variance in male healthy controls (IL-12: t = 3.54, p = .001; estradiol: t = -2.87, p = .007; prolactin: t = 2.79, p = .009; IL-10: t = 2.68, p = .02).

Summary : Lymphocyte data demonstrated,

(1) IL-13, IL-10, and age were the crucial contributors for lymphocyte IFN-γ variance in the whole control group. Nevertheless, no single essential "donor" of lymphocyte IFN-γ variance was found in the whole schizophrenic group.

(2) Prolactin, IL-10, and IL-12 were very important in forecasting lymphocyte IFN-γ in control males, while IL-4 was the only crucial factor in this regard in schizophrenic men.

(3) IL-13 and IL-10 were essential for lymphocyte IFN-γ production in healthy females, whereas none of the parameters measured made a significant contribution to lymphocyte IFN-γ in female schizophrenics.

6.6.2   IL-12: prolactin, IL-10 (lymphocyte)

The complete model facilitated reliable predictions of lymphocyte IL-12 production in the whole control group and male controls, however, not the other groups (whole CON: F = 5.64, p < .001; ♂CON: F = 5.38, p < .001; ♀CON: F = 2.04, p = .08; whole SCH: F = 1.39, p = .24; ♂SCH: F = 1.14, p = .40; ♀SCH: F = 3.21, p = .14).

Whole SCH vs. whole CON

A new model keeping IL-10, IL-4, IL-13, prolactin, testosterone, and age improved the reliability of predictor assembly in the whole schizophrenic group. It enlightened 20% and 39% lymphocyte IL-12 variance in the whole schizophrenic and control group, respectively (whole SCH: F = 2.59, p = .04; whole CON: F = 8.62, p < .001). IL-10 and age were the most dominant contributors for lymphocyte IL-12 release in controls; instead, prolactin was the only primary "donor" of IL-12 variance in schizophrenia (whole CON – IL-10: t = 4.25, p < .001; age: t = 3.12, p = .003; whole SCH – prolactin: t = 3.03, p = .005).

Male SCH vs. male CON

A reduced model including IFN-γ, IL-10, IL-4, testosterone, and age led to amelioration of predictive reliability of the model in schizophrenic males (F = 2.89, p = .04). The new model explicated 28% and 54% lymphocyte IL-12 variance in schizophrenic and control men, correspondingly. IL-10 offered the primary contribution to IL-12 variance in male schizophrenics; instead, the individual predictive powers of age and IFN-γ were significant in control men (♂SCH – IL-10: t = 2.40, p = .03; ♂CON – age: t = 2.33, p = .03; IFN-γ: t = 2.11,

p = .04). In addition, IL-10 tended to make a marked contribution to lymphocyte IL-12 secretion in control males as well (t = 1.91, p = .07).

Female SCH vs. female CON

Excluding IFN-γ, cortisol, and estradiol, the new model explained 69% and 26% of lymphocyte IL12 variance in control and schizophrenic females, respectively ($\female$CON: F = 5.38, p = .02; $\female$SCH: F = 2.68, p = .03). The primary contributor of IL-12 production was prolactin in schizophrenic females, but that in female controls was IL-10 ($\female$SCH – prolactin: t = 3.96, p = .005; $\female$CON – IL-10: t = 2.91, p = .007). Additionally, testosterone tended to have a marked contribution to IL-12 variance in schizophrenic women (t = -2.02, p = .08).

Summary :

(1) IL-10 and age were the essential contributors for lymphocyte IL-12 production in the whole control group, while prolactin made a marked contribution to IL-12 release in the whole schizophrenic patients.

(2) IFN-γ was the dominant influencing factor for lymphocyte IL-12 in male controls, while IL-10 significantly contributed to lymphocyte IL-12 variance in male schizophrenics.

(3) IL-10 was important in lymphocyte IL-12 production in female controls, while prolactin was essential in this regard in female schizophrenics.

6.6.3 **IL-2:** cortisol, estradiol, testosterone, IL-4 (serum), TNF-α (whole blood)

Serum data

For both the whole schizophrenic and control group as well as both female groups, no common model could be found due to having different influencing factors for serum IL-2 in these groups.

Whole SCH vs. whole CON

The complete model enabled an explication of 100% serum IL-2 variance in the whole control group, but not in the schizophrenic one (whole CON: F = 15375.15, p < .001; whole SCH: F = 1.17, p = .35). TNF-α dominated over the others included in the model and made a clear contribution to serum IL-2 variance in controls (t = 9.88, p < .001). Besides, IFN-γ tended to be able to predict serum IL-2 variance significantly in controls (t = 1.50, p = .08).

A model keeping TNF-α, cortisol, and estradiol showed that (1) cortisol was the primary contributor for serum IL-2 in schizophrenics and (2) estradiol also played a secondary role in

this regard, although the new model was only able to envisage 13% variance of serum IL-2 (whole SCH: F = 2.99, p = .04; cortisol: t = -2.44, p = .02; estradiol: t = -1.78, p = .08).

Male SCH vs. male CON

Similar findings were obtained for healthy males; (1) the model explained 100% serum IL-2 variance and (2) TNF-α was the primary influencing factor among those included in the model ($\male$CON: F = 16692.27, p < .001; TNF-α: t = 10.00, p < .001). In addition, IL-10 tended to have a noticeable effect on serum IL-2 in male controls (t = -1.85, p = .08). Nevertheless, no single parameter assessed in this study was able to make a significant prediction of serum IL-2 in male schizophrenics.

Female SCH vs. female CON

For both female groups, no common model could be found since serum IL-2 of both groups obviously had diverse influencing factors. However, an individual model dropping age for schizophrenic females showed that (1) estradiol, testosterone, cortisol, and IL-4 could significantly predict serum IL-2, (2) that IL-6 tended to be able to forecast IL-2 sufficiently, and (3) the model was able to explain 84% of serum IL-2 variance ($\female$SCH: F = 8.06, p = .03; estradiol: t = -6.17, p = .004; testosterone: t = 4.08, p = .02; cortisol: t = -3.52, p = .02; IL-4: t = 3.40, p = .03; IL-6: t = -2.51, p = .07). A model keeping IFN-γ, TNF-α, IL-4, cortisol, and testosterone for control females was found. It exhibited a single dominant predictor TNF-α for serum IL-2. However, the new model could explicate only 20% IL-2 variance ($\female$CON: F = 2.62, p = .05; TNF-α: t = 2.13, p = .04).

Summary :

(1) TNF-α was the dominant factor in the whole control group, whereas cortisol was the essential one in the whole schizophrenic group in envisaging serum IL-2. The explainable portion of serum IL-2 through the predictors included was only 13% in the schizophrenic group.

(2) TNF-α was the only important contributor of serum IL-2 in male controls, while none of those parameters assessed made a significant contribution to serum IL-2 in schizophrenic men. Serum IL-2 variance in male schizophrenics was impossible to be reliably explained by any of the parameters assessed in this study.

(3) TNF-α was crucial in predicting serum IL-2 in female controls, However, estradiol, testosterone, cortisol, and IL-4 were important in this regard in schizophrenic women. The explainable part of serum IL-2 variance was 84% in female schizophrenics.

The complete model could predict whole blood assay IL-2 variance reliably in all groups but female schizophrenics (whole SCH: F = 9.45, p < .001; whole CON: F = 4.06, p < .001; ♂SCH: F = 11.20, p < .001; ♂CON: F = 3.10, p = .03; ♀CON: F = 3.22, p = .009; ♀SCH: F = 2.04, p = .30).

Whole SCH vs. whole CON

TNF-α played a dominant role in envisaging whole blood IL-2 in vitro production in both the whole schizophrenic and control group (whole CON: t = 7.50, p < .001; whole SCH: t = 4.01, p < .001). IL-6 and age also tended to have marked impacts on whole blood assay IL-2 release in schizophrenics and controls, respectively (whole CON – IL-6: t = -1.81, p = .08; whole SCH – age: t = 1.83, p = .08). Furthermore, the explainable portions of whole blood assay IL-2 were 57% and 62% for schizophrenics and controls, correspondingly.

Male SCH vs. male CON

The results from male patients were similar to those of the whole schizophrenic group. TNF-α was the major contributor and age tended to make a significant contribution to whole blood assay IL-2 variance (TNF-α: t = 3.23, p < .001; age: t = 1.81, p = .09). In contrast to the findings in the whole control group, a series of variables were involved or tended to be involved in whole blood assay IL-2 release in male controls; they included TNF-α, IL-6, IFN-γ, testosterone, age, and SHBG. Nevertheless, the model explicated similar portions of IL-2 variance in both male groups (♂CON: 75%; ♂SCH: 72%).

Female SCH vs. female CON

Similar outcomes were gained from female controls; TNF-α was the primary "donor" of whole blood IL-2 in vitro secretion and, additionally, prolactin likewise tended to exert a marked influence on IL-2 (TNF-α: t = 4.03, p = .001; prolactin: t = 1.80, p = .09).

A reduced model dropping IL-6, age, and cortisol improved the envisaging reliability of the predictors in schizophrenic women. However, the model also led to a change in control females (♀SCH: F = 5.35, p = .03; ♀CON: F = 4.54, p = .002). The new model explicated, instead of 62% by the complete model, only 46% whole blood assay IL-2 variance in female controls. It explained, nevertheless, 71% of IL-2 variance in schizophrenic females. TNF-α kept its dominant role in the new model in healthy females, while no single predictor alone could significantly predict whole blood IL-2 in vitro production in female subjects with schizophrenia (♀CON – TNF-α: t = 4.10, p < .001).

<u>Summary</u> :

    (1) TNF-α seemed to play an essential role in predicting whole blood IL-2 in vitro production in all groups but female schizophrenics.

    (2) No single parameter was able to make a noticeable contribution to whole blood asssay IL-2 variance in schizophrenic women.

6.6.4 TNF-α: IL-4, testosterone, SHBG (serum), IL-2, IL-4 (whole blood)

Serum data

The complete model could make a reliable prediction of serum TNF-α in all groups but both schizophrenic gender subgroups and normal female subjects. It explained 100% serum TNF-α variance in the whole controls and healthy males (whole CON: R^2 = 1.00, F = 25598.08, p < .001; ♂CON: R^2 = .47, F = 38493.99, p < .001; whole SCH: F = 2.24, p = .04; ♂SCH: F = 1.40, p = .28; ♀SCH: F = .82, p = .65; ♀CON: F = 1.57, p = .18).

Whole SCH vs. whole CON

IL-2 was the primary source of serum TNF-α level in the whole control group. Instead, IL-4, testosterone, and SHBG offered the major contributions to serum TNF-α variance in the whole schizophrenic group (whole CON – IL-2: t = 9.88, p < .001; whole SCH – IL-4: t = 3.15, p < .001; testosterone: t = -2.69, p = .01; SHBG: t = -2.44, p = .02).

Male SCH vs. male CON

No common model was found for both male groups in order to compare the forecasting powers of diverse predictors. The major influencing factors of serum TNF-α in male controls consisted of IL-2, IL-10, and cortisol (♂CON – IL-2: t = 10.00, p < .001; IL-10: t = 2.54, p = .02; cortisol: t = -2.15, p = .04). A new model keeping IL-4, IL-10, and estradiol could explain 25% serum TNF-α variance in male schizophrenics (F= 3.67, p = .03). Among them, IL-4 and estradiol made significant contributions to the variance of serum TNF-α (♂SCH – IL-4: t = 2.77, p = .01; estradiol: t = -2.48, p = .02).

Female SCH vs. female CON

However, no reliable model could be found among the parameters assessed in this study for predict serum TNF-α in female schizophrenics. Nevertheless, a model dropping IL-10, testosterone, and age ameliorated the predicting reliability in female controls. It enlightened 25% serum TNF-α variance (F = 2.40, p = .05). IL-4 had a significant predicting power and IL-2 tended to have one in envisaging TNF-α variance in female controls (IL-4: t = 2.29, p = .03; IL-2: t = 2.00, p = .06).

Summary :

(1) IL-2 was essential in predicting serum TNF-α in the whole controls, while IL-4, testosterone, and SHBG were important in this respect in the whole schizophrenics. The explainable portion of serum TNF-α in schizophrenia was only 47%, in contrast to 100% in control.

(2) IL-2, IL-10, and cortisol were crucial for serum TNF-α in male controls, whereas IL-4 was the only primary influencing factor for serum TNF-α in male schizophrenics. The explainable part of serum TNF-α was only 25% in schizophrenic men.

(3) IL-4 was important to predict serum TNF-α in female controls, while no single variable could make a significant prediction of serum TNF-α in schizophrenic women. Serum TNF-α variance in female schizophrenics was impossible to be explicated by any variable assessed in this study.

Whole blood assay data

The complete model could predict whole blood assay TNF-α reliably in all groups but male schizophrenics (whole CON: F = 17.39, p < .001; whole SCH: F = 5.84, p < .001; ♂CON: F = 14.69, p < .001; ♂SCH: F = 2.56, p = .06; ♀CON: F = 5.31, p < .001; ♀SCH: F = 12.92, p = .03). It allowed to envisage 72%, 70%, 80%, 73%, and 98% whole blood assay TNF-α variance in the whole schizophrenic group, the whole controls, male controls, healthy females as well as female schizophrenics, respectively.

Whole SCH vs. whole CON

IL-2, IL-4, and IL-6 were the primary contributors for TNF-α variance in the whole control group, while IL-2 and age played dominant roles in envisaging whole blood TNF-α in vitro secretion in schizophrenics (whole CON – IL-2: t = 7.50, p < .001; IL-4: t = 3.30, p = .002; IL-6: t = 2.45, p = .02; whole SCH – IL-2: t = 4.01, p < .001; age: t = -2.05, p = .05).

Male SCH vs. male CON

Excluding estradiol out of the model improved the predictive reliability of parameters included in the model in male schizophrenics and raised only minor alterations in control men (♂SCH: F = 3.03, p = .03; ♂CON: F = 15.55, p < .001). IL-2 was the most essential factor in predicting whole blood TNF-α in vitro production in both male groups (♂CON – IL-2: t = 6.88, p < .001; ♂SCH – IL-2: t = 3.36, p = .005). In addition, IL-6 also made a significant contribution to TNF-α variance in control men (t = 2.36, p = .03).

Female SCH vs. female CON

In the female control group, IL-2 made a marked contribution to whole blood TNF-α in vitro secretion. IL-4, instead of IL-2, was the essential "donor" of TNF-α variance in the female schizophrenics ($\female$CON – IL-2: t = 4.03, p = .001; $\female$SCH – IL-2: t = 3.77, p = .03).

Summary : Generally, IL-2 appeared to be indispensable for predicting whole blood TNF-α in vitro production. In addition, IL-4 was also important in this regard in the whole control group and the female schizophrenic subgroup.

6.6.5 IL-4: TNF-α, estradiol, IL-2 (serum), IL-10, IFN-γ, TNF-α (whole blood)

Serum data

The complete model was not sufficient to make a reliable prediction for the variance of serum IL-4 in female controls, the whole schizophrenic group as well as both schizophrenic gender subgroups, although it could explain 98% serum IL-4 variance in the whole control group and healthy males (whole SCH: F = 1.86, p = .09; $\male$SCH: F = 1.64, p = .20; $\female$SCH: F = 2.25, p = .27; $\female$CON: F = .76, p = .68; whole CON: F = 258.05, p < .001; $\male$CON: F = 192.14, p < .001). However, the outcomes for the whole control group and normal males indicated no single dominant contributor for serum IL-4 variance. But all predictors together could envisage serum IL-4 variance reliably and sufficiently. Nevertheless, for female controls, it was not that case; neither essential nor reliable "donor" of serum IL-4 variance could be found among those parameters measured in this study.

Whole SCH vs. whole CON

An alternative model dropping both IL-6 and cortisol improved the reliability of the predictive power in the whole schizophrenic group without causing any major alteration in the whole control group (whole SCH: F = 2.43, p = .03; whole CON: F = 288.00, p < .001). The new model was capable of illuminating 25% and 98% serum IL-4 variance in schizophrenics and controls. As in the complete model, no single dominant contributor of IL-4 variance was observed in controls, while TNF-α and testosterone played dominant roles in predicting serum IL-4 in schizophrenics (whole SCH – TNF-α: t = 3.51, p = .001; testosterone: t = 3.15, p = .004). Additionally, SHBG in schizophrenia tended to make a significant contribution to IL-4 variance as well (t = 1.98, p = .06).

Male SCH vs. male CON

Dropping IL-2 and IL-10 out of the model, the reduced model could enlighten 35% and 98% serum IL-4 variance in male schizophrenics and controls, respectively ($\male$SCH: F = 2.65, p = .05; $\male$CON: F = 250.27, p < .001). For healthy control men existed no single dominant

contributor for serum IL-4 variance, whereas estradiol and TNF-α took over the crucial roles in predicting serum IL-4 in schizophrenic males (estradiol: t = 2.65, p = .02; TNF-α: t = 2.57, p = .02). Furthermore, prolactin in schizophrenic males likewise tended to make a noticeable contribution to IL-4 variance (t = 2.06, p = .06).

Female SCH vs. female CON

Various attempts did not succeed in finding a common reliable model for both female groups. A new model keeping IL-2, TNF-α, cortisol, prolactin, estradiol, and testosterone could make a reliable prediction for serum IL-4 variance. It elucidated 54% serum IL-4 variance in schizophrenic females (F = 3.77, p = .04).

Summary :

(1) No single dominant influencing factor was found for serum IL-4 in the whole control group and healthy males.

(2) TNF-α and testosterone were crucial for serum IL-4 in the whole schizophrenic group, while TNF-α and estradiol made significant contributions to serum IL-4 variance in the male schizophrenic group. The explainable portion of IL-4 variance was only 25% in the whole schizophrenic group.

(3) IL-2 was the primary predictor in female schizophrenics. Nevertheless, none of the variables measured in this study was able to make a reliable prediction of serum IL-4 in normal women.

Whole blood assay data

The complete model enabled a reliable prediction of whole blood assay IL-4 variance in the whole schizophrenic group, the whole control group, male controls, and female schizophrenics, nonetheless, not in male schizophrenics and female controls (whole SCH: R^2 = .55, F = 8.94, p < .001; whole CON: R^2 = .58, F = 3.56, p = .003; ♂CON: R^2 = .72, F = 9.57, p < .001; ♀SCH: R^2 = .98, F = 11.38, p = .04; ♂SCH: F = 1.17, p = .39; ♀CON: F = 1.47, p = .21).

Whole SCH vs. whole CON

IL-10 and IFN-γ dominated over the others in envisaging whole blood assay IL-4 variance in both schizophrenic and control group (whole CON – IL-10: t = 6.54, p < .001; IFN.γ: t = -3.05, p = .003; whole SCH – IL-10: t = 3.80, p = .001; IFN-γ: t = -2.12, p =.04). Compared to the control group, the influences of IL-10 and IFN-γ were much weaker in the whole schizophrenic group. Moreover, TNF-α also played a very important role in this regard in

controls, but it was not that case in the schizophrenic group (whole CON – TNF-α: t = 3.30, p = .002; whole SCH – TNF-α: t = 1.57, p = .13).

Male SCH vs. male CON

A new model keeping IFN-γ, TNF-α, IL-10, and IL-6 enabled a reliable prediction of whole blood assay IL-4 in male schizophrenics and led simply minor changes in control male subjects (♂SCH: F = 2.81, p = .05; ♂CON: F = 19.29, p < .001). It enlightened 71% whole blood assay IL-4 variance in control men, but only 27% in schizophrenic males. IL-10 was the most important, then IL-6, and finally IFN-γ, in envisaging whole blood assay IL-4 in male schizophrenics. The importance magnitudes of IL-6 and IFN-γ only showed a trend to be significant (♂SCH – whole blood assay IL-10: t = 2.93, p = .009; IL-6: t = 1.95, p = .07; IFN-γ: t = -1.78, p = .09). IL-10 was likewise the most dominant contributor of whole blood assay IL-4 variance in control males. Besides, TNF-α played an essential role in this regard as well (♂CON – IL-10: t = 6.45, p < .001; TNF-α: t = 2.47, p = .02).

Female SCH vs. female CON

Dropping IL-2, both sex hormones, and SHBG improved the predictive reliability of the remaining parameters in both female groups (♀SCH: F = 17.52, p < .001; ♀CON: F = 2.50, p = .05). The new model could explain 21%, however, 89% whole blood assay IL-4 variance in schizophrenic and control females, respectively. For the female controls, IFN-γ made an important contribution to whole blood IL-4 in vitro production and TNF-α showed such a tendency as well (IFN-γ: t = -2.17, p = .04; TNF-α: t = 2.00, p = .06). It's noteworthy that the new model changed the relative magnitudes of importance among the predictors in female schizophrenic patients. TNF-α was the most important factor in the complete model, while IL-10 dominated over the remaining parameters in the new one. However, the role of TNF-α in envisaging whole blood assay IL-4 in female patients remained noticeable in the new model (♀SCH – IL-10: t = 3.77, p = .005; TNF-α: t = 3.22, p = .01). Moreover, cortisol also tended to make a clear contribution to whole blood assay IL-4 variance in schizophrenic females (t = 1.90, p = .09).

Summary :

(1) Both IL-10 and IFN-γ were important in forecasting whole blood IL-4 in vitro production in both schizophrenic and control group.

(2) IL-10 was essential for envisaging whole blood assay IL-4 release in both male subgroups

(3) TNF-α made a significant contribution to whole blood assay IL-4 variance in female schizophrenics; nevertheless, no single dominant factor was found in healthy females.

Lymphocyte data

The complete model could generally predict lymphocyte IL-4 production reliably in distinct groups except the male controls (whole CON: F = 2.93, p = .005; whole SCH: F = 3.84, p = .002; ♂SCH: F = 3.60, p = .02; ♀SCH: F = 2.45, p = .04; ♀CON: F = 10.14, p = .02; ♂CON: F = 1.23, p = .31). It forecasted 22%, 57%, 72%, 52%, and 96% lymphocyte IL-4 variance in the whole control group, the whole schizophrenic group, male schizophrenics, female controls, and schizophrenic women, respectively. Various attempts failed to find any proper model to envisage lymphocyte IL-4 in control males.

Lymphocyte IL-13 was the most crucial contributor for lymphocyte IL-4 variance in both the whole schizophrenic group, the whole control group as well as both female subgroups (whole SCH: t = 3.40, p = .001; whole CON: t = 4.55, p < .001; ♀CON: t = 3.02, p = .006; ♀SCH: t = 4.19, p = .01). In addition, prolactin and age noticeably contributed to lymphocyte IL-4 variance in the whole schizophrenics and female patients as well (prolactin: t = 2.17, p = .03; age: t = -2.98, p = .04). However, for the male schizophrenics, instead of IL-13 and prolactin, IFN-γ and age were the first two dominant influencing factors for lymphocyte IL-4 (age: t = 3.00, p = .01; IFN-γ: t = 2.36, p = .03).

Summary :

(1) IL-13 was important in both schizophrenic and control group; additionally, prolactin was very important for predicting lymphocyte IL-4 in the whole control group.

(2) Prolactin played an important role in lymphocyte IL-4 secretion in male controls, while IFN-γ and age were essential in this regard in male schizophrenics.

(3) IL-13 was crucial in both female subgroups. Besides, age also made a significant contribution to lymphocyte IL-4 variance in female schizophrenic patients.

6.6.6 **IL-10:** IL-4, IFN-γ (whole blood)

Serum data

In general, the complete model was sufficient to make a reliable prediction of serum IL-10 in the whole schizophrenic group and male controls, however, not in the other groups (whole CON: F = 20654.58, p < .001; ♂CON: F = 20553.89, p < .001; whole SCH: F = .71, p = .72; ♂SCH: F = 1.11, p = .42; ♀SCH: F = .66, p = .74; ♀CON: F = 1.65, p = .15).

Whole SCH vs. whole CON

No further model out of the parameters assessed in this study was able to predict serum IL-10 reliably in the whole schizophrenic patients. For the whole controls, IL-6 and IFN-γ were the most essential predictors for serum IL-10; together with the others, they could explain 100% variance of serum IL-10 (whole CON – F = 20654.58, p < .001; serum IL-6: t = 6.27, p < .001; IFN-γ: t = 2.11, p = .04).

Male SCH vs. male CON

In the complete model, IL-6, TNF-α, and age were the first 3 dominant influencing factors for serum IL-10 in male controls (adjusted R^2 = 1.00, F = 20553.89, p < .001; serum IL-6: t = 6.16, p < .001; TNF-α: t = 2.54, p = .02; age: t = 2.03, p = .05). Nevertheless, no model could be found in this study enabling a reliable prediction of serum IL-10 in male schizophrenics.

Female SCH vs. female CON

Similarly, we failed to find any reliable model among the variables included in this study to predict serum IL-10 in the schizophrenic females. Nevertheless, dropping IL-2, TNF-α, and estradiol improved the model reliability; but it enlightened only 28% IL-10 variance in female controls (F = 2.57, p = .03). Among those parameters kept in the new model, SHBG, cortisol, IL-6, and IFN-γ significantly contributed to the variance of serum IL-10 in control women (SHBG: t = 2.83, p = .009; cortisol: t = -2.31, p = .03 ;IL-6: t = 2.07, p = .05; IFN-γ: t = 2.06, p = .05).

Summary :

(1) Apparently, the important influencing factors of serum IL-10 in all schizophrenic subgroups were beyond the scope of our assessments.

(2) IL-6 and IFN-γ in the whole control group, IL-6 and age in the male controls as well as SHBG, cortisol, IL-6, and IFN-γ in healthy women are likely crucial for serum IL-10 variance.

Whole blood assay data

The complete model was able to make a sufficient prediction for whole blood IL-10 in vitro production in the whole schizophrenic group, the whole control group as well as both control gender subgroups (whole SCH: F = 5.48, p < .001; whole CON: F = 12.99, p < .001; ♂CON: F = 10.56, p < .001; ♀CON: F = 4.21, p = .002). It explicated relatively similar portions of whole blood assay IL-10 variance in distinct groups; they were 68%, 65%, 74%, and 68% for

the whole schizophrenic patients, the whole controls, male controls, and normal females, correspondingly.

Whole SCH vs. whole CON

Both IL-4 and IFN-γ played dominant roles in envisaging whole blood assay IL-10 in the whole schizophrenic and control group (whole CON – IL-4: t = 6.54, p < .001; IFN-γ: t = 5.70, p < .001; whole SCH – IL-4: t = 3.80, p = .001; IFN-γ: t = 2.90, p = .007). Besides, SHBG also tended to make a marked contribution to whole blood assay IL-10 release in schizophrenia (t = 1.99, p = .06).

Male SCH vs. male CON

As in the whole control group, both IL-6 and IFN-γ took over the dominant roles in predicting whole blood assay IL-10 release in male controls, although the model failed to predict whole blood assay IL-10 reliably in schizophrenic men (♂CON – IL-6: t = 5.51, p < .001; IFN-γ: t = 3.25, p = .003; ♂SCH: F = .98, p = .51).

A new model keeping IFN-γ, TNF-α, IL-4, and SHBG led to improvement of the predictive power of the model in male schizophrenics (♂CON: F = 31.74, p < .001; ♂SCH: F = 2.94, p = .05). It explicated 77%, nevertheless, only 24% whole blood assay IL-10 variance in control and schizophrenic men, correspondingly. For both male groups, IL-4 and IFN-γ were the first two most essential contributors for whole blood assay IL-10, although the magnitudes of both parameters in envisaging IL-10 were much weaker in male patients (♂CON – IL-4: t = 7.14, p < .001; IFN-γ: t = 3.93, p < .001; ♂SCH – IL-4: t = 2.81, p = .01; IFN-γ: t = 2.00, p = .06). Furthermore, SHBG appeared to play an important role in predicting whole blood assay IL-10 release in healthy males as well (t = -2.24, p = .03).

Female SCH vs. female CON

Regardless of in the complete or reduced model, IFN-γ was the dominant contributor for whole blood assay IL-10 variance in female controls (complete model – IFN-γ: t = 3.45, p = .002; reduced model – IFN-γ: t = 3.36, p = .003). The complete model could explain 68%, while the reduced one only explicated 51% whole blood assay IL-10 variance in normal women (complete model: F = 4.21, p = .002; reduced model: F = 4.42, p = .002). However, the reduced model improved the predictive reliability of all included predictors and explicated 82% whole blood assay IL-10 variance in female schizophrenics (F = 7.38, p = .04). Nevertheless, no single dominant contributor was found in schizophrenic females.

Summary :

(1) IL-4 and IFN-γ were the dominant contributors for whole blood assay IL-10 release in the whole schizophrenic group, the whole control group, and male controls.

(2) IL-4 could have made a significant contribution to whole blood assay IL-10 variance in male schizophrenics.

(3) IFN-γ was the primary factor for predicting whole blood assay IL-10 in female controls, while no single dominant one was found in female schizophrenics.

Lymphocyte data

The complete model enabled a reliable prediction of lymphocyte IL-10 in all control groups, but not in any schizophrenic group (whole CON: $F = 5.44$, $p < .001$; ♂CON: $F = 3.36$, $p = .006$; ♀CON: $F = 3.04$, $p = .01$; whole SCH: $F = 1.52$, $p = .18$; ♂SCH: $F = 1.00$, $p = .49$; ♀SCH: $F = .52$, $p = .82$).

Whole SCH vs. whole CON

According to the complete model, only IFN-γ and IL-12 made significant contributions to lymphocyte IL-10 variance. Nevertheless, a new model dropping IL-4, testosterone, and age increased the importance of IL-13 in envisaging lymphocyte IL-10 in healthy subjects, in addition to IFN-γ and IL-12 (complete model – IFN-γ: $t = 3.14$, $p = .003$; IL-12: $t = 3.27$, $p = .002$; new model – IFN-γ: $t = 3.68$, $p < .001$; IL-12: $t = 3.49$, $p = .001$; IL-13: $t = -2.31$, $p = .02$). Although the new model ameliorated the predicting reliability in schizophrenia ($F = 3.38$, $p = .04$), no single predictor in the new model made a significant contribution except SHBG ($t = 2.02$, $p = .05$). Besides, the new model only explained 20% lymphocyte IL-10 variance in schizophrenia, whereas 40% in controls (SCH: $F = 2.38$, $p = .04$; CON: $F = 7.78$, $p < .001$).

Male SCH vs. male CON

A new model keeping IFN-γ, IL-12, IL-13, and cortisol enhanced the predictive reliability of the included parameters. It explicated 27% lymphocyte IL-10 variance in male schizophrenics ($F = 3.18$, $p = .04$). Among those predictors, IL-12 made a significant contribution to lymphocyte IL-10 production ($t = 2.64$, $p = .02$). On the contrary, IFN-γ played the primary role, while IL-12 was only secondary for lymphocyte IL-10 release in schizophrenic men (IFN-γ: $t = 2.46$, $p = .02$; IL-12: $t = 1.86$, $p = .07$).

Female SCH vs. female CON

Among the parameters involved in this study, no model was found to make a reliable prediction for lymphocyte IL-10 secretion in female schizophrenics.

134

The complete model exhibited that IL-12 and IFN-γ could significantly predict the lymphocyte IL-10 variance in control females. Additionally, IL-13 showed a trend to make a remarkable contribution as well (♀SCH – IL-12: t = 2.73, p = .01; IFN-γ: t = 2.09, p = .05; IL-13: t = -1.99, p = .06).

Summary:

(1) IFN-γ and IL-12 were the major contributors of lymphocyte IL-10 release in the whole control group, while no single dominant one was found in the schizophrenic group.

(2) IFN-γ was the primary influencing factor for lymphocyte IL-10 in male controls, whereas IL-12 had a noticeable impact on lymphocyte IL-10 in male schizophrenic patients.

(3) IFN-γ and IL-12 were also important for predicting lymphocyte IL-10 in female controls. However, the essential factors of lymphocyte IL-10 in female schizophrenics remained unknown.

6.6.7 IL-13: IL-4 (lymphocyte)

The complete model facilitated a reliable prediction of lymphocyte IL-13 production in both the whole schizophrenic and control group as well as both female groups, however, not both male subgroups (whole CON: R^2 = .26, F = 3.45, p = .001; whole SCH: R^2 = .48, F = 2.63, p = .02; ♀CON: R^2 = .69, F = 5.22, p = .001; ♀SCH: R^2 = .95, F = 7.60, p = .03; ♂CON: F = .86, p = .58; ♂SCH: F = 1.03, p = .47).

For the whole controls as well as healthy females, both IL-4 and IFN-γ made significant contributions to lymphocyte IL-13 variance, while in the whole schizophrenic group and female patients, IL-4 took over the only dominant role in predicting IL-13 release (whole CON – IFN-γ: t = 3.51, p = .001; IL-4: t = 3.40, p = .001; ♀CON – IFN-γ: t = 3.34, p = .003; IL-4: t = 3.02, p = .006; whole SCH – IL-4: t = 4.55, p < .001; ♀SCH – IL-4: t = 4.19, p = .01). Nonetheless, no reliable model within the measurements of this study could be found in order to forecast the lymphocyte IL-13 secretion in both male groups.

Summary:

(1) For both the whole control group and female controls, IL-4 and IFN-γ were essential for forecasting lymphocyte IL-13 production.

(2) Nevertheless, for both the whole schizophrenic group and female patients, IL-4 was the only dominant parameter in this regard.

(3) For both male subgroups, the essential contributors of lymphocyte IL-13 remained unclear.

6.6.8 IL-6: IFN-γ, TNF-α, estradiol, IL-4, IL-2 (whole blood)

Serum data

Multiple regression demonstrated that a model including IFN-γ, IL-2, TNF-α, IL-4, IL-10, various hormones, SHBG, and age allowed a reliable prediction of serum IL-6 in the whole control group and the healthy males, nevertheless, not the remaining groups (whole CON: F = 22507.37, p < .001; ♂CON: t = 22131.61, p < .001; ♀CON: t = 1.47, p = .21; whole SCH: F = .91, p = .55; ♂SCH: F = .85, p = .60; ♀SCH: F = .93, p = .60).

No proper model within our measurements could be generated to predict serum IL-6 reliably in all schizophrenic groups. However, the completed model explained 100% variance of serum IL-6 in both the whole control and male control group. Among the parameters assessed, the individual predictive powers of IL-10 and IFN-γ reached significance levels in both control groups stated above (whole CON – IL-10: t = 6.27, p < .001; IFN-γ: t = 2.97, p = .004; ♂CON – IL-10: t = 6.16, p < .001; IFN-γ: t = 2.21, p = .04). For female controls, a new model dropping TNF-α, IL-4, age, and both sex hormones was able to make a reliable prediction for serum IL-6. Nevertheless, it explicated only 23% serum IL-6 variance in female controls (F = 2.74, p = .04). Among the predictors, exclusively cortisol make a significant contribution to serum IL-6 variance and IL-10 tended to do so in healthy females (cortisol: t = 2.24, p = .03 ;IL-10: t = 2.00, p = .06).

Summary :

(1) The crucial factors involved in serum IL-6 variance remained unknown in all schizophrenic subgroups.

(2) For the whole control group and normal males, IL-10 and IFN-γ were essential to predict serum IL-6. Instead, cortisol could play an important role in this regard in female controls.

Whole SCH vs. whole CON

Comparisons between the whole schizophrenic and control group demonstrated that no single parameter made a substantial contribution to whole blood assay IL-6 variance in the whole schizophrenic group, while IFN-γ, testosterone, and TNF-α were the crucial influencing factors for whole blood IL-6 in vitro production in the whole control group (whole CON – IFN-γ: t = 3.09, p = .003; testosterone: t = -2.59, p = .01; TNF-α: t = 2.45, p = .02). In addition, IL-2 also showed a tendency to have a noticeable contribution to IL-6 in controls (t = -1.81, p = .08).

Male SCH vs. male CON

Dropping IL-10 and prolactin ameliorated the predicting reliability of the model in male schizophrenics. The model enlightened 39% whole blood assay IL-6 variance in schizophrenic men (F = 2.74, p = .04). Nevertheless, no single parameter was observed to make a significant contribution to the variance of whole blood assay IL-6 in schizophrenic males.

The new model improved the collective forecasting power of predictors and explained 27% whole blood assay IL-6 variance in healthy males (F = 2.51, p = .03). In contrast to the findings in male schizophrenics, there were a variety of parameters being able to predict whole blood assay IL-6 significantly. They included IFN-γ, IL-2, TNF-α, IL-4, cortisol, and estradiol ($\male$CON – IFN-γ: t = 3.30, p = .003; IL-2: t = -2.81, p = .009; TNF-α: t = 2.68, p = .01; IL-4: t = -2.31, p = .03; cortisol: t = -2.01, p = .05; estradiol: t = 1.78, p = .09).

Female SCH vs. female CON

The complete model allowed a reliable prediction of whole blood assay IL-6 in healthy female subjects, however, not in the female schizophrenic patients ($\female$CON: F = 2.99, p = .01; $\female$SCH: F = 1.65, p = .37). A model keeping IFN-γ, TNF-α, IL-4, cortisol, estradiol, and age ameliorated the predicting power of the model in schizophrenic women. The new model explained 58% and 40% whole blood assay IL-6 variance in schizophrenic and control females, correspondingly ($\female$CON: F = 4.17, p = .003; $\female$SCH: F = 4.23, p = .03). The essential predictors of whole blood assay IL-6 in schizophrenic women included TNF-α, estradiol, and IL-4, whereas those in healthy females contained IFN-γ, cortisol, and age ($\female$ CON – IFN-γ: t = 2.93, p = .007; cortisol: t = -2.67, p = .01; age: t = -2.16, p = .04; $\female$SCH – TNF-α: t = 3.62, p = .007; IL-4: t = -2.61, p = .03; estradiol: t = -2.81, p = .02).

Summary :

   (1) The essential predictors for whole blood assay IL-6 in the whole control group were IFN-γ, testosterone, and TNF-α, whereas IFN-γ was the only noticeable contributor in this regard in the whole schizophrenic group.

   (2) No single dominant influencing factor was found for male schizophrenics, although TNF-α, IL-2, and IFN-γ were shown to be essential in predicting whole blood assay IL-6 in normal men,

   (3) TNF-α, estradiol, and IL-4 were important for whole blood assay IL-6 production in female schizophrenics. In contrast, IFN-γ, cortisol, and age might play primary roles in predicting whole blood assay IL-6 release in female controls.

6.7 Correlations between Th1/Th2 cytokines/ratios and psychopathology in schizophrenic subjects

Since so far no theoretical or biological base indicates any relationship between cytokines and the scores on various PANSS scales (negative, positive, global), instead of multiple regression analysis, the Pearson-correlations between the scores on various PANSS scales and cytokines from distinct materials were calculated. The results are summarized in **Table 6-7**.

Serum data

Serum data included 48 schizophrenic patients who had information about serum cytokines, Th1/Th2 ratios as well as scores on various PANSS scales. They revealed no noteworthy correlation between any Th1/Th2 cytokine or ratio and any subscale of the PANSS. The only exception was the correlation between serum IL-2 and the score on the PANSS global subscale (PANSS positive – serum IFN-γ: r = .05, p = .73; IL-2: r = .01, p = .97; TNF-α: r = .04, p = .80; IL-4: r = -.06, p = .68; IL-10: r = -.02, p = .88; IL-6: r = .07, p = .64; IFN-y/IL-4: r = .06, p = .64; IFN-γ/IL-10: r = .10, p = .09; PANSS negative – serum IFN-γ: r = -.01, p = .96; IL-2: r = .22, p = .14; TNF-α: r = -.06, p = .71; IL-4: r = .01, p = .93; IL-10: r = -.13, p = .39; IL-6: r = .10, p = .50; IFN-γ/IL-4: r = .03, p = .82; IFN-γ/IL-10: r = .09, p = .53; PANSS global – serum IFN-γ: r = .15, p = .32; TNF-α: r = .12, p = .40; IL-4: r = -.06, p = .68; IL-10: r = .05, p = .75: IL-6: r = .16, p = .29; IFN-γ/IL-4: r = .22, p = .13; IFN-γ/IL-10: r = .13, p = .39). Serum IL-2 levels correlated positively with the scores on the PANSS global scale (r = .36, p = .01).

Summary of correlations between Th1/Th2 cytokines/ratios and psychopathology								
material	**Serum**			**Whole blood assay**			**lymphocyte**		
PANSS	PANSS			PANSS			PANSS		
subscale	Positive	Negative	Global	Positive	Negative	Global	Positive	Negative	Global
IFN-γ	.05	.01	.15	-.07	.32²	-.05	-.04	-.04	-.11
IL-12				*-.09*	*-.51**	*-.44*	-.02	.12	.05
IL-2	.01	.22	.36*	-.03	.17	.03			
TNF	.04	-.06	.12	.02	.26	.07			
IL-4	-.06	.01	-.06	-.38*	-.09	-.23	.06	.09	-.03
IL-10	-.02	-.13	.05	-.25	.04	-.04	*.02*	*.15*	*-.01*
IL-13				.14	.49	.56*	.06	.06	-.05
IL-6	.07	.10	.16	.02	.08	.01			
IFN4	.06	.03	.22	.16	.47**	.38*	.13	-.22	-.01
IFN10	.10	.09	.13	.07	.42*	.15	*-.05*	*-.18*	*-.03*
IFN13							-.01	-.20	-.07
N	48	48	48	*(18)*(13) 34	*(18)*(13) 34	*(18)*(13) 34	*(43)* 47	*(43)* 47	*(43)* 47
Note	IFN4 = IFN-γ/IL-4; IFN10 = IFN-γ/IL-10; IFN13 = IFN-γ/IL-13.								

Whole blood assay data

Totally, 34 schizophrenics had data for whole blood cytokine in vitro productions, Th1/Th2 ratios as well as diverse PANSS scores. Obvious associations were found (1) between whole blood IL-4 in vitro productions and scores on the PANSS positive scale, (2) between IFN-γ/IL-4 ratios and the PANSS negative scores, (3) between IFN-γ/IL-10 ratios and the PANSS negative scores as well as (4) between IFN-γ/IL-4 ratios and scores on the PANSS global subscale. The corresponding Pearson correlation coefficients were -.38 (p = .03), .47 (p = .006), .42 (p = .02), and .38 (p = .03). Furthermore, the relationship between IFN-γ and the PANSS negative scale tended to be significant as well (r = .32, p = .06). In addition to those significant findings stated above, no further remarkable correlation was observed between any other cytokine and score of any PANSS subscale (PANSS positive – whole blood assay IFN-γ: r = -.07, p = .68; IL-2: r = -.03, p = .89; TNF-α: r = .02, p = .90; IL-10: r = -.25, p = .16; IL-6: r = .02, p = .91; IFN-γ/IL-4: r = .16, p = .38; IFN-γ/IL-10: r = .07, p = .68; PANSS negative – whole blood assay IL-2: r = .17, p = .35; TNF-α: r = .26, p = .14; IL-4: r = -.09, p = .62; IL-10: r = .04, p = .81; IL-6: r = .08, p = .65; PANSS global – whole blood assay IFN-γ: r = -.05,

p = .79; IL-2: r = .03, p = .89; TNF-α: r = .07, p = .69; IL-4: r = -.23, p = .19; IL-10: r = -.04, p = .82; IL-6: r = .01, p = .96; IFN-γ/IL-10: r = .15, p = .40).

Moreover, 18 schizophrenic patients had whole blood assay IL-12 data (ELISA) and distinct PANSS scores. Thirteen schizophrenics had data regarding whole blood assay IL-13 (ELISA) and diverse PANSS scores. However, no noteworthy link was found between whole blood assay IL-12 or IL-13 and various PANSS scores except (1) that between scores on the PANSS negative scale and whole blood assay IL-12 (r = .51, p < .05) and (2) that between scores on the PANSS global scale and whole blood assay IL-13 (r = .56, p < .05).

Lymphocyte data

Nevertheless, the results from the 47 schizophrenics who had lymphocyte Th1/Th2 data and PANSS scores showed no marked correlation between any lymphocyte cytokine production or Th1/Th2 ratio and scores on any PANSS subscale (PANSS positive – lymphocyte IFN-γ: r = -.04, p = .81; IL-12: r = -.02, p = .92; IL-4: r = -.06, p = .71; IL-10: r = .02, p = .90; IL-13: r = .06, p = .70; IFN-γ/IL-4: r = .13, p = .38; IFN-γ/IL-10: r = -.06, p = .74; IFN-γ/IL-13: r = -.01, p = .93; PANSS negative – lymphocyte IFN-γ: r = -.04, p = .80; IL-2: r = .12, p = .45; IL-4: r = .09, p = .55; IL-10: r = .15, p = .34; IL-13: r = .06, p = .70; IFN-γ/IL-4: r = -.22, p = .13; IFN-γ/IL-10: r = -.18, p = .24; IFN-γ/IL-13: r = -.20, p = .19; PANSS global – lymphocyte IFN-γ: r = -.11, p = .45; IL-12: r = .05, p = .74; IL-4: r = -.03, p = .87; IL-10: r = -.01, p = .97; IL-13: r = -.05, p = .74; IFN-γ/IL-4: r = -.01, p = .95 ; IFN-γ/IL-10: r = -.03, p = .85; IFN-γ/IL-13: r = -.07, p = .62).

Others: In addition, (1) the scores on the CGI at admission correlated positively with serum IL-6 levels (N = 39, r = .36, p = .02) and (2) onset age was positively associated with lymphocyte IL-4 production and lymphocyte IFN-γ/IL-10 ratio (N = 24; IL-4: r = .41, p = .05; IFN-γ/IL-10: r = .41, p = .05).

7 Conclusion and discussion

A summary from epidemiological studies and the relevant literature indicates Th2-shift is possibly found in at least a subgroup of schizophrenia. The related literature includes (1) distinct immune dysfunctions described in schizophrenia, (2) the correlations between one part of schizophrenic patients and <u>viral infections during the pre-, peri-, or postnatal phase</u>, (3) the substantial role of the immune system in viral infections, and (4) the relationships between Th2-shift and some viral infections. Therefore, this study aimed at investigating Th2-shift in schizophrenia. Th2-shift was defined as a reduced IFN-γ/IL-4 or IFN-γ/IL-10 or IFN-γ/IL-13 ratio, compared to healthy controls.

Additionally, the close relationships between the immune and endocrine system have been suggested. Various hormones such as cortisol, prolactin, testosterone, and estradiol were shown to influence Th1/Th2 balance. In order to ensure Th1/Th2 imbalance in schizophrenia is rather a result of disease process than an outcome of distinct hormones, those hormones stated above were also included into the multi-variance analysis as co-variants. The purpose to include those endocrinological parameters into the analysis was (1) to control their effects on Th1/Th2 cytokines/ratios and (2) to examine their individual contributions towards Th1/Th2 imbalance in schizophrenia.

Th2-shift in schizophrenia was explored at serum, whole blood, and lymphocyte three different levels. The collected CPDA blood sample was partially used for whole blood assay and partially for lymphocyte isolation. Both <u>whole blood</u> and <u>lymphocyte</u>s were <u>stimulated</u> in vitro <u>with PHA</u> for 46 hours. The analysis methods contained Cytometric Bead Array (CBA), Enzyme-linked ImmunoSPOT (ELISPOT), and Enzyme-Linked ImmunoSorbent Assay (ELISA). CBA was applied to analyze IFN-γ, IL-2, TNF-α, IL-4, IL-10, and IL-6 in serum and stimulated whole blood, ELISA was for the analysis of IL-12 and IL-13 in whole blood after 46-hour PHA stimulation, while ELISPOT was used to detect IFN-γ, IL-12, IL-4, IL-13, and IL-10 in PHA-stimulated lymphocytes.

The key questions of this study were: (1) whether or not a Th2-shift occurred in any schizophrenic subgroup, (2) if it did, which of the epidemiological and clinical parameters

could characterize the specific schizophrenic subgroup(s), and (3) if it did, which of those immunological and endocrinological parameters measured in this study had contributed to the variance of the Th1/Th2 imbalance in schizophrenia. Our hypothesis was that Th2-shift was observed in at least a subgroup of schizophrenics. The results provided supporting evidence for Th2-shift hypothesis in at least one schizophrenic subgroup.

7.1 Primary findings of this study

The main results regarding Th2-shift in schizophrenia include:

➢ Significant serum Th2-shift**s** including reduced IFN-γ/IL-4 and IFN-γ/IL-10 occurred in schizophrenia;

➢ The whole female schizophrenics, whereas only one subgroup of male patients showed marked serum Th2-shifts. The whole group of male schizophrenics had only significantly reduced serum IFN-γ/IL-10 ratios;

➢ Serum Th2-shifts seemed to be schizophrenia-specific because no noticeable serum Th2-shift was observed in patients with schizophrenia-related disorders.

➢ Serum Th2-shift (either reduced IFN-γ/IL-4 or IFN-γ/IL-10) was also observed in distinct clinical subgroups except schizophrenics pre-dominated with positive symptoms;

➢ Whole blood assay Th2-shift**s** (significantly reduced whole blood assay IFN-γ/IL-4 and IFN-γ/IL-10 ratio) were related to no response towards treatments;

➢ Non-paranoid schizophrenic patients, chronic schizophrenics, patients having positive family psychiatric, and patients being drug-free for ≥ 3 months had markedly reduced whole blood assay IFN-γ/IL-10 ratios.

➢ Early-onset schizophrenics had significantly reduced serum IFN-γ/IL-4 and IFN-γ/IL-10, nonetheless, increased lymphocyte IFN-γ/IL-4 and IFN-γ/IL-13. Late-onset schizophrenics had noticeably decreased lymphocyte IFN-γ/IL-4 and IFN-γ/IL-13, but no clear alteration in serum IFN-γ/IL-4 and IFN-γ/IL-10 ratios if compared to normal subjects.

➢ Chronic schizophrenics had markedly reduced lymphocyte IFN-γ/IL-4 ratios.

Further major outcomes concerning the important influencing factors for the variances of diverse Th1/Th2 ratios in schizophrenia are as followed:

- IFN-γ, IL-4, IL-10, IL-6, and TNF-α were the essential contributors for (both serum and whole blood assay) Th1/Th2 ratios in the whole schizophrenic group;
- IFN-γ, IL-4, IL-10, SHBG, prolactin, age, IL-6, and testosterone had made substantial contributions to the variance of Th1/Th2 ratios in male schizophrenics;
- IFN-γ, IL-4, IL-10, IL-2, IL-6, cortisol, estradiol, testosterone, prolactin, and age could significantly predict Th1/Th2 ratios in schizophrenic women.

In addition, other principal findings regarding the relationships between various cytokine levels/productions, Th1/Th2 ratios, and psychopathology include:

- ❖ Scores on the PANSS positive scale were inversely related to whole blood assay IL-4;
- ❖ Scores on the PANSS negative scale correlated negatively with whole blood assay IL-12, however, positively with IFN-γ/IL-4 and IFN-γ/IL-10 ratio;
- ❖ Scores on the PANSS global scale associated positively with serum IL-2, whole blood assay IL-13, and IFN-γ/IL-4 ratio in schizophrenia.

7.2 Comparisons with other cytokine studies

Hitherto, no result was published regarding Th1/Th2 imbalance in schizophrenia. However, there are a number of studies investigating cytokine in vivo levels and in vitro productions in schizophrenics. In general, the variances of cytokine level/production in serum and in PHA-stimulated whole blood as well as Th1/Th2 ratios in healthy controls were greater than those in schizophrenia and these in males were greater than those in females.

7.2.1 IFN-γ: reduced in vitro IFN-γ in a schizophrenic subgroup

IFN-γ is the major Th1 cytokine. The main biological activity of IFN-γ in the CNS is to activate microglia, to induce and up-regulate MHC-II in glial cells. In the peripheral system, IFN-γ modulates T-cell growth and functional differentiation as well as inhibits the IL-4-induced B-cell growth.

In vivo IFN-γ levels

Serum IFN-γ has been rarely studied for the reason that its serum level is often under the detectable limitations of various available commercial analysis methods. Corresponding to the results of Gattaz et al. (1992) and Becker et al. (1990), we found no marked difference

between schizophrenics as a whole group and healthy controls. However, if excluding the only extreme control case out of the analysis according to the results of cluster-center analysis, schizophrenics as a whole group had significantly decreased serum IFN-γ if compared to normal controls. Furthermore, if male/female schizophrenic patients were compared to controls of their corresponding sex, then only the decrease in female schizophrenics reached a significance level, but not that in male patients.

In vitro IFN-γ productions

As the vast majority of studies investigating whole blood in vitro IFN-γ production, we also found that schizophrenics as a whole group had significantly reduced whole blood assay IFN-γ productions if compared to healthy subjects (Kaminska et al., 2001; Arolt et al., 2000; Rothermundt et al., 1998; Arolt et al., 1997; Wilke et al., 1996; Hornberg et al., 1995; Katila et al., 1989; Moises et al., 1985). In addition, the IFN-γ deficit in schizophrenia was observed not only at whole blood but also at lymphocyte level. Nevertheless, IFN-γ reductions at both whole blood and lymphocyte level also occurred in one subgroup of psychiatric patients having schizophrenia-related disorders. It might indicate that decreased whole blood assay and/or lymphocyte IFN-γ reduction is likely rather a common deficit among psychiatric patients with schizophrenia or schizophrenia-related diseases.

If both genders were compared separately, only the decrease in female schizophrenics remained significant. Male schizophrenic patients had lower whole blood assay IFN-γ productions than male controls; however, the decrease did not reach any statistic significance level. Similar findings exhibited in lymphocyte IFN-γ release; female patients had markedly reduced lymphocyte IFN-γ, while the reduction in male schizophrenics was not obvious if compared with their healthy counterparts of the same sex.

7.2.2 IL-2: likely decreased in a subgroup of schizophrenics

IL-2 is another typical Th1 cytokine. It is an antigen-unspecific T-cell growth factor in the peripheral system. In the CNS, IL-2 is able (1) to modulate NMDA receptor of mesolimibic neurons and (2) to damage the blood brain barrier (BBB).

In vivo serum IL-2 levels

Reports regarding serum IL-2 level in schizophrenia were not many also due to its undetectable in-vivo level in some subjects. Our study included 76 schizophrenics and 75 controls and had no missing data. Similar to the outcomes of Erbagci et al. (2001), Kaminska

et al. (2001), Theodoropoulou et al. (2001), Xu et al. (1994), and Gattaz et al. (1992) , nevertheless, in contrast to those of Zhang et al. (2002) and Kim et al. (1998), schizophrenics as a whole group were found to have decreased serum IL-2 levels in this study; however, the decrease was not significant . As a matter of fact, the study of Zhang et al (2002). included only treatment-resistant schizophrenics (criteria: no response to at least 3 anti-psychotics after a treatment for $\geq$ 3 months or over at full dose, equivalent to chlorpromazine 1000 mg/day). We did not have any data in this regard of our patients. The study of Kim et al. (1998) contained almost 50% (7 of 16) undifferentiated schizophrenics, while no single undifferentiated schizophrenic was recruited in our study. Furthermore, another reason why serum IL-2 was enhanced in Kim's study, but not ours, could be that the patients in Kim's study were either drug-naïve or had been drug-free for a longer time ($\geq$ 6 months) than ours ($\geq$ 3 days). We also found that drug-naïve schizophrenic patients or schizophrenics with longer washout periods had higher serum IL-2 than drug-free or patients with shorter washout periods, although the increases were not statistically significant.

In vitro IL-2 secretion

Similar to the findings of the majority of relevant studies (Kaminska et al., 2001; Kowalski et al., 2000; Arolt et al., 2000; Kim et al., 1998; Cazzullo et al., 1998; Rothermundt et al., 1998; Ausubel et al., 1997; Wilke et al., 1996; Hornberg et al., 1995; Ganguli et al., 1995; Ganguli et al., 1992; Ganguli et al., 1989; Villemain et al., 1987), we showed decreased whole blood IL-2 in vitro production in schizophrenia. Nevertheless, the reduction was not statistically significant. However, two further studies demonstrated increased whole blood assay IL-2 in schizophrenia (Cazzullo et al., 2002; Cazzullo et al., 2001). In these 2 studies, exclusively or almost only paranoid schizophrenics were recruited. Our study included nearly 2 to 4 times as many paranoid patients as those two studies mentioned above. Paranoid schizophrenics were found to produce more IL-2 in stimulated whole blood not only than their non-paranoid schizophrenic counterparts but also than normal subjects. However, the increases didn't reach any statistic significance level. The reason for the discrepancy might be that the drug-free-periods in both studies stated above were much longer ($\geq$ 2 weeks & < 104 weeks) than ours ($\geq$ 3 days). We compared schizophrenics with distinct drug-free-periods to controls and found the longer the drug-free period, the higher the whole blood IL-2 in vitro production.

Furthermore, as Arolt et al. (2000) reported, we also failed to find any noteworthy correlation between IFN-γ, IL-2 whole blood in vitro production with psychopathology measured with

the PANSS scale. Nonetheless, a significant correlation between serum IL-2 and scores on the PANSS global scale was shown in our schizophrenic patients.

7.2.3   **IL-12:** reduced in a female patient subgroup?

IL-12 is a Th1-cytokine and important for Th1-differentiation and IFN-γ production. Binding of IL-12 to IL-12R induces STAT4, which is particularly crucial for Th1-responses. The major biological activities of IL-12 contain inducing IFN-γ, activating NK cells, and promoting NK cytotoxicity.

The only report regarding IL-12 in schizophrenia was from Kim et al. (2000). Kim's study contained 25 male schizophrenic patients; 60% of them were drug-naïve and the remaining 40% patients had been free from neuroleptics for at least 6 months. They found no difference in plasma IL-12 between schizophrenics and healthy controls. We also replicated no marked disparity between schizophrenics and controls in both whole blood assay and lymphocyte IL-12 in vitro production. However, if males and females analyzed separately, no noticeable disparity in lymphocyte and whole blood assay IL-12 was found between both male groups as Kim et al. (2000) reported. Nevertheless, female schizophrenics tended to have lower whole blood assay IL-12 production than female controls.

7.2.4   **TNF-α:** unaltered

TNF-α can be produced by both Th1- and Th2-cells. It has effects on various immune and non-immune cells. On the endocrine system, TNF-α stimulates hormones such as ACTH, CRH, prolactin, but inhibits some others like TSH, FSH, and GH. On the CNS, it may alter the integrity of the BBB, stimulate glial cells, trigger apoptosis, and up-regulate different adhesion molecules.

In accordance with the relevant studies (Kaminska et al., 2001; Erbagci et al., 2001; Xu et al., 1994), we likewise found unaltered serum TNF-α level and whole blood TNF-α in vitro production in schizophrenia. Nevertheless, Theodoropoulou et al. (2001) reported of increased serum TNF-α in schizophrenia. Theodoropoulou's study included 53 drug-naïve, first-episode schizophrenics (ca. 65%) and further 29 being ill for at least 2 years. Our schizophrenic subjects exclusively contained less than 17% drug-naïve patients. Drug-naïve

schizophrenics were found to have higher serum TNF-α levels than drug-free patients in our study, although the enhancement was not statistically significant. Maybe, proportionally less drug-naïve schizophrenics in our study was the explanation for the diversity in serum TNF-α between our study and that of Theodoropoulou et al. (2001). Furthermore, Naudin et al. (1997) also found that enhanced serum TNF-α in 18 chronic schizophrenics (illness duration: range 5 – 20 years, M = 14.1 years); besides, 15 of them (81%) were <u>medicated</u>. However, no medicated schizophrenic patient was included in our study. We found that patients being drug-free for $\leq$ 1 week had higher serum TNF-α levels than those being free of neuroleptics for at least 3 months, although the increase was not significant. Likely, medication was the cause leading to the discrepancy between our study and that of Naudin et al. (1997).

7.2.5 IL-4: increased serum IL-4 in female schizophrenics

IL-4 is the key Th2 cytokine. IL-4 is important not only for Th2-differentiation but also for its development. IL-4 stimulates the synthesis of IgG1 and IgE, but inhibits that of IgG3, IgG2a, and IgG2b in activated B-cells. In addition, IL-4 exerts inhibitory effects on the pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, and TNF-α as well as IL-2-induced NK cell activation.

Reports concerning IL-4 in schizophrenia are rare. In line with the finding regarding paranoid schizophrenics in the study of Kaminska et al. (2001), we found no alteration in serum IL-4 level in either the whole group of schizophrenic patients or only paranoid schizophrenics, if compared to normal controls. However, we found that female schizophrenics had clearly higher serum IL-4 levels than their normal female counterparts. On the contrary, male patients had reduced serum IL-4 if compared to healthy males. Nevertheless, the decrease in serum IL-4 failed to reach any statistic significance level.

Corresponding to the result of Wilke et al. (1996), our data showed that schizophrenics as a whole group had increased whole blood IL-4 in vitro production than healthy subjects, despite of their genders. However, the augmentations in IL-4 whole blood in vitro production were statistically insignificant.

7.2.6 IL-10: unchanged

IL-10 can be secreted by Th1- and Th2-cells in humans. It is found as cytokine synthesis inhibitory factor and able to inhibit diverse immune cells, especially on T, B, NK, and monocytes/macrophages. On T cells, IL-10 inhibits the synthesis of Th1 cytokines such as IFN-γ, IL-2, and TNF-β.

In line with most findings regarding whole blood IL-10 in vitro production, we found that schizophrenics as a whole group and healthy controls released similar amounts of IL-10 (Cazzullo et al., 2002; Kaminska et al., 2001; Rothermundt et al., 1998). Hitherto, two studies examined serum IL-10 in schizophrenics; one found an increase in serum IL-10 in schizophrenics (Maes et al., 2002) and the other showed a reduction in paranoid patients (Kaminska et al., 2001). Our results revealed that schizophrenics (ca. 2/3 of them paranoid schizophrenics) as a whole group had decreased serum IL-10. However, if excluding these two extreme cases (one control and one schizophrenic), schizophrenics had higher serum IL-10 levels than normal subjects, although the increase did not reach any statistic significance level.

7.2.7 IL-6: possibly increased serum IL-6 in a schizophrenic subgroup

IL-6 is released by many cell-types including Th2-cells. But the major producing cells are monocytes/macrophages, fibroblasts, and endothelial cells. IL-6 is involved in various immune functions such as B- and cytotoxic T-cell differentiation, acute-phase reactions, and hematopoiesis. In addition, IL-6 exerts distinct effects on the CNS including activating the hypothalamic-pituitary-adrenal axis.

In accordance with the majority of reports concerning serum IL-6, our schizophrenics as a whole group had also higher serum IL-6 levels than controls if excluding extreme cases (Zhang et al., 2002; Kaminska et al., 2001; Maes et al., 2000; Lin et al., 1998; Naudin et al., 1997; Maes et al., 1996; Naudin et al., 1996; Xu et al., 1994; Maes et al., 1994; Ganguli et al., 1994; Shintani et al., 1991).

In fact, most of the studies stated above investigated only a schizophrenic subgroup with a certain clinical feature. For example, Kaminska et al. (2001) investigated only paranoid schizophrenics in their study. The studies of Zhang et al. (2002) and Maes et al. (2000) only contained treatment-resistant schizophrenics. These 2 studies of Naudin et al. (1996; 1997)

solely examined schizophrenics under neuroleptic medication and chronic patients, respectively. The study of Xu et al. (1994) merely included schizophrenics under neuroleptic medication and control subjects. The schizophrenic patients in the study of Shintani et al. (1991) were in remission. Lin et al. (1998) found that enhanced serum IL-6 was shown exclusively in treatment-resistant schizophrenics, but not in non-treatment-resistant patients. Furthermore, elevated serum IL-6 was only found in schizophrenics younger than 35 years old in the study of Maes et al. (1994). Therefore, taken together, increased serum IL-6 is supposed to be observed rather in one schizophrenic subgroup than the whole schizophrenic patients.

To date, solely Kaminska et al. (2001) reported of whole blood IL-6 in vitro production in paranoid schizophrenia; they found no alteration. However, our results revealed that schizophrenia as a whole group had highly reduced whole blood IL-6 in vitro production compared with controls. If only compared paranoid schizophrenics to healthy subjects, the reduction in whole blood IL-6 in vitro production only showed a trend to be significant.

7.2.8 IL-13: decreased in a female schizophrenic subgroup?

IL-13 is another major Th2 cytokine. The receptor of IL-13 (IL-13R) shares IL-4Rα, IL-2Rγ, and STAT6 with IL-4R. Thus, IL-13 and IL-4 have functional similarities such as inducing IgE switching in B-cells. But the principal functional difference between both cytokines lies in their effects on T cells. In contrast to IL-4, IL-13 has barely effects on T-cells because T-cells don't express IL-13R.

So far, no report regarding IL-13 in schizophrenia was published. We found that schizophrenics as a whole group had significantly reduced lymphocyte IL-13 in vitro production. However, at whole blood level, no marked diversity was found among those schizophrenics and controls whose IL-13 productions in PHA-stimulated whole blood were over the detectable limitation of ELISA. Nevertheless, there were clearly more controls than schizophrenics having undetectable whole blood assay IL-13. Besides, if both genders were analyzed separately, female schizophrenics exhibited a tendency to have higher whole blood IL-13 in vitro production than their healthy female counterparts.

7.3   *Interpretations of results regarding Th1/Th2 imbalance in schizophrenia*

In this study, Th2-shift was examined not only in the whole schizophrenic group but also in diverse schizophrenic subgroups because schizophrenia has been regarded as a very heterogeneous disease (Riley, 2004). Th1/Th2 imbalance described in the whole group of schizophrenic patients doesn't hint at mandatory alterations in all subgroups with diverse epidemiological or clinical features. The magnitudes of Th1/Th2 imbalance could vary with their characteristic features. Since distinct schizophrenic subgroups to identify in order to improve therapeutic effects is one of the goals in schizophrenia research, examining Th1/Th2 imbalance in diverse schizophrenic subgroups seems to be requisite. However, due to the **limit of space**, this report concerning statistic findings **focuses on gender subgroups**. The other important reason for focusing on gender schizophrenic subgroups was that the role of gender in the structures and functions of the CNS has been increasingly emphasized (Halbreich and Kahn, 2003; Hafner, 2003; Zubieta et al., 1999). Therefore, the test results for diverse schizophrenic clinical subgroups were only briefly presented. Nevertheless, they were also discussed in the following sections.

7.3.1 Serum Th2-shift found in most schizophrenic subgroups

Clear reduction in both serum IFN-γ/IL-4 and IFN-γ/IL-10 was observed in many schizophrenic subgroups as patients were hospitalized. Th2-shift in serum could represent only one of many common dysfunctions that led to schizophrenic symptoms or deterioration of symptoms in subjects with schizophrenia. Schizophrenia is regarded as a heterogeneous disorder with diversity in symptoms, course, prognosis, and probably etiology . Th2-shift per se could indicate a heterogeneous abnormality because either reduction in IFN-γ or elevation in IL-4, IL-10 or both could, but not necessarily, give rise to Th2-shift.

A few schizophrenic subgroups without serum Th2-shift

Although schizophrenics as a whole group had clearly reduced serum IFN-γ/IL-4 and IFN-γ/IL-10 ratio (Th2-shift**s**), there were also some schizophrenic subgroups in whom no noticeable serum Th2-shift (either reduced IFN-γ/IL-4 or IFN-γ/IL-10) was observed. Those subgroups include one subgroup of male patients, schizophrenics pre-dominated with positive symptoms, schizophrenic patients being drug-free for at least 3 months, and late-onset patients. However, if examining the means, standard deviations, and case numbers of those schizophrenic subgroups stated above with care, it might be more proper to claim that (1)

schizophrenics pre-dominated with positive symptoms did not have clear serum Th2-shifts and (2) one subgroup of male schizophrenics had even increased serum Th1/Th2 ratios.

<u>Schizophrenic patients dominated with positive symptoms</u>

The reason why schizophrenic patients dominated with positive symptoms had no Th2-shift is unknown. So far, no direct evidence in this regard was reported. Depressive or psychotic symptoms are well-known side effects of IFN therapy (Dobmeier et al., 2000). One decade ago, Inglot et al. demonstrated that the schizophrenic patients with high IFN response had dominant positive symptoms (Inglot et al., 1994). Nevertheless, in the patients with low IFN response dominated rather the negative symptoms. Paranoid schizophrenia was found to have significantly increased serum IL-6 and IFN-γ, but reduced IL-2, IL-4, and IFN-γ in PHA-stimulated or Newcastle virus-induced in vitro production (Kaminska et al., 2001). Accordingly, Th1-shift, Instead of serum Th2-shift, is likely observed in one subgroup of paranoid schizophrenia since increased serum IFN-γ was found in paranoid schizophrenia (Kaminska et al., 2001). Positive symptoms might dominate paranoid schizophrenia in the earlier period of hospitalization, but both are not identical (Loza et al., 2003). That subjects with schizophrenia dominated with positive symptoms had no clear Th2-shift was not the result of any endocrinological parameters measured in this study. Schizophrenic patients dominated and those not dominated with positive symptoms had comparable cortisol, prolactin, estradiol, testosterone, and SHBG levels. Schizophrenic patients not dominated with positive symptoms had, however, noticeably reduced serum IFN-γ/IL-4 and IFN-γ/IL-10, but not patients dominated with positive symptoms if compared with healthy subjects. Both schizophrenic subgroups had significantly lower testosterone levels than normal subjects. Schizophrenics dominated with positive symptoms had even lower testosterone than their schizophrenic counterparts not dominated with positive symptoms. Lower testosterone levels are supposed to favor Th2-shift since testosterone was found to inhibit Th2 cytokines (Huber et al., 1999). In contrast to schizophrenic patients not dominated with positive symptoms, those patients dominated with positive symptoms had no Th2-shift, despite of having lower testosterone levels. Schizophrenics not dominated with positive symptoms had extremely higher prolactin levels than controls, but not patients dominated with positive symptoms. Prolactin was found to shift the development of T-helper subpopulations towards Th1 (Matalka, 2003b). Schizophrenic patients dominated with positive symptoms had no Th2-shfit, despite of having lower prolactin levels than their schizophrenic counterparts not dominated with positive symptoms. Even prolactin exerts its effects on IFN-γ not monotonically as Matera and Mori (2000) reported, the outcomes regarding Th1/Th2

imbalance in schizophrenic subgroups dominated and not dominated with positive symptoms remained similar after considering the influences of prolactin.

In addition, there were proportionally more females in the subgroup dominated with positive symptoms than control subjects. The probability for females to have Th2-shift is higher (Giron-Gonzalez et al., 2000). So "gender" must be not the cause of lacking Th2-shift in schizophrenics dominated with positive symptoms. Th1/Th2 imbalance could be not involved in the occurrence of positive symptoms.

One subgroup of male schizophrenic patients

Further dividing the male patient subgroup resulted in one subgroup having significantly reduced serum IFN-γ/IL-4 and IFN-γ/IL-10 ratio and another subgroup having enhanced both Th1/Th2 ratios. However, none of the epidemiological and endocrinological measures obtained in this study was able to clearly distinguish both subgroups of male schizophrenic patients from each other. The subgroup of male patients having enhanced Th1/Th2 ratios was more heterogeneous than the other one with Th2-shifts regarding their Th1/Th2 ratios. There could exist one subgroup of male schizophrenics having a clear Th1-shift. Typical Th1 cytokines such as IFN-γ and IL-2 were ever found to be enhanced in schizophrenia or diverse schizophrenic subgroups in some studies (Zhang et al., 2002; Ebrinc et al., 2002; Kaminska et al., 2001; Kim et al., 2000; Hornberg et al., 1995). Among them, only Kim et al. (2000) investigated male schizophrenic patients. On the contrary, the major Th2 cytokines such as IL-4 and IL-10 were decreased in PHA-stimulated whole blood and serum, respectively (Kaminska et al., 2001). Whether or not one subgroup of male schizophrenics has Th1-shift requires further examination because the results from comparing both major cytokines of Th1/Th2 systems separately could be different from those investigating the ratios between both key cytokines.

Clear, but heterogeneous serum Th2-shift in female schizophrenia

In addition, although the female schizophrenic patients as a whole group had markedly reduced serum IFN-γ/IL-4 and IFN-γ/IL-10, they could be further divided into 2 very heterogeneous subgroups. The discrepancy between these two subgroups of female patients was even greater than that between the whole female schizophrenic group and healthy women. Similar to those two subgroups of male schizophrenics, no variable assessed in this study enabled a clear differentiation between both subgroups of female schizophrenic patients. Th2-shift in this study, defined as significantly reduced ratio between two major Th1/Th2 cytokines, could be raised by either (1) only markedly reduced serum IFN-γ or (2)

solely significantly enhanced IL-4, IL-10 or (3) both. The causes of Th2-shifts in female subjects with schizophrenia might contain at least 2 of the 3 possibilities stated above. Thus, 2 very heterogeneous subgroups could be gained within the female schizophrenic patients. In order to detect whether Th2-shift in female schizophrenic patients was caused by different abnormalities, multiple regression was conducted. Multiple regression analysis is used to examine the inter-correlations between a criterion and various predictors. The results regarding the causes of Th2-shift in female people with schizophrenia were discussed in a latter section.

More reduction in IFN-γ/IL-10 than in IFN-γ/IL-4

Moreover, the magnitudes of Th2-shifts were different in various subgroups as well as in distinct Th1/Th2 indicators (IFN-γ/IL-4, IFN-γ/IL-10). Generally, the reduction in serum IFN-γ/IL-10 was greater than that in serum IFN-γ/IL-4 ratio in most schizophrenic subgroups. The reason why the reduction in serum IFN-γ/IL-10 was normally more severe than that in serum IFN-γ/IL-4 could be that there are more cell types producing IL-10 than those producing IL-4 (Yssel et al., 1993; Yssel et al., 1992). The chance to enforce the positive feedback for IL-10 production was, hence, also increased. These findings further suggest that deficits in schizophrenia or in at least one subgroup of schizophrenics could be not limited within the Th1/Th2 systems. Schizophrenia has been suggested as a disorder with multiple abnormalities (Miyamoto et al., 2003). The other IL-10 producing cells such as Th0 or Th3 or macrophages/monocytes could have been involved in Th1/Th2-imbalance in at least one subgroup of schizophrenia. The exceptions were schizophrenics having positive family psychiatric history, patients with more severe symptoms at admission (Clinical Global Impressions **CGI** score ≥ 6), at discharge (CGI score ≥ 5) as well as those patients with barely changed symptoms after 8-week-treatment (mean CGI score difference between admission and discharge = .52, SD = .60).

Exception 1: schizophrenic patients having positive family psychiatric history

Schizophrenic patients with positive family psychiatric history had not only less significantly reduced serum IFN-γ/IL-10 but also IFN-γ/IL-4 than their schizophrenic counterparts with negative family history. The explanation might be that there were proportionally more women in the schizophrenic subgroup with negative family history than in the one with positive family history. In contrast to male subjects, the Th2-system usually dominates over the Th1 in females (Giron-Gonzalez et al., 2000).

Exception 2: schizophrenic patients having severe symptoms or non-responders

The outcomes from diverse CGI-variables showed that around 2/3 of the schizophrenics having severe symptoms at admission and discharge were males. More than 70% of the schizophrenic patients who did not respond to antipsychotic treatment were men.

The schizophrenic subgroups having higher CGI scores at admission and at discharge as well as the subgroup having less improvement in symptom severity (smaller CGI difference) contained proportionally more males if compared to the corresponding schizophrenic subgroup with the opposite clinical feature. The schizophrenic subgroup having smaller CGI-difference had particularly more men than the subgroup having greater CGI-difference between admission and discharge. Compared to females, males usually tend to have a shift towards Th1 (Giron-Gonzalez et al., 2000). Thus, those subgroups having more severe symptoms and the small CGI-difference schizophrenic subgroup had less pronounced reduction in both serum IFN-γ/IL-4 and IFN-γ/IL-10. Similar to the finding of Maes et al. (Maes et al., 2002), we found that patients having small CGI-difference between admission and discharge (non-responders) had relatively enhanced serum IL-10 (reduced serum IFN-γ/IL-10 ratios) if compared to healthy subjects.

The findings stated above could indicate that the sources of Th2-shift in the schizophrenic patients having severe symptoms (CGI scores ≥ 5) could be rather principally limited within the Th1/Th2-systems because they had comparable reductions in serum IFN-γ/IL-4 and IFN-γ/IL-10. The sources of Th2-shift in those schizophrenic subgroups mentioned above could be originated from the common producing cells of IL-4 and IL-10 in serum – Th2 and/or mast cells. Hence, although IL-10 is produced by much more cell-types in serum than IL-4 (Yssel et al., 1993; Yssel et al., 1992), the magnitudes of relative elevation in both Th2 cytokines were similar. In contrast, the low CGI patients or neuroleptic responders (higher CGI difference) likely had further deficits in other IL-10 producing systems such as Th0, Th3, macrophages/monocytes, or others. Therefore, the extent of the relative increase in serum IFN-γ/IL-10 was higher than that in IFN-γ/IL-4. Further deficiency in other IL-10-producing systems could lead to even more overproduction in IL-10 relative to IFN-γ. As Maes et al. (2002) suggested, schizophrenia may be accompanied by an activation of the monocytic and Th2 system as well as various alterations in the Th1 lymphocyte subpopulation. Macrophage-T-lymphocyte was once postulated to explicate how the schizophrenic symptoms occur (Smith, 1992). According to the macrophage-T-lymphocyte theory, chronic macrophage

154

activation with subsequent failure of activated macrophages to properly control T-lymphocyte secretion of IL-2 and IL-2R is proposed as the basic biological mechanism of schizophrenia.

7.3.2 Th2-shift = likely characteristic for schizophrenia

Schizophrenia-specific: reduced both serum Th1/Th2 ratios and whole blood assay IFN-γ/IL-10
Markedly reduced serum IFN-γ/IL-4, IFN-γ/IL-10, and whole blood assay IFN-γ/IL-10 ratio were possibly schizophrenia-specific since they were not observed in psychiatric patients having schizophrenia-related disorders. Schizophrenics might have either pro-Th2 or contra-Th1 or both factors, whereas patients having schizophrenia-related disorders possibly had either contra-IL-4 or pro-IFN-γ or both factors in serum and PHA-stimulated whole blood. So, people with schizophrenia had enhanced lymphocyte Th1/Th2 ratios, but significantly reduced serum und whole blood assay Th1/Th2 ratios. On the contrary, patients with schizophrenia-related disorders had reduced lymphocyte, whole blood assay, and serum Th1/Th2 ratios. However, the extents of reduction in whole blood assay and serum Th1/Th2 ratios were less evident than those in PHA-stimulated lymphocytes. Those pro- or contra-Th1 or Th2 factors could be endocrinological parameters such as prolactin, cortisol, testosterone, estradiol, and SHBG since they were reported to have impacts on Th1/Th2 cytokines (Protonotariou et al., 2004; Dimitrov et al., 2004; Elenkov, 2004; Matalka, 2003b; Xie et al., 2002; Elenkov and Chrousos, 2002; Miyaura and Iwata, 2002; Angele et al., 2001; Giltay et al., 2000; Franchimont et al., 1998). Nevertheless, both patient groups were relatively comparable in terms of those endocrinological variables mentioned above. The differences between both patient groups could be, hence, either raised by other unknown factors or disease process.

At lymphocyte levels, schizophrenic patients had significantly reduced IL-4 and IL-13, while patients having schizophrenia-related disorders had remarkably decreased IL-10. However, both patient groups had noticeably reduced IFN-γ if compared with healthy subjects. It could hint at that altered lymphocyte IL-4 and IL-13 are rather characteristic for schizophrenia, but changed IL-10 is more possibly found in schizophrenia-related disorders. Reduced IFN-γ could be just a common deficit of patients with schizophrenia and schizophrenia-related diseases. That is, the disparity in Th1/Th2 imbalance between patients with schizophrenia and those having schizophrenia-related disorders might be caused by the abnormalities in the Th2-sytem, but not in the Th1-subset.

Th2-shift defined as reduced IFN-γ/IL-4 ratio in PHA-stimulated whole blood was lately described in insomniac men (Sakami et al., 2002). Our study showed no significant reduction in IFN-γ/IL-4 ratio obtained from PHA-stimulated whole blood in the whole schizophrenic group. Among diverse schizophrenic subgroups, no subgroup but those having severe symptoms either at admission or discharge (CGI score ≥5) as well as those non-responders (less symptom improvement after 8-week-treatment) showed Th2-shift in PHA-stimulated whole blood. However, those 3 CGI schizophrenic subgroups in our study showed additionally Th1/Th2 aberrations if compared to insomniac men. They include significantly reduced IFN-γ/IL-4 and IFN-γ/IL-10 in serum as well as IFN-γ/IL-10 in PHA-stimulated whole blood. Besides, in our study, significantly reduced IFN-γ/IL-4 in stimulated whole blood in those CGI schizophrenic subgroups resulted from simultaneously considering the impacts of different hormones, whereas that of Sakami et al. (2002) did not. Lately, chronic alcohol use was reported to shift the development of T-helper lymphocytes towards Th2, using IgE and IFN-γ as Th2 and Th1 indicator (Starkenburg et al., 2001; Dominguez-Santalla et al., 2001). In our study, schizophrenic patients who had alcohol abuse or addiction were excluded. Thus, Th2-shift in schizophrenia found in this study was not a consequence of chronic alcohol consumption. Th2-shift was also found in phorbol myristate acetate (PMA)- and ionomycin-stimulated whole blood in autistic children (Gupta et al., 1998). The study of Gupta et al. (1998) and ours differ from each other in many aspects such as mitogen(s), mitogen concentration, incubation duration, and many other procedures. Most important of all, we calculated the ratio between IFN-γ and IL-4 to determine the balance between Th1 and Th2, but not Gupta et al. (1998). As shown in our results, the outcomes from examining IFN-γ and IL-4 separately and those from simultaneously considering both IFN-γ and IL-4 as a ratio could be different. Moreover, Gupta et al. (1998) did not demonstrate significantly reduced serum IFN-γ/IL-4 or IFN-γ/IL-10 or decreased IFN-γ/IL-10 in PHA-stimulated whole blood in autistic children. Reduction in whole blood assay IFN-γ/IL-4 doesn't mean a necessary decrease in serum IFN-γ/IL-4 because different endocrinological and immunological components are involved in Th1/Th2 balancing in serum and PHA-stimulated whole blood. So, significantly reduced IFN-γ/IL-4, IFN-γ/IL-10 in serum und IFN-γ/IL-10 in whole blood after 46-hour-PHA-stimulation could be rather specific for schizophrenia.

Lymphocyte IFN-γ/IL-10: no relationship with schizophrenia

Lymphocyte IFN-γ/IL-10 appeared not to be associated with any schizophrenic subgroup due to none of the schizophrenic subgroups showing any alteration in this regard. Altered IFN-γ/IL-10 in stimulated leukocytes could still occur in schizophrenia. Our study won't be able to offer any evidence in this respect. However, our results implicate that changes in IFN-γ/IL-10 obtained from serum und stimulated whole blood in schizophrenics could rather an interactive effect of diverse components at least between the immune and endocrine system than just a simple deficit at lymphocyte level. In fact, a few years ago a bio-pathogenetic hypothesis in schizophrenia was postulated (Altamura et al., 1999). According to the bio-pathogenetic hypothesis, dysfunctions in both the hypothalamic-pituitary-adrenal axis (HPA) and the inflammatory response system, especially cytokines, in schizophrenia were claimed. Endocrinological parameters such as prolactin, cortisol, estradiol, testosterone, and the sex hormone binding globuline (SHBG) could have been involved in the pathogenesis of schizophrenia. Those hormones were also measured in this study. The effects of those endocrinological parameters were discussed in a latter section "7.4. Possible causes of Th2-shift in schizophrenia".

Reduced lymphocyte IFN-γ/IL-4: shared by patients with schizophrenia and related disorders

Older schizophrenic subgroups such as chronic and late-onset schizophrenia were found to have significantly reduced lymphocyte IFN-γ/IL-4, especially chronic schizophrenics. Patients with schizophrenia-related diseases also showed a tendency to have reduced lymphocyte IFN-γ/IL-4 ratio. It might indicate that within the very heterogeneous patient group with schizophrenia-related disorders likely existed one subgroup sharing the deficit, reduced lymphocyte IFN-γ/IL-4, with chronic and late-onset schizophrenia. Some studies investigated Th1/Th2 balance in peripheral blood mononuclear cells and found that the magnitude of Th2-shift seems to correlate positively with age (Protonotariou et al., 2004; Gasparoni et al., 2003; Sandmand et al., 2002). Age could have contributed to lymphocyte Th2-shift in chronic and late-onset schizophrenia. However, age is not the only cause of Th2-shift in chronic and late-onset schizophrenia since lymphocyte Th2-shift remained after the effect of age was controlled. Th2-shift in PHA-stimulated lymphocytes in chronic and late-onset schizophrenia was not the result of age-induced alterations in various endocrinological parameters, either. The isolated lymphocytes examined in this study were not exposed to any endocrinological parameter as they were stimulated to secrete cytokines. Furthermore, in addition to reduced lymphocyte IFN-γ/IL-4, noticeably decreased IFN-γ/IL-4 and IFN-γ/IL-10 in serum und in PHA-stimulated whole blood were also observed in chronic schizophrenia, but not in schizophrenia-related disorders.

7.3.3   Gender differences in Th1/Th2 imbalance by schizophrenics

More marked serum Th2-shift in schizophrenic females

Female schizophrenics as a whole group had clear serum Th2-shifts, whereas rather only one subgroup than the whole male schizophrenics had Th2-shifts if both serum IFN-γ/IL-4 and IFN-γ/IL-10 taken into account. As a whole group, male schizophrenics had merely noticeably reduced serum IFN-γ/IL-10 ratios. In addition, there could be one subgroup of male schizophrenic patients having enhanced serum IFN-γ/IL-4 and IFN-γ/IL-10 ratios, but not female patients. However, those epidemiological and clinical data obtained in this study failed to characterize this subgroup of male patients. Furthermore, female patients had remarkably lower serum IFN-γ, but significantly higher serum IL-4 levels than their healthy female counterparts. In contrast, the whole male schizophrenic patients and normal males did not have obviously distinguishable serum IFN-γ and IL-4 levels. Those findings from multi-variance-analysis presented above appeared to suggest that the paradigm of Th1/Th2 antagonism (Wang et al., 2002) was observed in female schizophrenic patients, but not in male patients. Thus, significantly reduced serum IFN-γ in female schizophrenics seemed to be caused by noticeably enhanced IL-4. It's noteworthy that both significantly decreased IFN-γ und increased IL-4 in female schizophrenic patients were the results from comparing to the IFN-γ and IL-4 of healthy females <u>separately</u>. They are not the results from comparing the ratios between IFN-γ and IL-4. Thus, the outcomes from multi-variance analysis actually indicated that the paradigm of Th1/Th2 antagonism is likely to find in at least one subgroup of female patients, but not necessarily in the whole female schizophrenic group.

On the contrary, no significantly elevated serum IL-4 and no reduced IFN-γ in male schizophrenic patients don't obligatorily hint at "no Th1/Th2 antagonism in male patients". The reason is that <u>multi-variance-analysis</u> serves to <u>examine the diversities in certain variables between/among distinct groups</u>, but not to <u>detect the relationships among various parameters within the same group</u> as in the case of <u>multi-regression</u>. The differences in serum IFN-γ/IL-4 and IFN-γ/IL-10 between male and female schizophrenic patients could be raised by testosterone and estradiol because females usually have higher estradiol and lower testosterone levels than males. Testosterone was found to be more inhibitory to Th2 cytokines, whereas estradiol rather promoted them (Giltay et al., 2000; Huber et al., 1999). Males were, thus, found to have higher Th1/Th2 ratios, but females were shown to have lower

ones (Giron-Gonzalez et al., 2000). Therefore, Th2-shifts including decreased IFN-γ/IL-4 and IFN-γ/IL-10 were more explicit in female patients than in male schizophrenics.

Different influencing factors for serum Th1/Th2 imbalance

The results from multiple-regression showed which of IFN-γ, IL-2, TNF-α, IL-4, IL-10, IL-6, cortisol, prolactin, estradiol, testosterone, SHBG, and age could significantly predict the values of Th1/Th2 ratio (IFN-γ/IL-4 or IFN-γ/IL-10) within the same group of subjects and which could not. That is, which variables have more and which have less influence on the Th1/Th2 balance.

Multiple regression of serum Th1/Th2 ratios demonstrated that male schizophrenics had highly enhanced influences from IL-4, IL-10, and IFN-γ. In contrast, female patients had markedly reduced impacts from IL-4, IL-10, and IFN-γ on their Th1/Th2 ratios if compared to their healthy counterparts of the same sex. Additional essential influencing factors in male schizophrenics included SHBG and IL-10 for IFN-γ/IL-4 as well as age and prolactin for IFN-γ/IL-10. However, those important contributors for IFN-γ/IL-10 in female patients were estradiol, SHBG, IL-4, IL-2, IL-6, prolactin, and cortisol, while no single remarkable predictor was found for IFN-γ/IL-4.

Instead of both testosterone and estradiol, SHBG was important to predict IFN-γ/IL-4 in male schizophrenic patients. So far, no direct evidence showed a connection between SHBG and IFN-γ/IL-4. However, SHBG binds to both testosterone and estradiol (Anderson, 1974). Even if SHBG doesn't have direct influence on Th1/Th2 ratios, it's still possible for SHBG to exert its impacts indirectly through both sex hormones on Th1/Th2 balance. Our finding regarding SHBG implicated that it was rather the balance between estradiol and testosterone essential for generating IFN-γ/IL-4 in male schizophrenic patients, but not testosterone and estradiol separately. If combined the findings from both serum IFN-γ/IL-4 and IFN-γ/IL-10 in male schizophrenic patients together, they seem to indicate additional deficits in the hypothalamic-pituitary-adrenal (HPA) axis and gonadal functions. Deficiency in the pituitary-gonadal function was already described over 2 decades ago in male schizophrenic patients (Brambilla, 1980). In female schizophrenic patients, there were even more influencing factors contributed to the variance of IFN-γ/IL-10 than those in male schizophrenic patients. Except testosterone and TNF-α, the other immunological and endocrinological variables measured in this study were significantly correlated to IFN-γ/IL-10. For the variance of IFN-γ/IL-4, no single factor

assessed in our study alone was able to predict the measure of IFN-γ/IL-4 significantly. However, all the predictors together enabled a reliable prediction of IFN-γ/IL-4. The reason was that among those predictors IFN-γ, IL-2, TNF-α, IL-4, IL-10, IL-6, prolactin, cortisol, testosterone, estradiol, SHBG, and age existed very tangled inter-correlations in female subjects with schizophrenia. That is, serum Th1/Th2 imbalance in female patients was more a collective effect of many factors than of a few important factors. Another alternative explanation for the wide-spreading influencing factors for Th2-shift in female subjects with schizophrenia could be that Th2-shift in female schizophrenics was not homogeneous. Multiple regression concerning Th1/Th2 ratios in female schizophrenic patients also showed no or less evident correlations between IFN-γ, IL-4, IL-10, and IFN-γ/IL-4, IFN-γ/IL-10. These outcomes actually indicated that within the whole female schizophrenic group likely existed at least 3 different subgroups; one subgroup has only highly enhanced IL-4, a second one has only markedly reduced IFN-γ, and a third one had increased IL-4, IL-10 as well as decreased IFN-γ. So, if all 3 subgroups of female schizophrenic patients mixed together, the associations between the major Th1/Th2 cytokines and Th1/Th2 ratios become less clear. It could be the reason why there were so many important influencing factors or no clearly dominant influencing factor in female schizophrenics. Different influencing factors could have contributed to the variance of Th1/Th2 ratio in distinct subgroups. We also detected 2 very heterogeneous subgroups within the whole group of female patients. That schizophrenia is etiologically heterogeneous was already suggested long time ago (Kinney and Matthysse, 1978).

More Th1/Th2 cytokines less sufficiently explained in male schizophrenic patients

Multiple regression analysis at single cytokine level revealed that in male schizophrenics, (1) less than 40% of the IFN-γ, IL-12, TNF-α, and IL-4 variance could be explained and (2) those of IL-2, IL-6, and IL-10 were not explicable through the other parameters assessed in this study. Nevertheless, in female patients, the variance of IL-12 could not be explicated sufficiently, while those of TNF-α, IL-6, and IL-10 were unexplainable through the remaining variables measured. The variables including IFN-γ, IL-2, TNF-α, IL-4, IL-10, IL-6, prolactin, cortisol, estradiol, testosterone, SHBG, and age were only able to explain about 1/3 of the variance of the major Th1/Th2 cytokines including IFN-γ, IL-4, and IL-10 in male subjects with schizophrenia.

Taken together, similar to the findings of Pellegrini et al. (Pellegrini et al., 2003), our results lead us to the conclusion that the physiological network of Th1/Th2 cytokines regulating T-

helper polarization may be different in men and women. The diversities in this regard is schizophrenia appeared to be likely due to dysfunction in other Th1/Th2 related systems even greater and more complicated. Apart from testosterone and estradiol, there are many other factors believed to be able to induce gender difference in Th1/Th2 balance. One such example is progesterone; progesterone receptor-A/-B ratios were higher in the brains of males than in those of females at all ages (Camacho-Arroyo et al., 2003). Gender and endogenous sex steroids are, thus, thought to be responsible for neuroendocrine-immunological sexual dimorphism (Chisari et al., 2000).

7.3.4   Sources of Th2-shift in schizophrenia: beyond lymphocyte and whole blood

In our study, Th1/Th2 cytokines were examined at three different levels - serum, PHA-stimulated whole blood and lymphocytes. The findings from <u>lymphocyte</u> and <u>whole blood</u> were resulted from 46-hour stimulation with PHA; comparisons of the outcomes from both analysis materials offered a possibility to examine whether Th1/Th2 imbalance in schizophrenia was an aberration within the lymphocyte system or rather a deficit involved additional factors such as the endocrine system. Comparing the findings from <u>serum</u> to those from PHA-stimulated <u>whole blood</u> could further confirm whether the sources of Th2-shift in schizophrenia were limited within the whole blood system or more. The serum Th1/Th2 ratios likely represented an in-vivo equilibrium between both subsets of T-helper cells and others. However, the Th1/Th2 ratios in PHA-stimulated whole blood were rather the in vitro approximations of the Th1/Th2 balancing in vivo after 46-hour PHA-stimulation. The crucial diversities between the in-vitro whole blood and the in-vivo serum system were manifold. (1) First, the endocrinological (and other) system(s), due to excluding the involvement of the CNS and others, did not react dynamically, but likely remained constant as cytokines were produced in the whole blood system. (2) Secondly, different mitogens were shown to have distinct effects on lymphocytes (Sofuni and Yoshida, 1992; Stiernberg et al., 1987; Freund and Blair, 1982). The unspecific effects of mitogen such as PHA and those of natural stimuli in vivo might be different on diverse cell types or distinct cytokines. These two major reasons may have led to the discrepancies between the findings from serum and whole blood assay.

In the whole schizophrenic group and most schizophrenic subgroups, <u>lymphocyte</u> Th1/Th2 ratios including IFN-γ/IL-4, IFN-γ/IL-10, and IFN-γ/IL-13 were averagely enhanced if compared to normal subjects. The data from PHA-stimulated <u>whole blood</u> revealed,

nevertheless, opposite outcomes. Those findings indicated that the sources of Th2-shift in schizophrenia probably lied outside of lymphocytes. Th2-shift in schizophrenics is still likely to observe at leukocyte level. However, this study could not offer any evidence in this regard since only lymphocytes were analyzed. Comparisons between the results from <u>serum</u> and <u>whole blood</u> assay data among various schizophrenic subgroups revealed that Th2-shifts were more pronounced in serum than in whole blood assay. In addition to those reasons stated in the previous paragraph, they also suggested that there were more "pro-Th2" and/or "contra-Th1" factors in the in-vivo system than in the in-vitro whole blood system. That is, factors outside of the lymphocyte and whole blood system had noticeably contributed to the serum Th2-shift in schizophrenia. Schizophrenia has been implicated as much more likely to be a heterogeneous disorder resulting from interactions between multiple factors (Kirch, 1993). Our study also confirmed the statement of Kirch (1993). Some of those pro- and contra-Th1 or Th2 factors could be parameters from the endocrine system such as diverse hormones. The impacts of distinct hormones on Th1/Th2 balance were discussed in the section "7.4. Possible causes of Th2-shifts in schizophrenia".

7.3.5 Other essential factors than those measured involved in Th1/Th2 balancing in schizophrenic patients

Comparisons between the serum and whole blood assay data resulted from multiple regression in the <u>whole schizophrenic and control</u> group further implicated that there might be some other factors involving in Th1/Th2 balancing in stimulated whole blood. Those factors were, nevertheless, not examined in this study. Thus, the explainable portions of whole blood assay Th1/Th2 ratios in schizophrenics through the predictors included were lower than those in serum. However, it's not that case in normal subjects.

The data from both <u>male subgroups</u> also indicated the possible involvement of other crucial factors than those assessed in this study in the IFN-γ/IL-4 balancing in stimulated whole blood in schizophrenic men. That's why only barely 40% IFN-γ/IL-4 variance in stimulated whole blood, but over 90% serum IFN-γ/IL-4 variance was explainable through predictors included in this study in male schizophrenics. Contrarily, data regarding IFN-γ/IL-10 revealed that further factors took part into the process of serum IFN-γ/IL-10 balancing in controls; however, they were not investigated in this study and likely missing or "not required" in the balancing of serum IFN-γ/IL-10 in male schizophrenics. Therefore, 73% whole blood assay

IFN-γ/IL-10 variance, nonetheless, only 56% serum IFN-γ/IL-10 variance in male controls was explicable. But in schizophrenic males, the explainable portions of serum and whole blood assay IFN-γ/IL-10 were 98% and 80%, respectively. The lymphocyte data of male subjects further confirmed the insufficiencies of those variables assessed in this study to envisage the variances of Th1/Th2 ratios in schizophrenia. So, the explicable portions of lymphocyte IFN-γ/IL-4, IFN-γ/IL-10, and IFN-γ/IL-13 in schizophrenic men were 46%, 37%, and 45%, whereas those in male controls were 96%, 61%, and 81%, correspondingly.

Nevertheless, the comparisons between both <u>female subgroups</u> showed that there could be further factors which were involved in serum IFN-γ/IL-4 balancing in healthy females, but not "required/present" in IFN-γ/IL-4 balancing in female schizophrenics. Hence, the explainable parts of serum and whole blood assay IFN-γ/IL-4 in female controls were 76% and 67%, while those in female patients were 88% and 86%, correspondingly. Those factors could be not as many and important as those in male patients; so, the diversities in the explainable portions of Th1/Th2 ratios were smaller between both female groups. Nevertheless, those parameters included in the multiple regression model enabled a sufficient and reliable prediction of serum IFN-γ/IL-10 variance in female patients.

The possible candidates for other influencing factors of Th1/Th2 imbalance in schizophrenia than those hormones measured in this study could be catecholamines, progesterone, melatonin, dihydroepiandrostene sulphate (DHEAS), and human growth hormone (Yamashita et al., 2000; Petrovsky and Harrison, 1998). Catecholamines were lately found to inhibit Th1 cytokines such as IL-2, IL-12, and TNF-α, but to enhance Th2 cytokines like IL-4, IL-10, and IL-6 (Qiu et al., 2003). Progesterone was shown to be able to suppress Th1, but to enhance Th2 development directly (Miyaura and Iwata, 2002). Melatonin, the main hormone of the pineal gland, was found to be abnormal in schizophrenia (Jiang and Wang, 1998) and to have impacts on Th1/Th2 cell mediated immune responses (Raghavendra et al., 2001; Kuhlwein and Irwin, 2001).

7.3.6 Th2-shift = function of disease process, age-related changes, anti-psychotic medication, distinct hormones etc.

Disease process

Th2-shift appeared to be rather a result of disease process since drug-naïve schizophrenics also showed reduced serum and whole blood assay Th1/Th2 ratios, although drug-naïve

163

patients and healthy subjects were relatively comparable in terms of their ages, cortisol, prolactin, estradiol, testosterone, and SHBG levels. Additionally, many schizophrenic subgroups demonstrated reduced serum and whole blood assay IFN-γ/IL-4 and IFN-γ/IL-10 ratios after the effects of age and distinct hormones were controlled. Disease process per se could have contributed to Th2-shift in schizophrenia.

Neuroleptic treatments

Neuroleptic treatment could alter IFN-γ/IL-4 ratio since (1) Song et al. (2000) showed a bi-modal effect of clozapine on whole blood IFN-γ in vitro production and (2) Cazzullo et al. (2002) reported of time-dependent actions of risperidone on IFN-γ and IL-4 in vitro secretion. Our data implicated that anti-psychotics appeared to suppress both serum IFN-γ and IL-4 in schizophrenics. The reason was that pre-medicated schizophrenics had lower serum IFN-γ, IL-4, and serum Th1/Th2 ratios than drug-naïve patients. However, the repressive effects of neuroleptics on serum IL-4 seem to be weaker than those on IFN-γ because the reductions in both serum Th1/Th2 ratios were more evident in schizophrenics with pre-medication than in drug-naïve patients. That is, neuroleptic medication could have contributed to serum Th2-shift in schizophrenia. Nevertheless, neuroleptics appeared to have promoting effects on whole blood assay IFN-γ, but suppressive effects on whole blood assay IL-4 and IL-10 in favor of Th1-shift. So, pre-medicated patients had higher whole blood assay IFN-γ and Th1/Th2 ratios, however, lower IL-4 and IL-10 productions than drug-naïve schizophrenics. Diverse effects of neuroleptics on the same cytokine at serum and whole blood level were described; for example, clozapine was found to reduce IL-10 in vitro production (Song et al., 2000), however, to increase serum IL-10 (Maes et al., 2002). The effects of (some) neuroleptics on Th1/Th2 balance might be mediated primarily by prolactin (Meaney et al., 2004) and indirectly by gonadal hormones (Smith, 2003). The effects of these hormones on Th1/Th2 ratios were discussed in " 7.4. Possible causes of Th2-shift in schizophrenia".

Nevertheless, serum Th2-shifts in schizophrenia were not only the effects of neuroleptic treatment since pre-medicated schizophrenics still exhibited tremendously decreased serum Th1/Th2 ratios after excluding the effects of prolactin, a crucial indicator of neuroleptic medication, and testosterone. In addition, drug-naïve patients also had reduced serum Th1/Th2 ratios.

Age-related abnormalities

Younger schizophrenic subgroups such as drug-naïve and early-onset schizophrenics exhibited increased lymphocyte IFN-γ/IL-4 (Th1-shift), while older subgroups like chronic

and late-onset patients showed decreased lymphocyte IFN-γ/IL-4 ratios (Th2-shift). An age-related shift towards Th2 in peripheral blood mononuclear cells was lately described (Sandmand et al., 2002; Karanfilov et al., 1999). The productions of the major Th2 cytokines IL-4 and IL-10 were increased with age, nevertheless, no change was found in Th1 cytokines such as IFN-γ and IL-12 (Plackett et al., 2003). The lymphocyte Th1/Th2 imbalance in schizophrenia could be partly ascribed to the age-component. However, serum Th2-shift remained clearly observable in diverse schizophrenic subgroups after the effect of age was controlled.

Hormones

Distinct hormones were shown to have impacts on Th1/Th2 balance (Dimitrov et al., 2004; Zhang et al., 2004; Skjolaas et al., 2002; Huber et al., 1999). The influences of diverse hormones on Th1/Th2 balance were elucidated in a latter section "7.4 Possible causes of Th2-shift in schizophrenia".

7.3.7 Whole blood assay Th2-shifts reflect no response towards treatment?

In addition, data from diverse CGI (Clinical Global Impressions) subgroups pointed out that there was at least one schizophrenic subgroup whose symptoms at discharge remained as severe as they were hospitalized, despite of 8-week-treatment. This subgroup (ca. 1/3 – 2/5 of the whole schizophrenic subjects) had noticeably reduced whole blood assay Th1/Th2 ratios as well as, to a less extent, decreased serum Th1/Th2 ratios. Marked shifts in both whole blood assay Th1/Th2 ratios in the schizophrenic subgroup with low CGI-difference might implicate that Th2-shift in PHA-stimulated whole blood was either stronger and/or maintained longer ($\geq$ 46 hrs) than the other subgroup having high CGI-difference. Findings from serum/whole blood assay IL-10 and IL-4 also supported both explanations. The schizophrenics with low CGI-difference had higher whole blood IL-4 and IL-10 in vitro productions, nonetheless, lower serum IL-4 and IL-10 levels than their schizophrenic counterparts with high CGI-difference. The average whole blood assay IL-4 of the schizophrenics with low CGI-difference was even higher than that of normal subjects. Another alternative explanation is that there could be further essential, however, unknown "pro-Th2" and/or "contra-Th1" factors that triggered Th2 overproductions in stimulated whole blood. But those factors did not exist and/or worked differently due to interactions with other influencing factors in serum. Those factors could be diverse endocrinological parameters such as cortisol, prolactin, testosterone, and estradiol. Those hormones were

shown to influence Th1/T2 balance (Dimitrov et al., 2004; Elenkov, 2004; Skjolaas et al., 2002; Huber et al., 1999). They were discussed in the next sections. Until now, no examination investigates the relationship between treatment resistance in schizophrenia and Th1/Th2 balance. However, serum IL-10 was found to be elevated in schizophrenic patients resistant to treatment with neuroleptics (Maes et al., 2002). In line with the finding of Maes et al. (2002), our schizophrenic patients resistant towards neuroleptic treatments also showed a relative enhancement in serum IL-10.

7.4   *Possible causes of Th2-shift in schizophrenia*

In the following sections, the results from multi-variance analysis and multiple regression analysis were combined. Multiple regression analysis was used to detect the inter-variable relationships within the same group of subjects. The inter-variable correlations between the criterion Th1/Th2 ratios (either IFN-γ/IL-4 or IFN-γ/IL-10) and the predictors including IFN-γ, IL-2, TNF-α, IL-4, IL-10, IL-6, prolactin, cortisol, testosterone, estradiol, SHBG, and age were evaluated in schizophrenia and controls separately. They serve to examine the importance of each predictor in forecasting the measure of Th1/Th2 ratio within the same group. According to the findings from multiple regression, which of those predictors mentioned above significantly predicted the criterion (IFN-γ/IL-4, IFN-γ/IL-10) could be determined for schizophrenia and controls separately. The outcomes from multi-variance analysis revealed in which of the predictors and criteria stated above differentiated schizophrenic patients significantly from their healthy counterparts. If a predictor is essential in envisaging the variance of Th1/Th2 ratio(s) within the same group and is significantly different between schizophrenics and healthy subjects, then the predictor might be the cause of the Th1/Th2 imbalance in schizophrenia.

The findings discussed here are mainly originated from the serum data because they reflect the real, complex inter-correlations among those parameters stated above in vivo. In addition, serum Th2-shift was observed in the majority of schizophrenic subgroups examined in this study, but not whole blood assay or lymphocyte Th2-shift. Secondarily, whole blood assay data are considered for the following reasons: (1) the data from whole blood assay are better approximations of settings in vivo than lymphocyte data. (2) The serum and whole blood assay data were analyzed by the same analysis method (CBA). The comparability between the data from serum and whole blood assay is, hence, higher than those between serum or whole

blood assay and lymphocyte data. (3) Whole blood assay data could offer further information regarding reactions of Th1/Th2 and other system(s) in vitro towards short-term stimulation. In contrast, sera were obtained after at least 3-day washout period and likely gave a view at a different stage of Th1/Th2 balancing from that shown in whole blood assay. **Figure 7-4** gives a glance at the key causes of Th2-shifts in schizophrenia.

7.4.1 The whole group of schizophrenic patients

Disrupted Th1/Th2 antagonism in the whole schizophrenic group

The <u>serum</u> data in both schizophrenia and controls did show a noticeable <u>Th1/Th2 antagonism</u> as described by Mosmann and Coffman (1989). That is, IFN-γ positively correlated to the IFN-γ/IL-4 and IFN-γ/IL-10 ratio, while both IL-4 and IL-10 were negatively related to IFN-γ/IL-4 and IFN-γ/IL-10, respectively. The antagonism between IFN-γ and IL-4 as well as between IFN-γ and IL-10 in PHA-stimulated <u>whole blood</u> was still clearly to observe in healthy controls. In schizophrenia, the extent of antagonism between whole blood assay IFN-γ and IL-4 seemed to be reduced after 46-hour PHA-stimulation, but not that between IFN-γ and IL-10. Data from PHA-stimulated <u>lymphocytes</u> demonstrated antagonism between IFN-γ and IL-4 or IL-10 or IL-13 in healthy subjects. However, the antagonistic relationship between lymphocyte IFN-γ and IL-10 appeared to be disrupted in the whole schizophrenic group.

Less or insufficiently or unreliably explained Th1/Th2 ratios/cytokines in schizophrenia

In general, the explainable parts of both Th1/Th2 ratios through the predictors included in this study were similar in serum and PHA-stimulated whole blood in controls. Nevertheless, the explicable portion of the IFN-γ/IL-4 variance in stimulated whole blood was exclusively about 50% of that in serum IFN-γ/IL-4 ratio in schizophrenics. The explainable part of the whole blood assay IFN-γ/IL-10 variance was only around 80% of that in serum IFN-γ/IL-10 in schizophrenic patients. In addition, the predictors assessed in this study could either not sufficiently or not reliably envisage serum cytokine levels such as IFN-γ, IL-4, IL-10, and IL-6 in schizophrenia. On the contrary, it was not that case in controls.

Summarized findings from multiple regression and multi-variance analysis

<u>Multiple regression analysis</u> from both <u>serum</u> and <u>whole blood assay</u> data demonstrated that the major causes of Th1/Th2 imbalance in schizophrenia were primarily from IFN-γ, IL-4, and IL-10. IL-6, TNF-α, and age might be additionally involved in the whole blood assay

Th1/Th2 balancing in schizophrenics. The results at single cytokine level further pointed out that cortisol, prolactin, and testosterone/SHBG could indirectly play a role in Th2-shift in schizophrenics since they were significantly related to IFN-γ, IL-4, and IL-10.
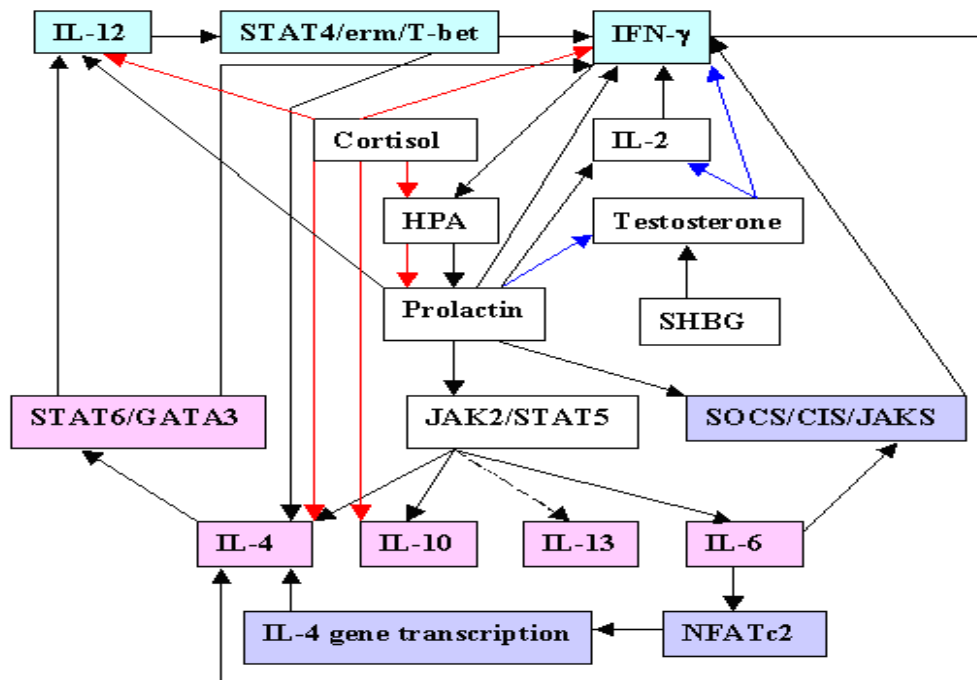
Figure 7-4: The major causes of Th1/Th2 imbalance in schizophrenia (the red arrows: specific for female schizophrenics; the blue arrows: characteristic for male schizophrenics; the black arrows: likely common for both genders).

Schizophrenics as a whole group had generally lower serum cytokine levels, Th1/Th2 ratios, SHBG, testosterone, and cortisol, however, higher prolactin levels than healthy controls. If excluding extreme values out of analysis, the whole schizophrenic group had significantly decreased serum IFN-γ, noticeably enhanced serum IL-4 and IL-6. The abnormalities described above and eventually Th2-shifts in schizophrenia could be caused by deficiencies in at least one of the following biological regulatory circuits: (1) the typical Th1/Th2 development/regulation pathways, (2) the newly described APC-IL-6/SOCS/CIS pathways (Diehl and Rincon, 2002), (3) the cytokines-HPA-prolactin-Th1/Th2 loop, (4) the cortisol-HPA-prolactin-Th1/Th2 circuit, and (5) the prolactin-SHBG-testosterone-Th1/Th2 connections. In addition, (6) CD30 was lately described as an important co-stimulator molecule being able to regulate the balance between Th1 and Th2 (Pellegrini et al., 2003).

Possible causes of Th2-shift in schizophrenia

Deficits in the IL-6/SOCS/CIS pathways

Since both key cytokines of the Th1/Th2 systems and serum IL-6 were remarkably altered in schizophrenia, they implicated the cause(s) of Th2-shifts was/were likely located at a site where both systems were involved. Newly, Diehl and Rincón (2002) suggested that APC-derived IL-6 has two faces on Th1/Th2 differentiation. On one hand, IL-6 is capable of activating Nuclear Factor of Activated T cells (NFAT), indirectly increasing IL-4 gene transcription and IL-4 production. On the other hand, APC-derived IL-6 is also able to inhibit IFN-γ releases through inducing SOCS1, SOCS3, and CIS of the SOCS/CIS family along the JAK1/STAT3 pathway. These two pathways are independent to each other. This route of the APC IL-6 to inhibit IFN-γ is not related to IL-12 or IL-4 (Diehl and Rincon, 2002). Since serum IL-6 was enhanced in the whole schizophrenic group in addition to IFN-γ and IL-4, possibly the APC IL-6 pathways to inhibit IFN-γ and to promote IL-4 were malfunctioning.

Deficiencies in the IL-12/STAT4/erm/T-bet &/or IL-4/STAT6/GATA3/c-maf pathway

Another alternative site(s) being able to cause abnormalities in both Th1/Th2 systems could be within the typical Th1/Th2 differentiation pathways because activating either pathway promotes the development of its own system, but inhibits that of the other. The classical Th1 developmental/regulatory route is through the IL-12/STAT4/erm/T-bet pathway (Agnello et al., 2003; Rao and Avni, 2000). The typical differentiation pathway for the Th2 system is via the IL-4/STAT6/GATA3/c-maf loop (Agnello et al., 2003; Rao and Avni, 2000). Particularly, T-bet and GATA-3 are major inducers of Th1 and Th2 differentiation by controlling the productions of their respective cytokines. Additionally, they are also involved in the commitment and stable maintenance of the Th1 and Th2 phenotypes (O'Garra and Arai, 2000). Therefore, maybe one or more components of these two classical pathways were defective in schizophrenia.

CD30 & Th1/Th2 balance

The CD30 antigen is a member of the TNF receptor superfamily (Gruss et al., 1994). The function of CD30 in mature peripheral T lymphocytes is unclear, but there is evidence that CD30 can act as a signal transduction molecule (Pellegrini et al., 2003). Ligation of CD30 by its ligand CD30L leads to rapid activation of the transcription factor NF-κB (linked to Bcl 2 expression) in T cells (McDonald et al., 1995). NF-κB was found to bind to IL-4 promoter in vivo upon T cell activation and is, therefore, directly involved in IL-4 transcription (Li-Weber et al., 2004). Besides, signaling through CD30 promotes the development of Th2 cells (Del Prete et al., 1995). So, CD30 is generally used as a marker for Th2 cell populations (Okumura

et al., 1999). Although the detailed working mechanisms remained unknown, CD30 likely triggered mechanisms which may regulate (1) the physiological balance between Th1 and Th2 functions by integrating Th1 and Th2 specific cytokine production including IFN-γ, IL-4, IL-10, IL-2, IL-12p40, IL-12p70, IL-5 and (2) Bcl 2 molecule expression (Pellegrini et al., 2003). Th2-shifts in schizophrenia could be raised by abnormality in CD30 since Th2-shifts in schizophrenia in this study resulted rather from over-production in IL-4 and IL-10 as well as from under-secretion of IFN-γ.

Diverse hormones and Th1/Th2 balance

In addition, the lymphocyte Th1/Th2 ratios including IFN-γ/IL-4, IFN-γ/IL-10, and IFN-γ/IL-13 were slightly enhanced in schizophrenia. However, the serum and whole blood assay IFN-γ/IL-4 and IFN-γ/IL-10 were (significantly) decreased if compared to healthy subjects. They indicated that some unknown "contra-Th1" and/or "pro-Th2" factors possibly had suppressed Th1 and/or promoted Th2 cytokines in serum and PHA-stimulated whole blood. Candidates being able to influence Th1/Th2 balance in schizophrenia might be prolactin, cortisol, and SHBG/testosterone according to the results of multiple regression and MANCOVA. The reason was that they were found to have impacts on Th1/Th2 balance and clearly distinguishable between schizophrenics and controls.

Prolactin & Th1/Th2

Highly elevated prolactin seemed to be an overwhelming aberration in schizophrenics. Enhanced prolactin levels in schizophrenic patients may be the results of anti-psychotic medication (Meaney et al., 2004). Nevertheless, prolactin abnormality was already described in un-medicated, drug-naive schizophrenics about one decade ago (Abel et al., 1996; Van Cauter et al., 1991). Hyperprolactin in schizophrenia is not an obligatory consequence of neuroleptic treatments. Apart from neuroleptic treatments, there are some other factors such as stress or cytokines (e.g. IFN-γ and TNF-α) that could have impacts on prolactin release. IFN-γ and TNF-α have the potential to act directly on anterior pituitary cells to slow the rate of prolactin release (Abel et al., 1996; Van Cauter et al., 1991; Walton and Cronin, 1990). The hyperprolactin in our patients could be partly due to decreased TNF-α and, particularly, IFN-γ. But both IFN-γ and TNF-α have no effect on the inhibition of prolactin release mediated by dopamine (Abel et al., 1996; Van Cauter et al., 1991; Walton and Cronin, 1990). The dopaminergic tuberoinfundibular pathway is responsible for dopamine-mediated prolactin release; this pathway is inhibited in acute stress, leading to increased prolactin levels. Over 2 decades ago, a model of schizophrenic susceptibility to environmental stress was constructed (Leff et al., 1983). The stress could be either acute stress in the form of life events and/or

chronic stress in the form of the emotional atmosphere in the patient's home (Leff, 1981). Increased prolactin levels in schizophrenia could be raised partially by acute stress. Another possible rationale for the hyperprolactin in our schizophrenics could be reduced cortisol since cortisol was newly found to have inhibitory effects on prolactin releases from pituitary (Hyde et al., 2004; Uchida et al., 2004).

Regardless of the resources of increased prolactin, prolactin was reported to amplify IFN-γ release (Hyde et al., 2004; Matalka, 2003a; Breidthardt et al., 2002; Rovensky et al., 1999). It was ever regarded as a Th1 phenotype due to its ability to activate IFN-γ and interact with IL-12 (Matera, 1997). Our schizophrenic patients had, despite of having higher prolactin levels than controls, reduced serum and whole blood assay Th1/Th2 ratios. The results implicated that the prolactin pathway to promote IFN-γ release could be somehow deficient, so leading to IFN-γ deficiency. Moreover, the impacts of prolactin on IFN-γ could be not monotonic. A high concentration of prolactin likely leads to rather a decrease than an increase of IL-2-induced IFN-γ synthesis (Matera and Mori, 2000). So, the tremendously reduced IFN-γ in schizophrenia could be induced by hyperprolactin. In addition, activation of T lymphocytes mediated by the interaction of prolactin, prolactin receptors, and the JAK2/STAT5 pathways leads to production and release of various Th2 cytokines including IL-4, IL-5, IL-6, and IL-10 (Vera-Lastra et al., 2002). Enhanced serum IL-4 and IL-6 in our schizophrenic patients was possibly, at least in part, attributed to hyperprolactin. Hence, it could be that either down-regulation in the IFN-γ promoting route and/or up-regulation in the Th2 cytokine-promoting pathway via prolactin occurred in schizophrenia. In addition, SOCS3 can be enhanced and CIS can be induced by prolactin (Dogusan et al., 2000). Highly reduced IFN-γ in schizophrenics could be additionally raised by hyperprolactinism via SOCS3 and CIS.

Prolactin appears to be able to exert its impacts on the Th1/Th2 system via distinct routes. Nevertheless, excluding the effect of prolactin, Th2-shifts in schizophrenia remained clear, especially, in serum.

Cortisol, SHBG/testosterone & Th1/Th2

Corresponding to the outcomes of Taherianfard and Shariaty (2004), we found that schizophrenics had lower cortisol, total testosterone, and estradiol levels. But the diversity in estradiol between schizophrenics and controls did not reach any significance level in our study. However, the sex-hormone binding globulin (SHBG), an agonist of estradiol, was found to be extremely lower in schizophrenics than in healthy subjects. Both markedly

reduced cortisol and SHBG appeared to be age-inconsistent abnormalities since they were lately found to vary positively with age in normal subjects (Purnell et al., 2004; Elmlinger et al., 2002).

Cortisol in humans can act as more than just stress hormones; they were newly shown (1) to have a significant influence on neuroenergetics as they could modulate activity-related changes in brain glycogen metabolism (Allaman et al., 2004) and (2) to be involved in modulation of hippocampal neuron excitability, memory formation, and neurodegeneration (Davies and MacKenzie, 2003). Moreover, DHEA/cortisol ratios (DHEA = dehydroepiandrosterone) were implicated to be able to identify a particularly impaired subgroup of medicated chronic schizophrenia (Harris et al., 2001). An attenuated cortisol response towards psychosocial stress was described in diverse patient groups (Buske-Kirschbaum et al., 2003). We also found markedly reduced cortisol levels in schizophrenia as a whole group if compared with healthy subjects. Decreased cortisol levels in our schizophrenic patients could be raised by psychosocial stress. However, reduced cortisol levels in our schizophrenic patients could also hint at an altered regulation of DHEA-S (Dehydroepiandrosterone sulphat) since DHEA-S may act as an anti-glucocorticoid agent (Marklund et al., 2004). Regardless of the possible sources of cortisol abnormality, cortisol was found to induce a shift from Th1 to Th2 (Visser et al., 1998; Franchimont et al., 1998). Despite that cortisol was shown to be reduced in schizophrenics in this study, our schizophrenic patients showed clear Th2-shifts in serum.

SHBG, a protein binding to testosterone with high affinity, was newly implicated in Alzheimer's disease (Hoskin et al., 2004). Testosterone was significantly associated with cognitive function in elderly males (Yaffe et al., 2002) and found to be lower in men with Alzheimer's disease (Hogervorst et al., 2003). Lower androgen levels are linked to increased plasma amyloid β peptide 40 in older men with memory loss or dementia (Gillett et al., 2003). The abnormalities in SHBG and testosterone likely suggested a cognitive alteration in schizophrenia. The reason for reduced SHBG levels in our schizophrenic subjects was unknown. SHBG normally responds to circulating testosterone and estrogen (Hoskin et al., 2004); therefore, reduced SHBG in schizophrenia suggested an abnormal decrease in its production and regulation. But decreased testosterone in our schizophrenic patients might be resulted from hyper-prolactin since prolactin-infusion led to reduction of testosterone (Romanowicz et al., 2004). Decreased testosterone could have somewhat diminished Th2-

shifts in the schizophrenics of this study since it was reported to inhibit Th2 and to promote Th1 cytokines (Huber et al., 1999). Up to now, no report concerning the influence of SHBG on Th1/Th2 balance was published. SHBG could, however, have impacts on Th1/Th2 balance at least indirectly via testosterone and estradiol since changes in SHBG levels lead to alterations in estrogen production and testosterone activity (Anderson, 1974). Nevertheless, excluding the effect of testosterone and SHBG, marked Th2-shifts were still observed in our schizophrenic patients.

Somehow, it's very difficult to differentiate causes and results in this case since Th1/Th2 cytokines, prolactin, SHBG/testosterone, and cortisol have influences on and are influenced by one another reciprocally. Not only diverse hormones have influences on cytokines. Cytokines such as IL-6, TNF, and IFN-γ could also play an important role in modulation of hormone secretion by directly influencing specific enzymes of steroid genesis in various endocrine cell types (Herrmann et al., 2002).

7.4.2 Male subjects with schizophrenia

Disturbed Th1/Th2 antagonism in male schizophrenic patients

Similar to the results in the whole group of healthy controls, all serum, whole blood assay, and lymphocyte Th1/Th2 ratios of control males showed clear antagonisms between IFN-γ and IL-4, IL-10, IL-13 according to the findings from multiple regression. That is, IFN-γ significantly positively correlated to IFN-γ/IL-4, IFN-γ/IL-10, and IFN-γ/IL-13 in healthy controls. In contrast, the major Th2 cytokines IL-4, IL-10, and IL-13 were markedly negatively associated with IFN-γ/IL-4, IFN-γ/IL-10, and IFN-γ/IL-13, correspondingly. However, the antagonism between whole blood assay IFN-γ/IL-4 and that between lymphocyte IFN-γ/IL-10 and IFN-γ/IL-13 were lacking in schizophrenic males. Male subjects with schizophrenia appeared to have a disturbed antagonistic mechanism between both T helper-systems in both in vitro systems.

Except cortisol, all parameters measured were involved in Th1/Th2 balance in male patients

Even though both the whole schizophrenic group and the male schizophrenic subgroup had a disrupted Th1/Th2 antagonism, the results concerning the possible causes of Th2-shift from multiple regression in male schizophrenic subjects showed a different picture from that in the whole schizophrenic group. Except cortisol, all the other parameters measured in this study such as SHBG, age, prolactin, estradiol, testosterone, and IL-6 might be directly involved in

serum and whole blood assay Th1/Th2 imbalance in male schizophrenics, in addition to the key factors IFN-γ, IL-4, and IL-10. In addition, IL-2 and TNF-α likely played an indirect role in the Th1/Th2 balancing in schizophrenic men because they were significantly related to IFN-γ and IL-4, respectively.

Involvement of various endocrinological parameters in male schizophrenic patients

Male schizophrenics had in general lower serum cytokine levels, testosterone, SHBG, however, higher prolactin levels than control men. In contrast to the results from the whole group of schizophrenic patients, all hormones except cortisol measured in this study exerted effects on the Th1/Th2 ratios in males with schizophrenia according to the findings from multiple regression analysis.

Generally, Th2-shift in male schizophrenic patients was less explicit than that in their female schizophrenic counterparts. Males were found to show a higher Th1/Th2 ratio compared with females (Giron-Gonzalez et al., 2000). The reason might be that males had higher levels of testosterone than females since testosterone was shown to favor Th1-shift (Huber et al., 1999). However, testosterone was not the explanation for the diversity in Th1/Th2 ratios between both male subject groups in our study because male subjects with schizophrenia had markedly lower testosterone levels than male controls. Reduced testosterone levels in male schizophrenic patients might be the outcomes of noticeably elevated prolactin since injection of prolactin resulted in decreased testosterone (Romanowicz et al., 2004). Various factors could have effects on prolactin; they include neuroleptic medication, cortisol, TNF-α, and IFN-γ (Hyde et al., 2004; Wallaschofski et al., 2003; Walton and Cronin, 1990). Higher prolactin in male schizophrenics probably was a result of neuroleptic medication. But it was unlikely an outcome of changes in cortisol, TNF-α, and IFN-γ, because male schizophrenic patients and controls had comparable levels in those parameters.

Summarized findings from multi-variance and multiple regression analysis in male patients

Schizophrenic men as a whole group had neither markedly higher serum IL-4 nor noticeably lower serum IFN-γ than male controls. The findings from multi-variance analysis in which IFN-γ and IL-4 were surveyed separately seemed to implicate that IFN-γ and IL-4 did not antagonize each other in male schizophrenics. Multi-variance analysis is applied to compare the differences in diverse variables between or among various groups. The outcomes from multi-variance analysis only suggested no marked diversity in IFN-γ, IL-4, and IFN-γ/IL-4 ratio between both male diagnostic subgroups. However, the findings from multiple regression of serum Th1/Th2 ratios revealed that IFN-γ correlated tremendously positively,

while IL-4 and IL-10 associated extremely negatively with IFN-γ/IL-4 and IFN-γ/IL-10 in schizophrenic men. That is, those male schizophrenics who had lower serum IFN-γ also had higher serum IL-4 and IL-10 and vice versa. Compared to female subjects with schizophrenia, male schizophrenic patients had less marked reductions in both serum IFN-γ/IL-4 and IFN-γ/IL-10 ratios. The reason could be that males had higher levels of testosterone than females since testosterone was shown to favor Th1-shift (Lambert et al., 2004; Burger and Dayer, 2002). Reduced testosterone levels in male schizophrenic patients could, therefore, have contributed to the less pronounced reductions in both serum IFN-γ/IL-4 and IFN-γ/IL-10 ratios. The explanation for why the reduction in serum IFN-γ/IL-4 in male subjects with schizophrenia was less obvious than in female schizophrenics could be that IL-4 mRNA expression were found to be lower in males than in females; testosterone, at least in part, may be responsible for the decreased Th2 cell responses in males in vivo (Hayashi et al., 2003).

Possible causes of Th2-shift in schizophrenic males

Further deficit(s) in the macrophage system in male schizophrenia?

Male schizophrenic patients had significantly reduced serum IFN-γ/IL-10, but not IFN-γ/IL-4 ratios if compared with healthy males. Deficits within the typical Th1/Th2 differentiation routes could be less explicit in schizophrenic males because IL-10 is not a typical Th2 cytokine. Th1/Th2 imbalance caused by deficits within the classical Th1/Th2 developmental pathways might have been compensated by testosterone since testosterone was found to favor Th1 development (Giltay et al., 2000). In addition, it could indicate further deficit(s) in other sites than the typical Th1/Th2 pathways. In humans, IL-10 is produced by activated CD8+ T cells, CD4+ T cells (Yssel et al., 1992), macrophages/monocytes, and mast cells (Verreck et al., 2004; Haddad et al., 2003). Reduced serum IFN-γ/IL-10, but not in whole blood assay IFN-γ/IL-10, could suggest that the sources of imbalance in serum IFN-γ/IL-10 lie at least partially in the factors which were excluded in the stimulated whole blood system. The candidate factors could be macrophages or mast cells since both cell types were not or less likely found in whole blood. Macrophages were more likely than mast cells as another possible sites of deficit in male subjects with schizophrenia because macrophages produce both IFN-γ and IL-10, but not IL-4 (Yanagawa et al., 1999; Havell and Spitalny, 1983). Over-activation in the macrophage/monocytes system could happen in male subjects with schizophrenia. Abnormality in macrophages was actually already observed in schizophrenia over 2 decades ago (Livni et al., 1979). Possible pathogenic mechanisms behind lymphocyte activation and macrophage dominance were once considered as the causes of acute psychotic symptoms (Nikkila et al., 2001). The macrophage-T-lymphocyte theory was ever postulated

as a possible etiological mechanism in schizophrenia (Smith and Maes, 1995). Accordingly, chronically activated macrophages and T-lymphocytes were previously proposed as the fundamental mediators of schizophrenia. Over-activation of macrophages could have led to over-production of IL-12 and IL-10. Additionally, a consistent hyper-production of IL-12 could again result in persistently elevated IL-10 and reduced IFN-γ, TNF-α, and IL-6 levels (Portielje et al., 2003).

Hormones: prolactin, SHBG/testosterone

Further factors such as prolactin and SHBG/testosterone could have partly contributed to Th1/Th2 imbalance in male schizophrenics since both male subgroups were remarkably different in those variables. They were elucidated in the previous section "7.4.1.The whole group of schizophrenic patients". The male schizophrenics of this study had evidently "accelerated aging" regarding their testosterone levels. Their testosterone levels were much lower than those in male controls, despite that both male subgroups were of similar ages. Our male patients had only about 2/3 of the average control testosterone level. The reduction ratio in testosterone of our male schizophrenics was similar to that of normal men at the age of 70 yrs (Vermeulen, 2003). Decreased testosterone levels in male people with schizophrenia may be caused by significantly elevated prolactin as prolactin injection led to reduction in testosterone (Romanowicz et al., 2004). The roles of gonadal steroids such as testosterone in neurite outgrowth, cell differentiation, cell death, synaptogenesis, and the determination of cell position in earlier developmental phases were reported over one decade ago (Tobet et al., 1994). Neuronal loss was prevented by simultaneous administration of testosterone in vivo and in vitro (Mizoguchi et al., 1992). Progressive and long-term sex hormone imbalance was found to lead to degenerative changes in the CNS (Danilovich et al., 2003). Markedly reduced testosterone and SHBG could hint at a neurodegenerative process occurring in one subgroup of male schizophrenic patients. In addition, they implicate possible cognitive dysfunctions in schizophrenic males since they were shown to be linked to cognitive function in Alzheimer's disease (Hoskin et al., 2004; Hogervorst et al., 2003). Moreover, reduced testosterone and SHBG likely explained why the Th1/Th2 ratios were less overt in male schizophrenics than in their female schizophrenic counterparts because testosterone is thought to shift the development of T-helper cells towards Th1 (Huber et al., 1999). Although elevated serum prolactin concentrations often correlate with abnormalities in immune responses (Brand et al., 2004), increased prolactin levels in male schizophrenic patients were probably raised by neuroleptic medication (Meaney et al., 2004). Prolactin was found to trigger the production of Th1 cytokines like IFN-γ, IL-12 and TNF-α, but not Th2 cytokines such as IL-6 and IL-10

(Carreno et al., 2004; Rovensky et al., 1999). Less clear Th2-shift in male people with schizophrenia could be ascribed to the elevation in prolactin levels.

Those endocrinological parameters might have direct or indirect impacts on Th1/Th2 balance. Nevertheless, they were not the only causes. Serum Th2-shift defined as significantly reduced IFN-γ/IL-10 ratio in schizophrenic males remained clear after the effects of those hormones mentioned above were controlled.

7.4.3   Females with schizophrenia

Disturbed Th1/Th2 antagonism at serum, whole blood, and lymphocyte levels

The results from <u>MANCOVA</u> showed that female schizophrenics had noticeably reduced serum IFN-γ, but enhanced IL-4 if compared to healthy women. They seemed to suggest Th1/Th2 antagonism in female subjects with schizophrenia. In contrast, the findings from <u>multiple regression</u> revealed no antagonism between both T-helper systems because IFN-γ did not significantly correlate to IFN-γ/IL-4, IFN-γ/IL-10, and IFN-γ/IL-13, while IL-4, IL-10, and IL-13 were not markedly related to the 3 Th1/Th2 ratios stated above, respectively. The findings from both MANCOVA and multiple regression together actually implicated that female schizophrenics could be further subdivided into at least 3 heterogeneous subgroups according to their Th1/Th2 ratios. One subgroup could only have highly elevated IL-4 or IL-13 or IL-10, a second one might have noticeably reduced IFN-γ, and a third one likely had abnormalities in the key cytokines of both Th1/Th2 systems. Th2-shift in female patients appeared to be more heterogeneous than that in their male schizophrenic counterparts.

Summarized findings from MANCOVA and multiple regression in schizophrenic females

<u>MANCOVA</u> demonstrated that schizophrenic females had generally (1) lower serum Th1/Th2 ratios, characteristic Th1 cytokines such as IFN-γ, IL-2, cortisol, and SHBG, however, (2) higher prolactin, Th2 cytokines such as IL-4, IL-6, as well as Th1/Th2 produced cytokines like IL-10 and TNF-α levels if compared to control females. Nevertheless, both female subgroups were quite comparable regarding testosterone and estradiol.

Nevertheless, the findings from <u>multiple regression analysis</u> of Th1/Th2 ratios in which both IFN-γ and IL-4 of the same subject were considered at the same time offered another picture. They demonstrated that all parameters assessed in this study except SHBG obviously had been involved in Th1/Th2 balancing. As elucidated above, superficially, female

177

schizophrenics appeared to be more homogenous regarding Th1/Th2 ratios; the whole female patients had clear Th2-shifts. If considering the causes of Th2-shifts, schizophrenic females were heterogeneous as a whole group; this could partially explain why the contributors of Th1/Th2 variances were so widespread in schizophrenic women.

Possible causes of Th2-shift in female schizophrenic patients

The APC IL-6/SOCS/CIS pathways

Thus, for those female patients who had primarily increased IL-4 or predominantly decreased IFN-γ, deficits in the APC IL-6/SOCS/CIS loops stated in the foregoing section were more likely because the pathways of IL-6 to inhibit IFN-γ and to promote IL-4 function independently (Diehl and Rincon, 2002). Maybe, the down-stream component(s) of the pathway to promote IL-4 or the route to inhibit IFN-γ was/were somehow up-regulated.

Typical routs for Th1/Th2 development

For those schizophrenic women who had alterations in IFN-γ and IL-4 simultaneously, shortages in both classical Th1/Th2 differentiation routs are also possible except the IL-6 alternative pathways. So, up-regulation of the Th2 system results in down-regulation of the Th1 system (Finkelman and Urban, Jr., 2001; Kroemer et al., 1996).

Stress hormones

Furthermore, hormones such as cortisol and prolactin could also have made their contribution to Th2-shift in schizophrenic women because they were shown to influence Th1/Th2 balance (Dimitrov et al., 2004; Elenkov, 2004) and significantly different between schizophrenic and control females.

Cortisol

Female schizophrenic patients as a whole group were found to have markedly reduced cortisol, but not males with schizophrenia if compared to healthy subjects of corresponding sex. Males have normally higher cortisol levels than females in response to stress (Sauro et al., 2003). Alterations in cortisol in female subjects with schizophrenia might be associated with stress (Raison and Miller, 2003). Nevertheless, cortisol can act more than just as a stress hormone. It was found to be able to modulate neuron activity-related changes in brain glycogen metabolism (Allaman et al., 2004). There is also evidence for a relationship between chronic changes in circulating cortisol and the memory impairments in schizophrenic patients (Walder et al., 2000; Newcomer et al., 1998). In addition, it was thought to (1) play a potential role in the expression of psychosis (Walder et al., 2000) and to (2) positively correlate with ratings of positive, disorganized, and overall symptom severity, but not with negative symptoms (Walder et al., 2000). The plasma cortisol response to apomorphine (a

dopamine agonist) and to ipsapirone (a 5HT1A partial agonist) were also found to be markedly blunted in patients with schizophrenia compared to normal controls (Lee and Meltzer, 2001; Meltzer et al., 2001). The finding concerning ipsapirone was only observed in female people with schizophrenia (Lee and Meltzer, 2001). Furthermore, neuroleptic responders were shown to have a higher cortisol response towards apomorphine compared to non-responders (Meltzer et al., 2001). Reduced cortisol levels in female schizophrenics of this study might be not only caused by stress but likely also by certain cognitive dysfunction; in addition, they could also implicate the responses of female schizophrenic patients to neuroleptic treatments.

Although Braun et al. (1997) found that Th1 and Th2 responses were equally affected by cortisol, evidence accumulated over the last 5-10 years indicates that glucocorticoids inhibit the production of IL-12, IFN-γ, IFN-α, and TNF-α by antigen-presenting cells and Th1 cells, but up-regulate the production of IL-4, IL- 10, and IL-13 by Th2 cells (Elenkov, 2004). Schizophrenic females had noticeably reduced cortisol that could have contributed to Th2-shift. No matter how cortisol impacts Th1/Th2 balance, after controlling the effects of cortisol, Th2-shift remained clear in female patients.

Prolactin

Elevated prolactin levels were also detected in female schizophrenic patients. Increased prolactin might be a result of neuroleptic medication (Meaney et al., 2004); however, it's not an obligatory consequence of anti-psychotic treatment. Prolactin abnormality was ever described in drug-naïve schizophrenics over 1 decade ago (Abel et al., 1996; Van Cauter et al., 1991). Prolactin was even found to be able to differentiate distinct schizophrenic subgroups such as paranoid and disorganized schizophrenia from each other (Segal et al., 2004). Schizophrenic patients reacted towards neuroleptic treatment with hyperprolactinaemia were found to have increased probability to have DRD2(*)A1allele (Young et al., 2004). Enhanced prolactin in schizophrenia could be partially resulted from the abnormal reaction to neuroleptic treatments that was caused by genetic deficits. In addition, many other factors could influence prolactin levels in schizophrenia. Examples include IFN-γ, TNF-α, and cortisol. They can slow down or inhibit the release of prolactin on anterior pituitary cells (Hyde et al., 2004; Uchida et al., 2004; Walton and Cronin, 1990).

Despite of the source and nature of elevated prolactin in schizophrenia, prolactin was shown to favor Th1 shift (Dimitrov et al., 2004). Prolactin could exert its effects on Th1/Th2 cells

via diverse pathways such as JAK2/STAT5, prolactin receptor, IL-2, SOCS3/CIS, NF-κB, and IRF-1 (interferon regulatory factor 1) (Brand et al., 2004; Vera-Lastra et al., 2002; Matera and Mori, 2000; Dogusan et al., 2000; Matera, 1997). Schizophrenic women had significantly reduced Th1/Th2 ratios, regardless of having higher prolactin than healthy females. Even if prolactin impacts IFN-γ in a bi-modal manner as Matera and Mori (2000) described, Th2-shift in female schizophrenic patients remained significant after the effects of prolactin were taken into account.

7.5   Strategies to restore Th1/Th2 balance in schizophrenia

In the subsequent sections, the strategies to re-balance Th1/Th2 are recommended within the framework of neuroleptic treatment in schizophrenia if Th2-shifts regarded as biological features of schizophrenics which might contribute to the outbreak of disease and therefore as a co-target of treatment.

7.5.1   The whole group of schizophrenic patients

The whole schizophrenic group had lower cortisol, SHBG, however, higher prolactin than the control group. In addition, they had significantly lower IFN-γ, nevertheless, higher IL-4 and IL-6 than controls if excluding extreme values. The key issues in conceptualizing pharmacological therapies for schizophrenic patients include: (1) increasing IFN-γ and lowering IL-4, IL-6 simultaneously, (3) down-regulating prolactin, (4) up-regulating cortisol and (5) SHBG/testosterone. Generally, the medicine administered to schizophrenic subjects in order to restore the balance between both Th1/Th2 systems has to promote IFN-γ and suppress IL-4 in both serum and whole blood assay. Additionally, it must promote IL-6 in whole blood assay, but suppress IL-6 in serum. To achieve the therapeutic issues stated above, further investigation using multiple regression analysis is required. More schizophrenic patients are also needed due to involvement of many essential predictors in Th1/Th2 balancing in schizophrenia and due to the complicated inter-correlations among those predictors.

To date, schizophrenics are treated principally with neuroleptics. However, so far, no study systematically investigates the effects of distinct medicines/neuroleptics on diverse serum and

whole blood assay Th1/Th2 ratios in schizophrenic patients and healthy controls, simultaneously considering the effects of diverse factors from the endocrine system. Which neuroleptic should be administered to schizophrenic patients in order to redirect the Th2-shift still remains unknown. Nevertheless, within the framework of present neuroleptic treatment in schizophrenia, generally speaking, anti-psychotics having high affinity for dopamine D2 receptors, particularly typical neuroleptics (Hall and Sallemark, 1987), are not suitable to treat schizophrenics with Th2-shift. Typical neuroleptics were found to have a rather long-lived prolactin increasing effect (Meltzer et al., 1989). Elevated prolactin is in turn not preferred. However, a newest study showed that haloperidol only led to a minor, non-significant increase in serum prolactin levels of schizophrenic and schizoaffective patients (Volavka et al., 2004).

According to available evidence, the effects of haloperidol on Th1 cytokines are controversial (Rudolf et al., 2002; Kowalski et al., 2000; Kim et al., 2000; Pollmacher et al., 1997; Boukhris et al., 1988). Nearly no report regarding the effects of haloperidol or other typical neuroleptics on Th2 cytokines was published. Only Pollmächer et al. (1997) showed no effect of haloperidol on plasma IL-6 at medium dosage. Therefore, haloperidol is likely due to its effect of provoking prolactin production and that of decreasing serum/whole blood assay IL-2 less suitable for schizophrenics having Th2-shift.

Atypical neuroleptic risperidone also caused significant elevation of prolactin levels that appeared to be dose-dependent (Volavka et al., 2004), although another study showed that the risperidone-induced hyperprolactin declined after 3-to-5-month treatment in children and adolescents (Findling et al., 2003). Risperidone was found, additionally, to increase serum IL-10, IL-6, sIL-2R as well as IL-10 and IL-2 in vitro production (Cazzullo et al., 2002; Maes et al., 2002; Maes et al., 1996). Besides, risperidone had impacts on both IFN-γ and IL-4 production in a time-dependent manner (firstly reduced and later increased) (Cazzullo et al., 2002). The effects of risperidone on Th1/Th2 cytokines seem to be highly complicated. But another atypical neuroleptic clozapine demonstrated prolactin-sparing effects due to its low affinity to dopamine D2 receptors in the dopaminergic tuberoinfundibular pathway (Hamner, 2002). Moreover, the relevant literature mostly suggests that clozapine increases several Th1 cytokines and/or their receptors including IFN-γ, IL-2 in vitro production, plasma TNF-α, sTNF-Rp55, sTNF-Rp75, and sIL-2R (Rudolf et al., 2002; Song et al., 2000; Haack et al., 1999; Hinze-Selch et al., 1998; Muller et al., 1997; Maes et al., 1996; Pollmacher et al., 1995;

Maes et al., 1994). However, clozapine was also found to increase serum and plasma IL-6 in (acute) schizophrenics (Maes et al., 2002; Maes et al., 1997). That is, it's possible for clozapine to cause Th2-shift via the APC IL-6/SOCS/CIS pathways (Diehl and Rincon, 2002). Nevertheless, clozapine is <u>relatively more suitable</u> than the other neuroleptics to treat schizophrenics with Th2-shift because it increases Th1 cytokines and has less hyperprolactin effect.

7.5.2 Male schizophrenic patients

Male schizophrenics had in general lower serum cytokine levels, testosterone, SHBG, however, higher prolactin than control men. The major concerns to redirect Th2-shifts in male patients contain: (1) increasing IFN-γ, (2) lowering prolactin, and (3) increasing testosterone/SHBG. Clozapine, supplemented with testosterone, might be beneficial in schizophrenic males having Th2-shift for the reasons that (1) clozapine could increase IFN-γ, IL-2, IL-6, TNF-α (Rudolf et al., 2002; Song et al., 2000; Haack et al., 1999; Hinze-Selch et al., 1998; Maes et al., 1994), (2) that clozapine has less prolactin-provoking effects (Volavka et al., 2004), and (3) that testosterone promotes Th1, while inhibits Th2 cytokines (Giltay et al., 2000; Huber et al., 1999). Elderly men ($\geq$ 70 years) who had a total testosterone level lower than 3 ng/ml were suggested to undertake testosterone replacement (Basaria et al., 2002). The average total testosterone level of our male schizophrenic patients was 4.56 ng/ml (SD = 2.13 ng/ml). That is, one subgroup of our male schizophrenic patients had extremely low testosterone levels; their testosterone levels were comparable to those of males older than 70 years old. This subgroup of male patients may profit from testosterone supplement. However, whether or not a combination with testosterone is proper to treat male schizophrenic patients required further examinations because (1) Th2 cytokines were lower in male schizophrenics and (2) use of testosterone could lead to further decrease in SHBG which might be not favorable due to noticeably decreased SHBG in male schizophrenics. In addition, one subgroup of male schizophrenic patients had rather enhanced Th1/Th2 ratios compared to healthy male subjects; for this subgroup, additional supplement of testosterone could be detrimental.

7.5.3 Female patients with schizophrenia

In female schizophrenics, all parameters measured obviously had participated in Th1/Th2 balancing in accordance with the findings from multiple regression analysis. Additionally, the major cause of Th1/Th2 imbalance in female patients was either extremely reduced IFN-γ or highly enhanced IL-4 or both. Schizophrenic females had generally lower serum IFN-γ, IL-2, cortisol, SHBG, and both serum Th1/Th2 ratios, however, higher prolactin, TNF-α, IL-4, IL-6, and IL-10 levels if compared to control females. Female schizophrenic patients had the lowest serum and whole blood assay IFN-γ, IL-2, nonetheless, the highest whole blood assay IL-4 and serum prolactin level among these 4 gender subgroups.

Medicines administered to female schizophrenics having Th2-shifts had to enhance serum/whole blood assay IFN-γ and/or decrease serum/whole blood assay IL-4, however, decrease serum IL-6, but increase whole blood assay IL-6, depending on their Th1/Th2 deficits. As elucidated in the section "7.5.1. The whole group of schizophrenic patients", it requires further examinations in order to find out which medicine/neuroleptic can achieve such therapeutic effects. Hitherto, investigations in this regard are lacking. Before conducting such a study, systematic examinations of diverse neuroleptics on distinct Th1/Th2 ratios under the consideration of various endocrinological parameters in schizophrenia and controls are firstly needed. In the following sections, recommendations are made within the framework of present anti-psychotic treatment because neuroleptics are currently the most often used psychotic agents in schizophrenia.

As the results from MANCOVA and multiple regression analysis shown, Th2-shift in schizophrenic women is very heterogeneous. So, it's very essential to ascertain which factors could contribute to Th2-shfit of which subgroup of female patients. That is, which of those predictors contribute to Th2-shift mainly resulted from reduced IFN-γ, which influencing factors contribute to Th2-shift primarily originated from elevated IL-4, and which parameters contribute to Th2-shift caused by both decreased IFN-γ and augmented IL-4.

So far, clozapine appears to have advantages over the other neuroleptics in terms of restoring Th1/Th2 balance. Clozapine was shown to (1) increase various Th1 cytokine productions such as IFN-γ and IL-2 (Rudolf et al., 2002; Song et al., 2000; Haack et al., 1999; Hinze-Selch et al., 1998; Muller et al., 1997; Maes et al., 1996; Pollmacher et al., 1995; Maes et al., 1994) and (2) to have less hyperprolactin effects (Volavka et al., 2004), although it was found to increase serum IL-6 (Maes et al., 2002; Maes et al., 1997) which is not desired.

Furthermore, it's noteworthy that among distinct cytokines, prolactin, cortisol, estradiol, testosterone, and SHBG existed very tangled inter-correlations in schizophrenic females as shown in the results from multiple regression. Changes in one variable could lead to alterations in many other factors. So, if restoration of Th1/Th2 is considered as a co-target of treatment in female schizophrenics, then how those factors vary with one another might be crucial to achieve favorable effects and require further examinations.

Currently, due to the neuroprotective role of estrogen (Sortino et al., 2004; Alexaki et al., 2004), there is so-called estrogen hypothesis (Salokangas, 2004). The estrogen hypothesis proposes that the lower need for neuroleptic drugs in female schizophrenia patients is caused by the antidopaminergic effect of estrogens, and that when estrogen production decreases at menopause, the need for neuroleptic drugs increases in female schizophrenia patients (Salokangas, 2004). Estrogen add-on therapy is, therefore, deemed as promising (Moller, 2003) because it can augment the treatment effects of antipsychotic medication (Liao et al., 2002; Grigoriadis and Seeman, 2002). **If** Th2-shifts are some of the many primary biological defects leading to (certain) schizophrenic symptoms, then the supplement could be harmful for those schizophrenic women having Th2-shift since (1) estradiol was found to exert rather inhibitory effects on Th1 cytokines, but promoting effects on Th2 cytokines and (2) schizophrenic and control women had relatively comparable estradiol levels (Lambert et al., 2004; Burger and Dayer, 2002; McMurray et al., 2001; Huber et al., 1999; Salem et al., 1999). In addition, the use of estrogen replacement therapy in conjunction with antipsychotic medication in postmenopausal women with schizophrenia has its limit. It may help reduce negative, but not positive, symptoms (Lindamer et al., 2001). Besides, there is individual variability for response to estrogen supplementation, possibly associated with onset age (Liao et al., 2002). Probably, estrogen supplement can be applied to female schizophrenic subjects having late onset because late-onset schizophrenic patients did not have serum Th2-shift. Neuroleptic combined with estrogen won't deteriorate serum Th2-shift in schizophrenic women and could be, thus, helpful for this subgroup of female schizophrenic patients.

7.6 *Critics and questions for further investigations*

7.6.1 Critics on this study

In this study, the major cytokine levels in serum and productions in stimulated whole blood were assessed with Cytometric Bead Array (CBA). The biggest advantage of CBA is that

both Th1 and Th2 key cytokines of the same individual were measured together with the same material (serum/supernatant). Unlike in conventional ELISA, for each single cytokine, a certain amount of material is required to measure each cytokine separately. Therefore, the comparability of the indicators of both Th1/Th2 systems in the same subject is very high due to no inter-assay variance among distinct cytokines within the same participant.

The principal shortage of this study concerns the lymphocyte data (ELISPOT). Using the separating isotonic solution with a density of 1.077, not only lymphocytes but also 8-9% of monocytes and 1-2% of mast cells/basophils were isolated. It would be better if either only lymphocytes or complete leukocytes were separated, but not cells mixed with a small amount of different cell types. It's harder to draw a clear conclusion using partly mixed cell types for analysis than just using one or all leukocytes. Another drawback of this study is that no other psychiatric patients with the same disorder (e.g. patients only with schizoaffective disorder or patients only having bipolar disorder) were included as reference group to compare with the schizophrenics. Comparisons with a patient group having mixed diseases won't be able to clarify whether or not schizophrenics share which deficit(s) with which disorder(s) due to the heterogeneity in a patient group with mixed illnesses. Additionally, the case numbers of diverse schizophrenic clinical subgroups are relatively small. Some schizophrenic subgroups had only about 1/13 of the cases in healthy controls. Particularly for multiple regression analysis, it would be better to have a higher number of schizophrenic cases because there were so many predictors involved. Other drawbacks include no data regarding the menstrual cycles of females with schizophrenia and no information relating to precise washout periods. Nevertheless, the purpose to obtain the menstrual cycles was to control their effects on Th1/Th2 balance in female patients. In this study, serum estradiol levels were measured and included as a possible covariant of Th1/Th2 imbalance in schizophrenic women. It's rather a biological parameter like estradiol than a clinical variable such as menstrual cycle exerting direct effects on Th1/Th2 ratios. Therefore, including estradiol into the study should have compensated the shortage of no data concerning the menstrual cycles of female subjects. In addition, the precise washout periods were missing in some patients. The aim to obtain washout periods is also to control their effects on Th1/Th2 balance. Serum prolactin is an indicator of neuroleptic treatment and was assessed in this study. As stated above, it's rather biological parameters like prolactin having direct impacts on a biological process such as Th1/Th2 balancing. It's not a clinical variable like washout period having direct influences on Th1/Th2 cytokines. So, inclusion of prolactin as a co-variant into the analysis should have

countervailed the drawback of not having completed data regarding washout period in schizophrenic patients.

7.6.2   Questions for further investigations

Th2-shifts appeared to be schizophrenia-specific immuno-endocrinological deficits. However, in this study, we won't be able to detect which schizophrenic symptoms are Th2-shifts related to. We only found out that Th2-shifts had no obvious connection with positive symptoms. So the first question is: which schizophrenic symptom(s) is/are Th2-shift-relevant?

Neurotransmitter, particularly dopamine, hypothesis of schizophrenia has been dominating this field for many decades. Despite of the insufficiency of dopamine hypothesis to elucidate all schizophrenic cases, it may be able to illuminate the pathogenesis of at least one schizophrenic subgroup and therefore remains as the focus of neurochemical research in the past five years. Since (1) we found serum Th2-shifts were observed in most schizophrenic subgroups and (2) various elements in the dopamine synthesis were lately found to have diverse impacts on distinct Th1/Th2 cytokines (Carr et al., 2003; Ghosh et al., 2003; Alaniz et al., 1999), possibly there is one schizophrenic subgroup having both dopamine and Th1/Th2 abnormalities. Therefore, the second question is: is there any connection between dopamine abnormalities and Th2-shifts in schizophrenia? If there is, what kind of relationship exists between both biological features in schizophrenic patients? Clarifying questions of this kind might be helpful to select "proper patients" for "suitable neuroleptics targeting the dopamine system" and thus avoid diverse side effects since neuroleptics are frequently administered to schizophrenic patients.

To the end, some more questions are addressed for investigations in the future:
(1) In this study, serum/whole blood assay/lymphocyte IFN-γ/IL-4, IFN-γ/IL-10, and lymphocyte IFN-γ/IL-13 ratio were used to indicate Th1/Th2 balance in schizophrenia. We found that one subgroup of schizophrenics who had markedly reduced whole blood assay IFN-γ/IL-4 and IFN-γ/IL-10 ratio might be treatment-resistant due to barely change in severity of symptoms after 8-week-treatment. In addition, we also found early-onset schizophrenics had reduced lymphocyte IFN-γ/IL-4 and IFN-γ/IL-13, while late-onset schizophrenics had enhanced both lymphocyte

Th1/Th2 ratios. Are these three Th1/Th2 indicators together or separately able to differentiate distinct schizophrenic subgroups from one another?

(2) Obviously, neuroleptics have direct or indirect impacts on Th1/Th2 ratios. Since (a) neuroleptics belong to the psychotic agents often prescribed to schizophrenics and (b) Th1/Th2 imbalance has been associated with a variety of diseases, it might be important to clarify which neuroleptic has what kind of effect on which (Th1/Th2) cytokine under what kind of experimental condition in order to optimized therapeutic effects.

(3) Hyperprolactin is frequently associated to anti-psychotic treatment. But according to the latest literature, it is not necessary the result of neuroleptic therapies. Since prolactin could influence Th1/Th2 cytokines via distinct routes, it could be also indispensable to disentangle the relationships between prolactin and diverse Th1/Th2 cytokines in order to (a) restore Th1/Th2 balance and (b) to avoid prolactin-induced side effects such as sexual dysfunction in schizophrenics.

Summary

Schizophrenia is a complex disease, in which except a genetic component external influencing factors also play an important role. Epidemiological data further indicate a possible role of viral infection as an environmental factor in the etiology of schizophrenia. Th2-shift has been associated with distinct viral infections. Diverse lines of immunological evidence point out that a Th2-dominated condition could be observed in at least one subgroup of schizophrenia.

(1) **Aims**: This study is designed to explore (a) the balance between Th1/Th2 systems and (b) eventually to detect the possible causes of Th1/Th2 imbalance among the parameters investigated in this study in schizophrenic patients.

(2) **Questions**:

(a) Is a significant Th2-shift observed in any schizophrenic subgroup after the effects of diverse endocrinological parameters are taken into account?

(b) If yes, can this schizophrenic subgroup be characterized through any clinical or epidemiological variable?

(c) If yes, which of those immunological and endocrinological parameter(s) assessed in this study could make remarkable contribution(s) to the variances of the Th1/Th2 ratios in schizophrenia?

(3) **Hypothesis**:

(a) To question (2a), A Th2-shift is supposed to occur in at least one subgroup of schizophrenics; that is, the Th1/Th2 ratio(s) is/are significantly reduced. The IFN-γ/IL-4, IFN-γ/IL-10, and IFN-γ/IL-13 ratio are used as indicators of Th1/Th2 balance.

(b) Question (2b) and (2c) are open questions; no hypothesis in these regards is assumed.

(4) **Methods**:

(a) *Analysis materials* include serum, whole blood, and isolated lymphocytes. "Total serum data" means that all data for serum cytokine levels, serum Th1/Th2 ratios, hormones, SHBG, gender, and age were available. Similarly, "complete whole blood assay data" means that all data for whole blood assay cytokine productions, whole blood assay Th1/Th2 ratios, hormones, SHBG, gender, and age were obtained. "Entire lymphocyte data" means that all data for lymphocyte cytokine secretions, Th1/Th2 ratios, hormones, SHBG, gender, and age were collected.

(b) *Subjects*: Totally, 114 schizophrenic patients and 101 healthy subjects had participated in this study. Among them, 76 schizophrenics and 75 normal subjects had total serum data, 44 patients and 76 normal controls had complete whole blood assay data as well as 72 schizophrenic patients and 98 controls had entire lymphocyte data.

(c) *Variables* comprised <u>immunological</u>, <u>endocrinological</u>, and diverse <u>clinical</u> parameters. The immunological variables consisted of Th1 cytokines such as IFN-γ, IL-12, IL-2, and TNF-α as well as Th2 cytokines including IL-4, IL-10, IL-13, and IL-6. The endocrinological parameters contained two stress-hormones cortisol and prolactin, two sex-hormones estradiol and testosterone as well as the sex hormone binding globulin (SHBG). Clinical data included information regarding clinical diagnostic subgroups, family psychiatric history, pre-medication, disease episode, drug-naïve/drug-free, washout-period, onset age, illness duration, scores on the CGI (at admission & discharge) as well as on diverse subscales of the PANSS (positive, negative, global).

(d) *Analysis methods* contained Cytometric Bead Array (<u>CBA</u>), <u>ELISA</u>, and <u>ELISPOT</u>. CBA was used to measure serum and whole blood assay IFN-γ, IL-2, TNF-α, IL-4, IL-10, and IL-6. ELISA was applied to assess whole blood assay IL-12 and IL-13, while ELISPOT was utilized to detect IFN-γ, IL-4, IL-10, IL-13, and IL-12 production at lymphocyte level. Furthermore, hormone levels in serum such as cortisol, prolactin, estradiol, testosterone, and SHBG were obtained by using corresponding Elecsys Kit.

(e) *Design*: All schizophrenic patients were firstly analyzed as a whole group and then divided into distinct subgroups according to their genders and various clinical features; these were the <u>independent variables</u>. The main <u>dependent variables</u> were Th1/Th2 ratios including IFN-γ/IL-4, IFN-γ/IL-10 (serum, whole blood, lymphocyte), and IFN-γ/IL-13 (lymphocyte). If any marked disparity in age, various hormones, and SHBG between any schizophrenic subgroup and controls, they were included into the analysis as <u>co-variants</u> in order to control their effects on Th1/Th2 balance in the corresponding index-groups.

(f) *Major statistics*: MAN(C)OVA and multiple regression analysis. MAN(C)OVA was applied to unravel the question (2a) and (2b), while multiple regression analysis was utilized to solve the question (2c).

(5) **Primary results**:

(a) The results supported our hypothesis "Th2-shift in at least one schizophrenic subgroup".

(b) Findings regarding Th1/Th2 imbalance in schizophrenia (outcomes from MANCOVA):

- The in vivo serum data indicated a clear Th2-shift in schizophrenia as a whole group <u>after excluding the effects of age and various hormones (particularly prolactin)</u>.

- Serum Th2-shift appeared to be <u>schizophrenia-specific</u> as the data shown no Th2-shift in patients with schizophrenia-related disorders.

- Female schizophrenics as a whole group had significantly decreased serum IFN-γ/IL-4 and IFN-γ/IL-10 ratios, whereas male schizophrenics as a whole group had only noticeably reduced serum IFN-γ/IL-10 ratios if compared to the corresponding sex of controls.

- Reduced serum IFN-γ/IL-4 and IFN-γ/IL-10 were likewise observed in various clinical schizophrenic subgroups with noticeably increased probabilities except schizophrenics pre-dominated with positive symptoms.

- Significantly reduced whole blood assay IFN-γ/IL-10 ratios were found in non-paranoid, chronic schizophrenics, patients with positive family history or being drug-free for longer than 3 months, and schizophrenics having lower scores on the PANSS negative scale. It's worthy to note that drug-naïve schizophrenics also showed a tendency to have decreased whole blood assay IFN-γ/IL-10 ratios.

- Those schizophrenics whose symptom severities remained nearly unaltered after 8-week treatment had tremendously lower whole blood assay IFN-γ/IL-4 and IFN-γ/IL-10 ratios than their healthy counterparts.

- Early-onset schizophrenics had noticeably reduced serum IFN-γ/IL-4 and IFN-γ/IL-10, nonetheless, enhanced lymphocyte IFN-γ/IL-4 and IFN-γ/IL-13 ratios. Late-onset schizophrenic patients did not have markedly decreased serum Th1/Th2 ratios. But they did show significantly reduced lymphocyte IFN-γ/IL-4 and IFN-γ/IL-13 ratios.

(c) The possible causes of Th1/Th2 imbalance in schizophrenia (results from multiple regression):

- For schizophrenics as a whole group, mainly IFN-γ, IL-4, and IL-10 had been involved in serum Th1/Th2 balancing. IL-6 and TNF-α could have contributed to the balancing between IFN-γ and IL-4 in whole blood assay, while IL-4 and age

might have impacts on the whole blood assay IFN-γ/IL-10 imbalance in schizophrenia.

- For schizophrenic females, although the variables measured in this study were able to predict serum IFN-γ/IL-4 sufficiently ($\geq$ 67% or 2/3), no clear source for the variance of serum IFN-γ/IL-4 could be found. On the contrary, the balance between whole blood assay IFN-γ and IL-4 was markedly influenced by a variety of parameters including IFN-γ, IL-4, TNF-α, IL-6, prolactin, estradiol, testosterone, and age. Similarly, numerous variables could significantly predict the serum and whole blood assay IFN-γ/IL-10 in schizophrenic women. Among those predictors existed very tangled inter-correlations, in contrast to those in female controls.

- For the whole group of male schizophrenics, there were likely some other crucial factors than those measured in this study involved in the IFN-γ/IL-10 balancing. Age, prolactin, and estradiol were important for the variance of serum IFN-γ/IL-10, while IL-6 was essential for the whole blood assay IFN-γ/IL-10 in male patients, in addition to IFN-γ and IL-10. Furthermore, no significant influence from testosterone, SHBG, and estradiol on the whole blood assay IFN-γ/IL-10 was found in schizophrenic men as in the case of male controls.

(d) Psychopathology and Th1/Th2 ratios in schizophrenia: The scores on the PANSS negative subscale were found to correlate positively with the whole blood assay IFN-γ/IL-4 and IFN-γ/IL-10 ratios. In addition, the scores on the PANSS global scale also had a positive association with the whole blood assay IFN-γ/IL-4.

(6) **Conclusion and Discussion**:

(a) The results of this study revealed clear serum Th2-shifts in distinct schizophrenic subgroups and offered supporting evidence for the Th2-shift hypothesis of schizophrenia.

(b) Th2-shifts in schizophrenia seemed to be rather the consequences of complicated interactions among disease process, age-related abnormalities (particularly in hormones), and anti-psychotic medication. But they were not simply the results of anti-psychotic treatment and/or aging-induced alterations.

Zusammenfassung

Die Schizophrenie ist eine komplexe Erkrankung, bei der neben einer genetischen Komponente äußere Einflussfaktoren eine wichtige Rolle spielen. Epidemiologische Studien weisen auf eine mögliche Rolle von Virusinfektionen als Umwelt-Faktor in der Ätiologie der Schizophrenie hin. Eine Verschiebung der spezifischen Immunantwort in Richtung T-helfer-2-Antwort (ein sogenannter Th2-shift) wurde bei verschiedenen Virusinfektionen beobachtet. Einige immunologische Untersuchungen weisen auch zumindest bei einer Subgruppe der Schizophrenie auf einen Th2-shift hin.

(1) **Ziele**: Diese Studie dient (a) der Untersuchung der Th1/Th2-Balance der spezifischen Immunantwort unter Berücksichtigung der Effekte verschiedener endokrinologischer Parameter und (b) der Identifizierung der möglichen Ursachen des gestörten Th1/Th2-Gleichgewichts; die hier untersuchten Einflussgrößen beziehen sich auf unterschiedliche Hormone.

(2) **Fragestellungen**:

(a) Lässt sich eine Th2-Verschiebung bei einer Subgruppe der Schizophrenie beobachten, nachdem die Einflüsse diverser endokrinologischer Parameter mitberücksichtigt worden sind?

(b) Wenn ja, ist diese Subgruppe durch klinische oder epidemiologische Variablen charakterisierbar?

(c) Wenn ja, welcher oder welche der untersuchten immunologischen und endokrinologischen Parameter tragen zur Streuung des Th1/Th2-Verhältnises bei schizophrenen Patienten bei?

(3) **Hypothese**:

(a) Zur Frage (2a) ist eine Th2-Verschiebung angenommen; d.h., die Th1/Th2-Quotienten sind deutlich reduziert. Die Quotienten IFN-γ/IL-4, IFN-γ/IL-10 und IFN-γ/IL-13 wurden als Indikatoren der Th1/Th2-Balance betrachtet.

(b) Frage (2b) und (2c) sind offene Fragen, weshalb keine Hypothese im Bezug auf diese beiden Fragen gestellt wurde.

(4) **Methoden**:

(a) *Analyse-Materialien* schließen Serum, Voll-Blut und isolierte Lymphozyten ein. „Vollständige Serum-Daten" bedeutet, dass alle Daten für Serum-Zytokin-Konzentrationen, Serum Th1/Th2-Quotienten, Hormone, SHBG (Sexhormon-

bindendes Globulin), Geschlecht und Alter vorhanden waren. Ebenso bedeutet „vollständige Voll-Blut-Daten", dass alle Daten bezüglich der in vitro Zytokin-Produktion im Voll-Blut nach einer 46-stündigen PHA-Stimulation, Voll-Blut-Th1/Th2 Quotienten, Hormone, SHBG, Geschlecht und Alter erhoben wurden. „Vollständige Lymphozyten-Daten" bedeutet, dass alle Daten hinsichtlich der in-vitro Zytokin-Freisetzung bei Lymphozyten, Th1/Th2-Quotienten, Hormone, Geschlecht und Alter verfügbar waren.

(b) *Studien-Teilnehmer*: Insgesamt nahmen 114 schizophrene Patienten und 101 gesunde Probanden an die Studie teil. Unter ihnen hatten 76 schizophrene Patienten und 75 Kontrollen vollständige Serum-Daten, 44 Patienten und 76 normale Kontrollen hatten vollständige Voll-Blut-Daten, 72 schizophrene Patienten und 98 gesunde Teilnehmer hatten vollständige Lymphozyten-Daten.

(c) *Variablen* umfassen hauptsächlich immunologische, endokrinologische und verschiedene klinische Parameter. Die immunologischen Variablen bestehen aus Th1-Zytokinen wie IFN-γ, IL-12, IL-2, TNF-α und Th2-Zytokinen einschließlich IL-4, IL-10, IL-13 und IL-6. Die endokrinologischen Kenngrößen setzen sich aus den folgenden Parametern zusammen: zwei Stress-Hormone Cortisol und Prolactin, zwei Geschlechts-Hormone Östradiol und Testosteron, sowie das Geschlechts-Hormon-bindende Globulin (SHBG). Die erhobenen klinischen Daten schließen die Folgenden ein: klinische diagnostische Subgruppen, Familienanamnese bezüglich psychiatrischer Erkrankungen, Medikation vor der Aufnahme, Krankheitsepisode, Antipsychotika-frei/Antipsychotika-naiv, Wash-out-Periode, Erstmanifestationsalter der Erkrankung, Krankheitsdauer, CGI-Werte bei der Aufnahme und Entlassung (CGI = Clinical Global Impressions), sowie die verschieden PANSS Subskalen (Negativ-Symptomatik, Positiv-Symptomatik und Globale Symtpomatik; PANSS = Posivtive and Negative Syndrome Scale).

(d) *Analyse-Methoden* enthalten Cytometric Bead Array (CBA), ELISA und ELISPOT. CBA wurde zur Messung von IFN-γ, IL-2, TNF-α, IL-4, IL-10 und IL-6 im Zellkulturüberstand des Voll-Blut-Assays und im Serum verwendet, ELISA wurde zur Bestimmung der IL-12- und IL-13-Produktion im PHA-stimulierten Voll-Blut-Assay eingesetzt, während ELISPOT zum Erfassen der in-vitro-Produktion von IFN-γ, IL-12, IL-4, IL-13 und IL-10 bei Lymphozyten benutzt wurde. Die Serumkonzentrationen der Hormone Prolactin, Cortisol, Östradiol, Testosteron, sowie

SHBG wurden mit entsprechenden Reagenzienkits am Analysenautomaten Elecsys 2010 erhoben.

(e) *Auswertung*: Die schizophrenen Patienten wurden zuerst als eine ganze Gruppe untersucht, danach nach Geschlecht und verschiedenen klinischen Eigenschaften in unterschiedliche Subgruppen eingeteilt; die so gebildeten verschiedenen Subgruppen sind die unabhängigen Variablen. Die wichtigen abhängigen Variablen sind Th1/Th2-Quotienten einschließlich IFN-γ/IL-4, IFN-γ/IL-10 (Serum, Voll-Blut-Assay, Lymphozyten) und IFN-γ/IL-13 (Lymphozyten). Bei auffälligen Unterschied(en) bezüglich Alter, oder Hormonkonzentrationen und SHBG zwischen einer schizophrenen Subgruppe und den entsprechenden Kontrollen wurden diese Parameter als Kovarianten in die Analyse eingeschlossen, um ihre Effekte auf die Th1/Th2-Balance bei den zu vergleichenden Gruppen zu kontrollieren.

(f) *Statistik*: MAN(C)OVA und Multiple Regression. MAN(C)OVA wurde verwendet, um die Fragestellung (2a) und (2b) zu untersuchen, während Multiple Regression zur Beantwortung der Fragestellung (2c) diente.

(5) **Primäre Ergebnisse**:

(a) Die Ergebnisse dieser Studie unterstützen unsere Hypothese einer Th2-Verschiebung zumindest bei einer Subgruppe der Schizophrenie.

(b) Befunde bezüglich der Th1/Th2-Balance in Schizophrenie (Resultate der MAN(C)OVA):

- Die Serum-Daten deuteten auf eine eindeutige Th2-Verschiebung bei schizophrenen Patienten als Gesamtgruppe hin, nachdem die Effekte von Alter und verschiedener Hormone (insbesondere Prolactin) ausgeschlossen worden waren.

- Die Th2-Verschiebung im Serum scheint Schizophrenie-spezifisch zu sein, wie die Daten der Patienten mit schizophrenie-ähnlicher Symptomatik zeigen.

- Im geschlechts-spezifischen Vergleich zu gesunden Probanden hatten weibliche schizophrene Patienten signifikant reduzierte Quotient sowohl für Serum IFN-γ/IL-4 als auch für IFN-γ/IL-10, während männliche Patienten ausschließlich einen deutlich verminderten Serum IFN-γ/IL-10 Quotient zeigten.

- Reduzierte Serum IFN-γ/IL-4- und IFN-γ/IL-10-Quotienten wurden ebenfalls bei diversen klinischen Subgruppen beobachtet außer bei schizophrenen Patienten mit vorwiegender Positivsymptomatik.

- Ein deutlich reduzierter IFN-γ/IL-10-Quotient im PHA-stimulierten Voll-Blut wurde (a) bei Nicht-Paranoid oder chronischen schizophrenen Patienten gezeigt, bei

194

Patienten, die (b) eine positive psychiatrische Familienanamnese hatten und (c) vor Einschluss in die Studie länger als 3 Monate Antipsychotika-frei gewesen waren oder (d) bei Aufnahme in die stationär-psychiatrische Behandlung niedrigere Werte auf der PANSS-Negativ-Skala hatten. Bemerkenswerter weise zeigten auch Antipsychotika-naive Patienten mit Schizophrenie tendenziell einen beträchtlich reduzierten IFN-γ/IL-10-Quotient im Voll-Blut.

- Die schizophrenen Patienten, deren Symptome nach einer 8-wöchigen Behandlung fast unverändert blieben, hatten auffallend niedrigere IFN-γ/IL-4- und IFN-γ/IL-10-Quotienten im Voll-Blut als die gesunden Probanden.

- Die schizophrenen Patienten mit einem frühen Krankheitsausbruch hatten außergewöhnlich reduzierte Serum IFN-γ/IL-4- und IFN-γ/IL-10-Quotienten, aber einen erhöhten IFN-γ/IL-4 und IFN-γ/IL-13 in PHA-stimulierten Lymphozyten. Im Gegensatz zeigten diejenigen mit einem späten Ausbruch keine Änderung der beiden Serum Th1/Th2-Quotienten, jedoch auffallend reduzierte IFN-γ/IL-4- und IFN-γ/IL-13-Quotienten bei in-vitro stimulierten Lymphozyten.

(c) Die möglichen Ursachen der Th1/Th2-Dysbalance bei Schizophrenie-Patienten (Ergebnisse von Multiple-Regression):

- Für die schizophrenen Patienten als ganze Gruppe waren vorwiegend IFN-γ, IL-4 und IL-10 an die Balance zwischen dem Th1- und Th2-System beteiligt. IL-6 und TNF-α könnten zur Balance zwischen IFN-γ und IL-4 im PHA-stimulierten Voll-Blut beigetragen haben, während IL-4 und das Alter offensichtliche Einflüsse auf die Balance zwischen IFN-γ und IL-10 im Voll-Blut bei Patienten mit Schizophrenie gehabt haben dürften.

- Für die schizophrenen Patientinnen wurde keine eindeutige Quelle für das Ausbalancieren zwischen Serum IFN-γ und IL-4 gefunden, obwohl die gemessenen Variablen in der Lage waren, die IFN-γ/IL-4-Varianz zuverlässig vorherzusagen (d.h. ≥67% oder 2/3 der Varianz waren dadurch erklärbar). Das Abgleichen zwischen IFN-γ und IL-4 im Voll-Blut nach PHA-Stimulation wurde eher von den komplexen wechselseitigen Korrelationen unter IFN-γ, IL-4, TNF-α, IL-6, Prolactin, Östradiol, Testosteron und Alter beeinflusst. Ähnlich komplexe Inter-Korrelationen unter diesen obengenannten Kenngrößen wurden ebenfalls beim Ausgleichen zwischen IFN-γ und IL-10 sowohl im Serum als auch im PHA-stimulierten Voll-Blut beobachtet.

- Für männliche schizophrene Patienten gab es vermutlich einige andere entscheidende Faktoren, welche in dieser Studie nicht geprüft worden waren, die jedoch an der Balancierung zwischen IFN-γ und IL-10 im Voll-Blut beteiligt gewesen waren. Im Gegensatz zu gesunden Probanden könnten Alter, Prolactin und Östradiol zusätzlich am Abgleichen von Serum IFN-γ/IL-10 beteiligt gewesen sein. Hingegen war IL-6 am Abgleichen von IFN-γ/IL-10 Voll-Blut-Assay bei männlichen schizophrenen Patienten beteiligt. Beachtenswerte Beiträge von Testosteron, SHBG und Östradiol zur Balancierung vom Voll-Blut IFN-γ/IL-10 wie im Fall der Kontrollen waren bei männlichen Patienten mit Schizophrenie nicht zu beobachten.

(d) Psychopathologie und Th1/Th2-Quotienten: Der durchschnittliche Messwert auf der PANSS-Negativ-Skala korrelierte positiv mit Voll-Blut-Assay IFN-γ/IL-4 und IFN-γ/IL-10. Außerdem war der Mittelwert auf der PANSS Global Skala ebenfalls positiv mit Voll-Blut IFN-γ/IL-4 assoziiert.

(6) **Schlussfolgerung und Diskussion**:

(a) Die Ergebnisse dieser Studie zeigen deutliche Th2-Verschiebungen im Serum bei verschiedenen schizophrenen Subgruppen und bieten einen eher unterstützenden Hinweis für die Hypothese der Th2-Verschiebung von Schizophrenie.

(b) Th2-Verschiebungen bei schizophrenen Patienten scheinen eine komplexe Folge von Wechselwirkungen von Krankheitsprozess, Hormonen und antipsychotischer Medikation, jedoch wahrscheinlich nicht nur ein Resultat der antipsychotischen Behandlung oder der durch Alterung ausgelösten Veränderungen zu sein.

References

Abbas,A.K. (2003). The control of T cell activation vs. tolerance. Autoimmun. Rev *2*, 115-118.

Abel,K.M., O'Keane,V., and Murray,R.M. (1996). Enhancement of the prolactin response to d-fenfluramine in drug-naive schizophrenic patients. Br J Psychiatry *168*, 57-60.

Adams,W., Kendell,R.E., Hare,E.H., and Munk-Jorgensen,P. (1993). Epidemiological evidence that maternal influenza contributes to the aetiology of schizophrenia. An analysis of Scottish, English, and Danish data. Br J Psychiatry *163*, 522-534.

Aderka,D., Engelmann,H., Wysenbeek,A.J., and Levo,Y. (1992). The possible role of tumor necrosis factor (TNF) and its natural inhibitors, the soluble-TNF receptors, in autoimmune diseases. Isr. J Med Sci *28*, 126-130.

Aggarwal,B.B. (1992). Comparative analysis of the structure and function of TNF-alpha and TNF-beta. Immunol Ser. *56*, 61-78.

Agnello,D., Lankford,C.S., Bream,J., Morinobu,A., Gadina,M., O'Shea,J.J., and Frucht,D.M. (2003). Cytokines and transcription factors that regulate T helper cell differentiation: new players and new insights. J Clin Immunol *23*, 147-161.

Airoldi,I., Gri,G., Marshall,J.D., Corcione,A., Facchetti,P., Guglielmino,R., Trinchieri,G., and Pistoia,V. (2000). Expression and function of IL-12 and IL-18 receptors on human tonsillar B cells. J Immunol *165*, 6880-6888.

Akaboshi,I., Nagayoshi,I., Omura,M., and Iwaki,K. (2001). Elevated serum levels of interleukin-10 in children with acute rubella infection. Scand. J Infect. Dis. *33*, 462-465.

Akpek,E.K. and Gottsch,J.D. (2003). Immune defense at the ocular surface. Eye *17*, 949-956.

Alaniz,R.C., Thomas,S.A., Perez-Melgosa,M., Mueller,K., Farr,A.G., Palmiter,R.D., and Wilson,C.B. (1999). Dopamine beta-hydroxylase deficiency impairs cellular immunity. Proc. Natl. Acad Sci U. S A *96*, 2274-2278.

Alas,S., Emmanouilides,C., and Bonavida,B. (2001). Inhibition of interleukin 10 by rituximab results in down-regulation of bcl-2 and sensitization of B-cell non-Hodgkin's lymphoma to apoptosis. Clin Cancer Res. *7*, 709-723.

Alexaki,V.I., Charalampopoulos,I., Kampa,M., Vassalou,H., Theodoropoulos,P., Stathopoulos,E.N., Hatzoglou,A., Gravanis,A., and Castanas,E. (2004). Estrogen exerts neuroprotective effects via membrane estrogen receptors and rapid Akt/NOS activation. FASEB J.

Allaman,I., Pellerin,L., and Magistretti,P.J. (2004). Glucocorticoids modulate neurotransmitter-induced glycogen metabolism in cultured cortical astrocytes. J Neurochem. *88*, 900-908.

Almanzar,G., Schwaiger,S., Jenewein,B., Keller,M., Grubeck-Loebenstein,B., Wurzner,R., and Schonitzer,D. (2004). IFN-gamma production by CMV-specific CD8+ T cells is high in elderly donors. Exp Gerontol. *39*, 863-865.

Aloisi,F., Ria,F., Penna,G., and Adorini,L. (1998). Microglia are more efficient than astrocytes in antigen processing and in Th1 but not Th2 cell activation. J Immunol *160*, 4671-4680.

Altamura,A.C., Boin,F., and Maes,M. (1999). HPA axis and cytokines dysregulation in schizophrenia: potential implications for the antipsychotic treatment. Eur Neuropsychopharmacol *10*, 1-4.

American Psychiatric Association (1994). Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition., American Psychiatric Association, ed. (Washington, DC: American Psychiatric Association).

Anderson,D.C. (1974). Sex-hormone-binding globulin. Clin Endocrinol. (Oxf) *3*, 69-96.

Anderson,P., Sundstedt,A., Li,L., O'Neill,E.J., Li,S., Wraith,D.C., and Wang,P. (2003). Differential activation of signal transducer and activator of transcription (STAT)3 and STAT5 and induction of suppressors of cytokine signalling in T(h)1 and T(h)2 cells. Int Immunol *15*, 1309-1317.

Andreasen,N.C. (1995). Symptoms, signs, and diagnosis of schizophrenia. Lancet *346*, 477-481.

Angele,M.K., Knoferl,M.W., Ayala,A., Bland,K.I., and Chaudry,I.H. (2001). Testosterone and estrogen differently effect Th1 and Th2 cytokine release following trauma-haemorrhage. Cytokine *16*, 22-30.

Araujo,D.M., Lapchak,P.A., Collier,B., and Quirion,R. (1989). Localization of interleukin-2 immunoreactivity and interleukin-2 receptors in the rat brain: interaction with the cholinergic system. Brain Res. *498*, 257-266.

Armitage,R.J., Macduff,B.M., Spriggs,M.K., and Fanslow,W.C. (1993). Human B cell proliferation and Ig secretion induced by recombinant CD40 ligand are modulated by soluble cytokines. J Immunol *150*, 3671-3680.

Arolt,V., Rothermundt,M., Wandinger,K.P., and Kirchner,H. (2000). Decreased in vitro production of interferon-gamma and interleukin-2 in whole blood of patients with schizophrenia during treatment. Mol Psychiatry *5*, 150-158.

Arolt,V., Weitzsch,C., Wilke,I., Nolte,A., Pinnow,M., Rothermundt,M., and Kirchner,H. (1997). Production of interferon-gamma in families with multiple occurrence of schizophrenia. Psychiatry Res. *66*, 145-152.

Aste-Amezaga,M., D'Andrea,A., Kubin,M., and Trinchieri,G. (1994). Cooperation of natural killer cell stimulatory factor/interleukin-12 with other stimuli in the induction of cytokines and cytotoxic cell-associated molecules in human T and NK cells. Cell Immunol *156*, 480-492.

Austin,A.S., Haas,K.M., Naugler,S.M., Bajer,A.A., Garcia-Tapia,D., and Estes,D.M. (2003). Identification and characterization of a novel regulatory factor: IgA-inducing protein. J Immunol *171*, 1336-1342.

Ausubel,L.J., Krieger,J.I., and Hafler,D.A. (1997). Changes in cytokine secretion induced by altered peptide ligands of myelin basic protein peptide 85-99. J Immunol *159*, 2502-2512.

Avery,P.R. and Hoover,E.A. (2004). Gamma interferon/interleukin 10 balance in tissue lymphocytes correlates with down modulation of mucosal feline immunodeficiency virus infection. J Virol. *78*, 4011-4019.

Azzolina,A., Bongiovanni,A., and Lampiasi,N. (2003). Substance P induces TNF-alpha and IL-6 production through NF kappa B in peritoneal mast cells. Biochim. Biophys. Acta *1643*, 75-83.

Bach,E.A., Aguet,M., and Schreiber,R.D. (1997). The IFN gamma receptor: a paradigm for cytokine receptor signaling. Annu. Rev Immunol *15*, 563-591.

Ballestas,M.E. and Benveniste,E.N. (1995). Interleukin 1-beta- and tumor necrosis factor-alpha-mediated regulation of ICAM-1 gene expression in astrocytes requires protein kinase C activity. Glia *14*, 267-278.

Barak,V., Barak,Y., Levine,J., Nisman,B., and Roisman,I. (1995). Changes in interleukin-1 beta and soluble interleukin-2 receptor levels in CSF and serum of schizophrenic patients. J Basic Clin Physiol Pharmacol. *6*, 61-69.

Barcena,A., Toribio,M.L., Gutierrez-Ramos,J.C., Kroemer,G., and Martinez,C. (1991). Interplay between IL-2 and IL-4 in human thymocyte differentiation: antagonism or agonism. Int Immunol *3*, 419-425.

Barish,M.E., Mansdorf,N.B., and Raissdana,S.S. (1991). Gamma-interferon promotes differentiation of cultured cortical and hippocampal neurons. Dev. Biol *144*, 412-423.

Barna,B.P., Mattera,R., Jacobs,B.S., Drazba,J., Estes,M.E., Prayson,R.A., and Barnett,G.H. (2001). Epidermal growth factor regulates astrocyte expression of the interleukin-4 receptor via a MAPK-independent pathway. Cell Immunol *208*, 18-24.

Barr,C.E., Mednick,S.A., and Munk-Jorgensen,P. (1990). Exposure to influenza epidemics during gestation and adult schizophrenia. A 40-year study. Arch Gen Psychiatry *47*, 869-874.

Barten,D.M. and Ruddle,N.H. (1994). Vascular cell adhesion molecule-1 modulation by tumor necrosis factor in experimental allergic encephalomyelitis. J Neuroimmunol. *51*, 123-133.

Barton,B.E. (1997). IL-6: insights into novel biological activities. Clin Immunol Immunopathol. *85*, 16-20.

Bartova,L., Rajcani,J., and Pogady,J. (1987). Herpes simplex virus antibodies in the cerebrospinal fluid of schizophrenic patients. Acta Virol. *31*, 443-446.

Basaria,S., Nguyen,T., Rosenson,R.S., and Dobs,A.S. (2002). Effect of methyl testosterone administration on plasma viscosity in postmenopausal women. Clin Endocrinol. (Oxf) *57*, 209-214.

Bazzoni,F. and Beutler,B. (1996). The tumor necrosis factor ligand and receptor families. N Engl. J Med *334*, 1717-1725.

Becker,D., Kritschmann,E., Floru,S., Shlomo-David,Y., and Gotlieb-Stematsky,T. (1990). Serum interferon in first psychotic attack. Br J Psychiatry *157*, 136-138.

Ben Sasson,S.Z., Gerstel,R., Hu-Li,J., and Paul,W.E. (2001). Cell division is not a "clock" measuring acquisition of competence to produce IFN-gamma or IL-4. J Immunol *166*, 112-120.

Beniguel,L., Diallo,T.O., Remoue,F., Williams,D.L., Cognasse,F., Charrier-Mze,N., N'Diaye,A.A., Perraut,R., Capron,M., Riveau,G., and Garraud,O. (2003). Differential production in vitro of antigen specific IgG1, IgG3 and IgA: a study in Schistosoma haematobium infected individuals. Parasite Immunol *25*, 39-44.

Benveniste,E.N. (1997). Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis. J Mol Med *75*, 165-173.

Benveniste,E.N. and Merrill,J.E. (1986). Stimulation of oligodendroglial proliferation and maturation by interleukin-2. Nature *321*, 610-613.

Bernardes-Silva,M., Anthony,D.C., Issekutz,A.C., and Perry,V.H. (2001). Recruitment of neutrophils across the blood-brain barrier: the role of E- and P-selectins. J Cereb. Blood Flow Metab *21*, 1115-1124.

Bernardini,R., Kamilaris,T.C., Calogero,A.E., Johnson,E.O., Gomez,M.T., Gold,P.W., and Chrousos,G.P. (1990). Interactions between tumor necrosis factor-alpha, hypothalamic corticotropin-releasing hormone, and adrenocorticotropin secretion in the rat. Endocrinology *126*, 2876-2881.

Biernacki,K., Prat,A., Blain,M., and Antel,J.P. (2001). Regulation of Th1 and Th2 lymphocyte migration by human adult brain endothelial cells. J Neuropathol. Exp Neurol. *60*, 1127-1136.

Bilici,M., Tekelioglu,Y., Efendioglu,S., Ovali,E., and Ulgen,M. (2003). The influence of olanzapine on immune cells in patients with schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry *27*, 483-485.

Blanchard,C., Durual,S., Estienne,M., Bouzakri,K., Heim,M.H., Blin,N., and Cuber,J.C. (2004). IL-4 and IL-13 up-regulate intestinal trefoil factor expression: requirement for STAT6 and de novo protein synthesis. J Immunol *172*, 3775-3783.

Bluthe,R.M., Bristow,A., Lestage,J., Imbs,C., and Dantzer,R. (2001). Central injection of interleukin-13 potentiates LPS-induced sickness behavior in rats. Neuroreport *12*, 3979-3983.

Bochner,B.S., Klunk,D.A., Sterbinsky,S.A., Coffman,R.L., and Schleimer,R.P. (1995). IL-13 selectively induces vascular cell adhesion molecule-1 expression in human endothelial cells. J Immunol *154*, 799-803.

Boehm,U., Klamp,T., Groot,M., and Howard,J.C. (1997). Cellular responses to interferon-gamma. Annu. Rev Immunol *15*, 749-795.

Bondy,B. (2002). [Clinical and neurological aspects of schizophrenia. The disease structure of schizophrenia]. Pharm. Unserer Zeit *31*, 530-536.

Booth,M., Mwatha,J.K., Joseph,S., Jones,F.M., Kadzo,H., Ireri,E., Kazibwe,F., Kemijumbi,J., Kariuki,C., Kimani,G., Ouma,J.H., Kabatereine,N.B., Vennervald,B.J., and Dunne,D.W. (2004). Periportal fibrosis in

human Schistosoma mansoni infection is associated with low IL-10, low IFN-gamma, high TNF-alpha, or low RANTES, depending on age and gender. J Immunol *172*, 1295-1303.

Boukhris,W., Kouassi,E., and Revillard,J.P. (1988). Differential effect of mixed D1/D2 and selective D2 dopaminergic antagonists on mouse T and B lymphocyte proliferation and interleukin production in vitro. Immunopharmacol. Immunotoxicol. *10*, 501-512.

Braff,D.L., Heaton,R., Kuck,J., Cullum,M., Moranville,J., Grant,I., and Zisook,S. (1991). The generalized pattern of neuropsychological deficits in outpatients with chronic schizophrenia with heterogeneous Wisconsin Card Sorting Test results. Arch Gen Psychiatry *48*, 891-898.

Brakenhoff,J.P., de Hon,F.D., and Aarden,L.A. (1995). Development of human IL-6 receptor antagonists. Ann N Y Acad Sci *762*, 129-134.

Brambilla,F. (1980). Neuroendocrine function in schizophrenia. Acta Psychiatr Belg. *80*, 421-435.

Brand,J.M., Frohn,C., Cziupka,K., Brockmann,C., Kirchner,H., and Luhm,J. (2004). Prolactin triggers pro-inflammatory immune responses in peripheral immune cells. Eur Cytokine Netw. *15*, 99-104.

Braun,C.M., Huang,S.K., Bashian,G.G., Kagey-Sobotka,A., Lichtenstein,L.M., and Essayan,D.M. (1997). Corticosteroid modulation of human, antigen-specific Th1 and Th2 responses. J Allergy Clin Immunol *100*, 400-407.

Breidthardt,T., Frohn,C., Luhm,J., Kirchner,H., and Brand,J.M. (2002). Prolactin induces enhanced interferon gamma release in peripheral whole blood after stimulation with either PHA or LPS. Immunobiology *206*, 424-431.

Breytenbach,U., Clark,A., Lamprecht,J., and Bouic,P. (2001). Flow cytometric analysis of the Th1-Th2 balance in healthy individuals and patients infected with the human immunodeficiency virus (HIV) receiving a plant sterol/sterolin mixture. Cell Biol Int *25*, 43-49.

Brodie,C., Goldreich,N., Haiman,T., and Kazimirsky,G. (1998). Functional IL-4 receptors on mouse astrocytes: IL-4 inhibits astrocyte activation and induces NGF secretion. J Neuroimmunol. *81*, 20-30.

Brosnan,C.F., Cannella,B., Battistini,L., and Raine,C.S. (1995). Cytokine localization in multiple sclerosis lesions: correlation with adhesion molecule expression and reactive nitrogen species. Neurology *45*, S16-S21.

Brown,A.S. and Susser,E.S. (2002). In utero infection and adult schizophrenia. Ment Retard. Dev. Disabil. Res. Rev *8*, 51-57.

Brunner,T., Heusser,C.H., and Dahinden,C.A. (1993). Human peripheral blood basophils primed by interleukin 3 (IL-3) produce IL-4 in response to immunoglobulin E receptor stimulation. J Exp Med *177*, 605-611.

Bullens,D.M., Kasran,A., Thielemans,K., Bakkus,M., and Ceuppens,J.L. (2001). CD40L-induced IL-12 production is further enhanced by the Th2 cytokines IL-4 and IL-13. Scand. J Immunol *53*, 455-463.

Burd,P.R., Thompson,W.C., Max,E.E., and Mills,F.C. (1995). Activated mast cells produce interleukin 13. J Exp Med *181*, 1373-1380.

Burger,D. and Dayer,J.M. (2002). Cytokines, acute-phase proteins, and hormones: IL-1 and TNF-alpha production in contact-mediated activation of monocytes by T lymphocytes. Ann N Y Acad Sci *966*, 464-473.

Buske-Kirschbaum,A., von Auer,K., Krieger,S., Weis,S., Rauh,W., and Hellhammer,D. (2003). Blunted cortisol responses to psychosocial stress in asthmatic children: a general feature of atopic disease? Psychosom. Med *65*, 806-810.

Cai,G., Kastelein,R.A., and Hunter,C.A. (1999). IL-10 enhances NK cell proliferation, cytotoxicity and production of IFN-gamma when combined with IL-18. Eur J Immunol *29*, 2658-2665.

Callard,R.E., Matthews,D.J., and Hibbert,L. (1996). IL-4 and IL-13 receptors: are they one and the same? Immunol Today *17*, 108-110.

Camacho-Arroyo,I., Gonzalez-Arenas,A., Gonzalez-Aguero,G., Guerra-Araiza,C., and Gonzalez-Moran,G. (2003). Changes in the content of progesterone receptor isoforms and estrogen receptor alpha in the chick brain during embryonic development. Comp Biochem. Physiol A Mol Integr. Physiol *136*, 447-452.

Carballido,J.M., Aversa,G., Kaltoft,K., Cocks,B.G., Punnonen,J., Yssel,H., Thestrup-Pedersen,K., and de Vries,J.E. (1997). Reversal of human allergic T helper 2 responses by engagement of signaling lymphocytic activation molecule. J Immunol *159*, 4316-4321.

Carpenter,W.T., Jr. and Buchanan,R.W. (1994). Schizophrenia. N Engl. J Med *330*, 681-690.

Carr,L., Tucker,A., and Fernandez-Botran,R. (2003). The enhancement of T cell proliferation by L-dopa is mediated peripherally and does not involve interleukin-2. J Neuroimmunol. *142*, 166-169.

Carreno,P.C., Jimenez,E., Sacedon,R., Vicente,A., and Zapata,A.G. (2004). Prolactin stimulates maturation and function of rat thymic dendritic cells. J Neuroimmunol. *153*, 83-90.

Carson,W.E., Lindemann,M.J., Baiocchi,R., Linett,M., Tan,J.C., Chou,C.C., Narula,S., and Caligiuri,M.A. (1995). The functional characterization of interleukin-10 receptor expression on human natural killer cells. Blood *85*, 3577-3585.

Cassatella,M.A., Meda,L., Gasperini,S., D'Andrea,A., Ma,X., and Trinchieri,G. (1995). Interleukin-12 production by human polymorphonuclear leukocytes. Eur J Immunol *25*, 1-5.

Cavaillon,J.M. (1994). Cytokines and macrophages. Biomed. Pharmacother. *48*, 445-453.

Cazzullo,C.L., Sacchetti,E., Galluzzo,A., Panariello,A., Adorni,A., Pegoraro,M., Bosis,S., Colombo,F., Trabattoni,D., Zagliani,A., and Clerici,M. (2002). Cytokine profiles in schizophrenic patients treated with risperidone: a 3-month follow-up study. Prog Neuropsychopharmacol Biol Psychiatry *26*, 33-39.

Cazzullo,C.L., Sacchetti,E., Galluzzo,A., Panariello,A., Colombo,F., Zagliani,A., and Clerici,M. (2001). Cytokine profiles in drug-naive schizophrenic patients. Schizophr Res. *47*, 293-298.

Cazzullo,C.L., Scarone,S., Grassi,B., Vismara,C., Trabattoni,D., Clerici,M., and Clerici,M. (1998). Cytokines production in chronic schizophrenia patients with or without paranoid behaviour. Prog Neuropsychopharmacol Biol Psychiatry *22*, 947-957.

Chadban,S.J., Tesch,G.H., Foti,R., Lan,H.Y., Atkins,R.C., and Nikolic-Paterson,D.J. (1998). Interleukin-10 differentially modulates MHC class II expression by mesangial cells and macrophages in vitro and in vivo. Immunology *94*, 72-78.

Che,S. and Huston,D.P. (1994). Natural killer cell suppression of IgM production. Nat Immun *13*, 258-269.

Chen,C.H., Chiu,Y.L., Wei,F.C., Koong,F.J., Liu,H.C., Shaw,C.K., Hwu,H.G., and Hsiao,K.J. (1999). High seroprevalence of Borna virus infection in schizophrenic patients, family members and mental health workers in Taiwan. Mol Psychiatry *4*, 33-38.

Chen,J., Huang,C., Xiao,D., Chen,H.P., and Cheng,J.S. (2003). Expression of interleukin-6 mRNA in ischemic rat brain after electroacupuncture stimulation. Acupunct. Electrother. Res. *28*, 157-166.

Chen,S., Hu,D., Shi,X., Shen,N., Gu,Y., and Bao,C. (2000). The relationship between Th1/Th2-type cells and disease activity in patients with systemic lupus erythematosus. Chin Med J (Engl.) *113*, 877-880.

Chisari,A.N., Gaillard,R.C., Giovambattista,A., Voirol,M.-J., Piermaria,J., and Spinedi,E. (2000). Sexual dimorphism in the hypothalamo-pituitary-adrenal (HPA) axis and TNFalpha responses to phospholipase A2-related neurotoxin (from crotalus durissus terrifcus) challenge. J Endocrinol. Invest *23*, 440-448.

Chomarat,P., Rissoan,M.C., Banchereau,J., and Miossec,P. (1993). Interferon gamma inhibits interleukin 10 production by monocytes. J Exp Med *177*, 523-527.

Chung,I.Y. and Benveniste,E.N. (1990). Tumor necrosis factor-alpha production by astrocytes. Induction by lipopolysaccharide, IFN-gamma, and IL-1 beta. J Immunol *144*, 2999-3007.

Clark,B.R. (1995). Fate of intercellular MHC-peptide-T-cell receptor complexes during T-cell activation. J Mol Recognit. *8*, 63-66.

Clarke,C.J., Hales,A., Hunt,A., and Foxwell,B.M. (1998). IL-10-mediated suppression of TNF-alpha production is independent of its ability to inhibit NF kappa B activity. Eur J Immunol *28*, 1719-1726.

Coil,J., Tam,E., and Waterfield,J.D. (2004). Proinflammatory cytokine profiles in pulp fibroblasts stimulated with lipopolysaccharide and methyl mercaptan. J Endod. *30*, 88-91.

Collins,J.T. and Dunnick,W.A. (1993). Germline transcripts of the murine immunoglobulin gamma 2a gene: structure and induction by IFN-gamma. Int Immunol *5*, 885-891.

Colton,C.A., Yao,J., Keri,J.E., and Gilbert,D. (1992). Regulation of microglial function by interferons. J Neuroimmunol. *40*, 89-98.

Conti,P., Kempuraj,D., Di Gioacchino,M., Boucher,W., Letourneau,R., Kandere,K., Barbacane,R.C., Reale,M., Felaco,M., Frydas,S., and Theoharides,T.C. (2002). Interleukin-6 and mast cells. Allergy Asthma Proc. *23*, 331-335.

Cornblatt,B., Obuchowski,M., Roberts,S., Pollack,S., and Erlenmeyer-Kimling,L. (1999). Cognitive and behavioral precursors of schizophrenia. Dev. Psychopathol. *11*, 487-508.

Cosenza,M.A., Zhao,M.L., Si,Q., and Lee,S.C. (2002). Human brain parenchymal microglia express CD14 and CD45 and are productively infected by HIV-1 in HIV-1 encephalitis. Brain Pathol. *12*, 442-455.

Croitoru-Lamoury,J., Guillemin,G.J., Boussin,F.D., Mognetti,B., Gigout,L.I., Cheret,A., Vaslin,B., Le Grand,R., Brew,B.J., and Dormont,D. (2003). Expression of chemokines and their receptors in human and simian astrocytes: evidence for a central role of TNF alpha and IFN gamma in CXCR4 and CCR5 modulation. Glia *41*, 354-370.

D'Andrea,A., Aste-Amezaga,M., Valiante,N.M., Ma,X., Kubin,M., and Trinchieri,G. (1993). Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. J Exp Med *178*, 1041-1048.

D'Andrea,A., Ma,X., Aste-Amezaga,M., Paganin,C., and Trinchieri,G. (1995). Stimulatory and inhibitory effects of interleukin (IL)-4 and IL-13 on the production of cytokines by human peripheral blood mononuclear cells: priming for IL-12 and tumor necrosis factor alpha production. J Exp Med *181*, 537-546.

Dalal,S., Parkin,S.M., Homer-Vanniasinkam,S., and Nicolaou,A. (2003). Effect of homocysteine on cytokine production by human endothelial cells and monocytes. Ann Clin Biochem. *40*, 534-541.

Danilovich,N., Harada,N., Sairam,M.R., and Maysinger,D. (2003). Age-related neurodegenerative changes in the central nervous system of estrogen-deficient follitropin receptor knockout mice. Exp Neurol. *183*, 559-572.

Davies,E. and MacKenzie,S.M. (2003). Extra-adrenal production of corticosteroids. Clin Exp Pharmacol. Physiol *30*, 437-445.

De Smedt,T., Van Mechelen,M., De Becker,G., Urbain,J., Leo,O., and Moser,M. (1997). Effect of interleukin-10 on dendritic cell maturation and function. Eur J Immunol *27*, 1229-1235.

de Waal,M.R., Abrams,J., Bennett,B., Figdor,C.G., and de Vries,J.E. (1991). Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med *174*, 1209-1220.

de Waal,M.R., Figdor,C.G., and de Vries,J.E. (1993a). Effects of interleukin 4 on monocyte functions: comparison to interleukin 13. Res. Immunol *144*, 629-633.

de Waal,M.R., Figdor,C.G., Huijbens,R., Mohan-Peterson,S., Bennett,B., Culpepper,J., Dang,W., Zurawski,G., and de Vries,J.E. (1993b). Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes. Comparison with IL-4 and modulation by IFN-gamma or IL-10. J Immunol *151*, 6370-6381.

de Waal,M.R., Yssel,H., and de Vries,J.E. (1993c). Direct effects of IL-10 on subsets of human CD4+ T cell clones and resting T cells. Specific inhibition of IL-2 production and proliferation. J Immunol *150*, 4754-4765.

Del Prete,G., De Carli,M., Almerigogna,F., Daniel,C.K., D'Elios,M.M., Zancuoghi,G., Vinante,F., Pizzolo,G., and Romagnani,S. (1995). Preferential expression of CD30 by human CD4+ T cells producing Th2-type cytokines. FASEB J *9*, 81-86.

Delclaux,C. and Azoulay,E. (2003). Inflammatory response to infectious pulmonary injury. Eur Respir. J Suppl *42*, 10s-14s.

Delgado,A.V., McManus,A.T., and Chambers,J.P. (2003). Production of tumor necrosis factor-alpha, interleukin 1-beta, interleukin 2, and interleukin 6 by rat leukocyte subpopulations after exposure to substance P. Neuropeptides *37*, 355-361.

Dell'Albani,P., Santangelo,R., Torrisi,L., Nicoletti,V.G., de Vellis,J., and Giuffrida Stella,A.M. (2001). JAK/STAT signaling pathway mediates cytokine-induced iNOS expression in primary astroglial cell cultures. J Neurosci. Res. *65*, 417-424.

Della,B.S., Nicola,S., Riva,A., Biasin,M., Clerici,M., and Villa,M.L. (2004). Functional repertoire of dendritic cells generated in granulocyte macrophage-colony stimulating factor and interferon-alpha. J Leukoc. Biol *75*, 106-116.

Descamps,L., Coisne,C., Dehouck,B., Cecchelli,R., and Torpier,G. (2003). Protective effect of glial cells against lipopolysaccharide-mediated blood-brain barrier injury. Glia *42*, 46-58.

Dhiman,N., Ovsyannikova,I.G., Howe,R.C., Ryan,J.E., Jacobson,R.M., and Poland,G.A. (2004). Interleukin-4 induced by measles virus and measles-derived peptides as measured by IL-4 receptor-blocking ELISA. J Immunol Methods *287*, 217-225.

Dickerson,F.B., Boronow,J.J., Stallings,C.R., Origoni,A.E., and Yolken,R.H. (2003). Reduction of symptoms by valacyclovir in cytomegalovirus-seropositive individuals with schizophrenia. Am J Psychiatry *160*, 2234-2236.

Diehl,S. and Rincon,M. (2002). The two faces of IL-6 on Th1/Th2 differentiation. Mol Immunol *39*, 531-536.

Dimitrov,S., Lange,T., Fehm,H.L., and Born,J. (2004a). A regulatory role of prolactin, growth hormone, and corticosteroids for human T-cell production of cytokines. Brain Behav Immun *18*, 368-374.

Dimitrov,S., Lange,T., Tieken,S., Fehm,H.L., and Born,J. (2004b). Sleep associated regulation of T helper 1/T helper 2 cytokine balance in humans. Brain Behav Immun *18*, 341-348.

Dobmeier,M., Frick,E., Frank,S., Franke,C., and Wolfersdorf,M. (2000). Schizophrenic psychosis: a contraindication for treatment of hepatitis C with interferon alpha? Pharmacopsychiatry *33*, 72-74.

Dogusan,Z., Book,M.L., Verdood,P., Yu-Lee,L.Y., and Hooghe-Peters,E.L. (2000). Prolactin activates interferon regulatory factor-1 expression in normal lympho-hemopoietic cells. Eur Cytokine Netw. *11*, 435-442.

Dolganov,G., Bort,S., Lovett,M., Burr,J., Schubert,L., Short,D., McGurn,M., Gibson,C., and Lewis,D.B. (1996). Coexpression of the interleukin-13 and interleukin-4 genes correlates with their physical linkage in the cytokine gene cluster on human chromosome 5q23-31. Blood *87*, 3316-3326.

Dominguez-Santalla,M.J., Vidal,C., Vinuela,J., Perez,L.F., and Gonzalez-Quintela,A. (2001). Increased serum IgE in alcoholics: relationship with Th1/Th2 cytokine production by stimulated blood mononuclear cells. Alcohol Clin Exp Res. *25*, 1198-1205.

Dong,Y. and Benveniste,E.N. (2001). Immune function of astrocytes. Glia *36*, 180-190.

Durali,D., de Goer de Herve MG, Giron-Michel,J., Azzarone,B., Delfraissy,J.F., and Taoufik,Y. (2003). In human B cells, IL-12 triggers a cascade of molecular events similar to Th1 commitment. Blood *102*, 4084-4089.

Ebrinc,S., Top,C., Oncul,O., Basoglu,C., Cavuslu,S., and Cetin,M. (2002). Serum interleukin 1 alpha and interleukin 2 levels in patients with schizophrenia. J Int Med Res. *30*, 314-317.

Egger,J. (1992). [From psychobiological stress research to neuropsychoimmunology]. Padiatr. Padol. *27*, 91-96.

el Mallakh,R.S., Suddath,R.L., and Wyatt,R.J. (1993). Interleukin-1 alpha and interleukin-2 in cerebrospinal fluid of schizophrenic subjects. Prog Neuropsychopharmacol Biol Psychiatry *17*, 383-391.

Elenkov,I.J. (2004). Glucocorticoids and the Th1/Th2 balance. Ann N Y Acad Sci *1024*, 138-146.

Elenkov,I.J. and Chrousos,G.P. (2002). Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity. Ann N Y Acad Sci *966*, 290-303.

Ellerin,T., Rubin,R.H., and Weinblatt,M.E. (2003). Infections and anti-tumor necrosis factor alpha therapy. Arthritis Rheum. *48*, 3013-3022.

Ellison,M.D., Povlishock,J.T., and Merchant,R.E. (1987). Blood-brain barrier dysfunction in cats following recombinant interleukin-2 infusion. Cancer Res. *47*, 5765-5770.

Elmlinger,M.W., Kuhnel,W., and Ranke,M.B. (2002). Reference ranges for serum concentrations of lutropin (LH), follitropin (FSH), estradiol (E2), prolactin, progesterone, sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), cortisol and ferritin in neonates, children and young adults. Clin Chem. Lab Med *40*, 1151-1160.

Engelhardt,B., Conley,F.K., Kilshaw,P.J., and Butcher,E.C. (1995). Lymphocytes infiltrating the CNS during inflammation display a distinctive phenotype and bind to VCAM-1 but not to MAdCAM-1. Int Immunol *7*, 481-491.

Engelhardt,B., Vestweber,D., Hallmann,R., and Schulz,M. (1997). E- and P-selectin are not involved in the recruitment of inflammatory cells across the blood-brain barrier in experimental autoimmune encephalomyelitis. Blood *90*, 4459-4472.

Erbagci,A.B., Herken,H., Koyluoglu,O., Yilmaz,N., and Tarakcioglu,M. (2001). Serum IL-1beta, sIL-2R, IL-6, IL-8 and TNF-alpha in schizophrenic patients, relation with symptomatology and responsiveness to risperidone treatment. Mediators Inflamm. *10*, 109-115.

Essner,R., Huynh,Y., Nguyen,T., Rose,M., Kojima,M., and Hoon,D.S. (2001). Functional interleukin-4 receptor and interleukin-2 receptor common gamma chain in human gastric carcinoma: a possible mechanism for cytokine-based therapy. J Gastrointest. Surg. *5*, 81-90.

Fabbri,M., Smart,C., and Pardi,R. (2003). T lymphocytes. Int J Biochem. Cell Biol *35*, 1004-1008.

Farrar,J.D., Asnagli,H., and Murphy,K.M. (2002). T helper subset development: roles of instruction, selection, and transcription. J Clin Invest *109*, 431-435.

Ferguson-Smith,A.C., Chen,Y.F., Newman,M.S., May,L.T., Sehgal,P.B., and Ruddle,F.H. (1988). Regional localization of the interferon-beta 2/B-cell stimulatory factor 2/hepatocyte stimulating factor gene to human chromosome 7p15-p21. Genomics *2*, 203-208.

Ferrer,J.M., Plaza,A., Kreisler,M., and Diaz-Espada,F. (1992). Differential interleukin secretion by in vitro activated human CD45RA and CD45RO CD4+ T cell subsets. Cell Immunol *141*, 10-20.

Ferrero,E. (2004). Assessment of TNFalpha-induced endothelial damage through the loss of its barrier function. Methods Mol Med *98*, 127-136.

Findling,R.L., Kusumakar,V., Daneman,D., Moshang,T., De Smedt,G., and Binder,C. (2003). Prolactin levels during long-term risperidone treatment in children and adolescents. J Clin Psychiatry *64*, 1362-1369.

Finkelman,F.D. and Urban,J.F., Jr. (2001). The other side of the coin: the protective role of the TH2 cytokines. J Allergy Clin Immunol *107*, 772-780.

Franchimont,D., Louis,E., Dewe,W., Martens,H., Vrindts-Gevaert,Y., De Groote,D., Belaiche,J., and Geenen,V. (1998). Effects of dexamethasone on the profile of cytokine secretion in human whole blood cell cultures. Regul. Pept. *73*, 59-65.

Frei,K., Malipiero,U.V., Leist,T.P., Zinkernagel,R.M., Schwab,M.E., and Fontana,A. (1989). On the cellular source and function of interleukin 6 produced in the central nervous system in viral diseases. Eur J Immunol *19*, 689-694.

Freund,Y.R. and Blair,P.B. (1982). Depression of natural killer activity and mitogen responsiveness in mice treated with pristane. J Immunol *129*, 2826-2830.

Frommberger,U.H., Bauer,J., Haselbauer,P., Fraulin,A., Riemann,D., and Berger,M. (1997). Interleukin-6-(IL-6) plasma levels in depression and schizophrenia: comparison between the acute state and after remission. Eur Arch Psychiatry Clin Neurosci. *247*, 228-233.

Fujii-Maeda,S., Kajiwara,K., Ikizawa,K., Shinazawa,M., Yu,B., Koga,T., Furue,M., and Yanagihara,Y. (2004). Reciprocal regulation of thymus and activation-regulated chemokine/macrophage-derived chemokine production by interleukin (IL)-4/IL-13 and interferon-gamma in HaCaT keratinocytes is mediated by alternations in E-cadherin distribution. J Invest Dermatol. *122* , 20-28.

Fujisawa,M., Okuda,Y., Fujioka,H., and Kamidono,S. (2002). Expression and regulation of gp130 messenger ribonucleic acid in cultured immature rat Sertoli cells. Endocr. Res. *28*, 1-8.

Gadient,R.A. and Otten,U. (1994). Expression of interleukin-6 (IL-6) and interleukin-6 receptor (IL-6R) mRNAs in rat brain during postnatal development. Brain Res. *637*, 10-14.

Gallagher,G., Dickensheets,H., Eskdale,J., Izotova,L.S., Mirochnitchenko,O.V., Peat,J.D., Vazquez,N., Pestka,S., Donnelly,R.P., and Kotenko,S.V. (2000). Cloning, expression and initial characterization of interleukin-19 (IL-19), a novel homologue of human interleukin-10 (IL-10). Genes Immun *1*, 442-450.

Ganguli,R., Brar,J.S., Chengappa,K.R., DeLeo,M., Yang,Z.W., Shurin,G., and Rabin,B.S. (1995). Mitogen-stimulated interleukin-2 production in never-medicated, first-episode schizophrenic patients. The influence of age at onset and negative symptoms. Arch Gen Psychiatry *52*, 668-672.

Ganguli,R., Brar,J.S., Solomon,W., Chengappa,K.N., and Rabin,B.S. (1992). Altered interleukin-2 production in schizophrenia: association between clinical state and autoantibody production. Psychiatry Res. *44*, 113-123.

Ganguli,R. and Rabin,B.S. (1989). Increased serum interleukin 2 receptor concentration in schizophrenic and brain-damaged subjects. Arch Gen Psychiatry *46*, 292.

Ganguli,R., Rabin,B.S., and Belle,S.H. (1989). Decreased interleukin-2 production in schizophrenic patients. Biol Psychiatry *26*, 427-430.

Ganguli,R., Yang,Z., Shurin,G., Chengappa,K.N., Brar,J.S., Gubbi,A.V., and Rabin,B.S. (1994). Serum interleukin-6 concentration in schizophrenia: elevation associated with duration of illness. Psychiatry Res. *51*, 1-10.

Garver,D.L., Tamas,R.L., and Holcomb,J.A. (2003). Elevated interleukin-6 in the cerebrospinal fluid of a previously delineated schizophrenia subtype. Neuropsychopharmacology *28*, 1515-1520.

Gasparoni,A., Ciardelli,L., Avanzini,A., Castellazzi,A.M., Carini,R., Rondini,G., and Chirico,G. (2003). Age-related changes in intracellular TH1/TH2 cytokine production, immunoproliferative T lymphocyte response and natural killer cell activity in newborns, children and adults. Biol Neonate *84*, 297-303.

Gattaz,W.F., Dalgalarrondo,P., and Schroder,H.C. (1992). Abnormalities in serum concentrations of interleukin-2, interferon-alpha and interferon-gamma in schizophrenia not detected. Schizophr Res. *6*, 237-241.

Gauchat,J.F., Henchoz,S., Mazzei,G., Aubry,J.P., Brunner,T., Blasey,H., Life,P., Talabot,D., Flores-Romo,L., Thompson,J., and . (1993). Induction of human IgE synthesis in B cells by mast cells and basophils. Nature *365*, 340-343.

Gaughran,F. (2002). Immunity and schizophrenia: autoimmunity, cytokines, and immune responses. Int Rev Neurobiol. *52*, 275-302.

Gaughran,F., O'Neill,E., Cole,M., Collins,K., Daly,R.J., and Shanahan,F. (1998). Increased soluble interleukin 2 receptor levels in schizophrenia. Schizophr Res. *29*, 263-267.

Gazzinelli,R.T., Wysocka,M., Hayashi,S., Denkers,E.Y., Hieny,S., Caspar,P., Trinchieri,G., and Sher,A. (1994). Parasite-induced IL-12 stimulates early IFN-gamma synthesis and resistance during acute infection with Toxoplasma gondii. J Immunol *153*, 2533-2543.

Gehrmann,J. (1995). Colony-stimulating factors regulate programmed cell death of rat microglia/brain macrophages in vitro. J Neuroimmunol. *63*, 55-61.

Germann,T., Gately,M.K., Schoenhaut,D.S., Lohoff,M., Mattner,F., Fischer,S., Jin,S.C., Schmitt,E., and Rude,E. (1993). Interleukin-12/T cell stimulating factor, a cytokine with multiple effects on T helper type 1 (Th1) but not on Th2 cells. Eur J Immunol *23*, 1762-1770.

Gesser,B., Leffers,H., Jinquan,T., Vestergaard,C., Kirstein,N., Sindet-Pedersen,S., Jensen,S.L., Thestrup-Pedersen,K., and Larsen,C.G. (1997). Identification of functional domains on human interleukin 10. Proc. Natl. Acad Sci U. S A *94*, 14620-14625.

Ghosh,M.C., Mondal,A.C., Basu,S., Banerjee,S., Majumder,J., Bhattacharya,D., and Dasgupta,P.S. (2003). Dopamine inhibits cytokine release and expression of tyrosine kinases, Lck and Fyn in activated T cells. Int Immunopharmacol. *3*, 1019-1026.

Giannakoulas,N.C., Karakantza,M., Theodorou,G.L., Pagoni,M., Galanopoulos,A., Kakagianni,T., Kouraklis-Symeonidis,A., Matsouka,P., Maniatis,A., and Zoumbos,N.C. (2004). Clinical relevance of balance between type 1 and type 2 immune responses of lymphocyte subpopulations in aplastic anaemia patients. Br J Haematol. *124*, 97-105.

Gibbs,B.F., Haas,H., Falcone,F.H., Albrecht,C., Vollrath,I.B., Noll,T., Wolff,H.H., and Amon,U. (1996). Purified human peripheral blood basophils release interleukin-13 and preformed interleukin-4 following immunological activation. Eur J Immunol *26*, 2493-2498.

Gillett,M.J., Martins,R.N., Clarnette,R.M., Chubb,S.A., Bruce,D.G., and Yeap,B.B. (2003). Relationship between testosterone, sex hormone binding globulin and plasma amyloid beta peptide 40 in older men with subjective memory loss or dementia. J Alzheimers. Dis. *5*, 267-269.

Giltay,E.J., Fonk,J.C., von Blomberg,B.M., Drexhage,H.A., Schalkwijk,C., and Gooren,L.J. (2000). In vivo effects of sex steroids on lymphocyte responsiveness and immunoglobulin levels in humans. J Clin Endocrinol. Metab *85*, 1648-1657.

Giron-Gonzalez,J.A., Moral,F.J., Elvira,J., Garcia-Gil,D., Guerrero,F., Gavilan,I., and Escobar,L. (2000). Consistent production of a higher TH1:TH2 cytokine ratio by stimulated T cells in men compared with women. Eur J Endocrinol. *143*, 31-36.

Godbout,J.P. and Johnson,R.W. (2004). Interleukin-6 in the aging brain. J Neuroimmunol. *147*, 141-144.

Golding,B. and Scott,D.E. (1995). Vaccine strategies: targeting helper T cell responses. Ann N Y Acad Sci *754*, 126-137.

Gould,D.J., Bright,C., and Chernajovsky,Y. (2004). Inhibition of established collagen-induced arthritis with a tumour necrosis factor-alpha inhibitor expressed from a self-contained doxycycline regulated plasmid. Arthritis Res. Ther *6*, R103-R113.

Green,M.F. (1996). What are the functional consequences of neurocognitive deficits in schizophrenia? Am J Psychiatry *153*, 321-330.

Grigoriadis,S. and Seeman,M.V. (2002). The role of estrogen in schizophrenia: implications for schizophrenia practice guidelines for women. Can J Psychiatry *47*, 437-442.

Grohmann,U., Bianchi,R., Belladonna,M.L., Vacca,C., Silla,S., Ayroldi,E., Fioretti,M.C., and Puccetti,P. (1999). IL-12 acts selectively on CD8 alpha- dendritic cells to enhance presentation of a tumor peptide in vivo. J Immunol *163*, 3100-3105.

Grunewald,S.M., Werthmann,A., Schnarr,B., Klein,C.E., Brocker,E.B., Mohrs,M., Brombacher,F., Sebald,W., and Duschl,A. (1998). An antagonistic IL-4 mutant prevents type I allergy in the mouse: inhibition of the IL-4/IL-13 receptor system completely abrogates humoral immune response to allergen and development of allergic symptoms in vivo. J Immunol *160*, 4004-4009.

Gruss,H.J., Boiani,N., Williams,D.E., Armitage,R.J., Smith,C.A., and Goodwin,R.G. (1994). Pleiotropic effects of the CD30 ligand on CD30-expressing cells and lymphoma cell lines. Blood *83*, 2045-2056.

Gupta,S., Aggarwal,S., Rashanravan,B., and Lee,T. (1998). Th1- and Th2-like cytokines in CD4+ and CD8+ T cells in autism. J Neuroimmunol. *85*, 106-109.

Haack,M., Hinze-Selch,D., Fenzel,T., Kraus,T., Kuhn,M., Schuld,A., and Pollmacher,T. (1999). Plasma levels of cytokines and soluble cytokine receptors in psychiatric patients upon hospital admission: effects of confounding factors and diagnosis. J Psychiatr Res. *33*, 407-418.

Haddad,J.J., Saade,N.E., and Safieh-Garabedian,B. (2003). Interleukin-10 and the regulation of mitogen-activated protein kinases: are these signalling modules targets for the anti-inflammatory action of this cytokine? Cell Signal. *15*, 255-267.

Hafner,H. (2003). Gender differences in schizophrenia. Psychoneuroendocrinology *28 Suppl 2*, 17-54.

Hailer,N.P., Heppner,F.L., Haas,D., and Nitsch,R. (1998). Astrocytic factors deactivate antigen presenting cells that invade the central nervous system. Brain Pathol. *8*, 459-474.

Halbreich,U. and Kahn,L.S. (2003). Hormonal aspects of schizophrenias: an overview. Psychoneuroendocrinology *28 Suppl 2*, 1-16.

Hale,K.K., Smith,C.G., Baker,S.L., Vanderslice,R.W., Squires,C.H., Gleason,T.M., Tucker,K.K., Kohno,T., and Russell,D.A. (1995). Multifunctional regulation of the biological effects of TNF-alpha by the soluble type I and type II TNF receptors. Cytokine *7*, 26-38.

Hall,H. and Sallemark,M. (1987). Effects of chronic neuroleptic treatment on agonist affinity states of the dopamine-D2 receptor in the rat brain. Pharmacol. Toxicol. *60*, 359-363.

Hama,T., Kushima,Y., Miyamoto,M., Kubota,M., Takei,N., and Hatanaka,H. (1991). Interleukin-6 improves the survival of mesencephalic catecholaminergic and septal cholinergic neurons from postnatal, two-week-old rats in cultures. Neuroscience *40*, 445-452.

Hamner,M. (2002). The effects of atypical antipsychotics on serum prolactin levels. Ann Clin Psychiatry *14*, 163-173.

Harris,D.S., Wolkowitz,O.M., and Reus,V.I. (2001). Movement disorder, memory, psychiatric symptoms and serum DHEA levels in schizophrenic and schizoaffective patients. World J Biol Psychiatry *2*, 99-102.

Hart,P.H., Vitti,G.F., Burgess,D.R., Whitty,G.A., Piccoli,D.S., and Hamilton,J.A. (1989). Potential antiinflammatory effects of interleukin 4: suppression of human monocyte tumor necrosis factor alpha, interleukin 1, and prostaglandin E2. Proc. Natl. Acad Sci U. S A *86*, 3803-3807.

Havell,E.A. and Spitalny,G.L. (1983). Endotoxin-induced interferon synthesis in macrophage cultures. J Reticuloendothel. Soc. *33*, 369-380.

Hayashi,T., Adachi,Y., Hasegawa,K., and Morimoto,M. (2003). Less sensitivity for late airway inflammation in males than females in BALB/c mice. Scand. J Immunol *57*, 562-567.

Heaton,R., Paulsen,J.S., McAdams,L.A., Kuck,J., Zisook,S., Braff,D., Harris,J., and Jeste,D.V. (1994). Neuropsychological deficits in schizophrenics. Relationship to age, chronicity, and dementia. Arch Gen Psychiatry *51*, 469-476.

Heinrich,P.C., Behrmann,I., Muller-Newen,G., Schaper,F., and Graeve,L. (1998). Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem. J *334 (Pt 2)*, 297-314.

Heller,R.F., Wheeler,M.J., Micallef,J., Miller,N.E., and Lewis,B. (1983). Relationship of high density lipoprotein cholesterol with total and free testosterone and sex hormone binding globulin. Acta Endocrinol. (Copenh) *104*, 253-256.

Herrmann,M., Scholmerich,J., and Straub,R.H. (2002). Influence of cytokines and growth factors on distinct steroidogenic enzymes in vitro: a short tabular data collection. Ann N Y Acad Sci *966*, 166-186.

Hery,C., Sebire,G., Peudenier,S., and Tardieu,M. (1995). Adhesion to human neurons and astrocytes of monocytes: the role of interaction of CR3 and ICAM-1 and modulation by cytokines. J Neuroimmunol. *57*, 101-109.

Heufler,C., Koch,F., Stanzl,U., Topar,G., Wysocka,M., Trinchieri,G., Enk,A., Steinman,R.M., Romani,N., and Schuler,G. (1996). Interleukin-12 is produced by dendritic cells and mediates T helper 1 development as well as interferon-gamma production by T helper 1 cells. Eur J Immunol *26* , 659-668.

Hewett,S.J., Corbett,J.A., McDaniel,M.L., and Choi,D.W. (1993). Interferon-gamma and interleukin-1 beta induce nitric oxide formation from primary mouse astrocytes. Neurosci. Lett. *164*, 229-232.

Hinze-Selch,D., Becker,E.W., Stein,G.M., Berg,P.A., Mullington,J., Holsboer,F., and Pollmacher,T. (1998). Effects of clozapine on in vitro immune parameters: a longitudinal study in clozapine-treated schizophrenic patients. Neuropsychopharmacology *19*, 114-122.

Hinze-Selch,D., Mullington,J., and Pollmacher,T. (1996). [Clozapine, cytokines, fever and sleep]. Wien. Med Wochenschr. *146*, 311-313.

Hinze-Selch,D. and Pollmacher,T. (2001). In vitro cytokine secretion in individuals with schizophrenia: results, confounding factors, and implications for further research. Brain Behav Immun *15*, 282-318.

Hirano,T. (1998). Interleukin 6 and its receptor: ten years later. Int Rev Immunol *16*, 249-284.

Hoenstein,R., Admon,D., Solomon,A., Norris,A., Moqbel,R., and Levi-Schaffer,F. (2001). Interleukin-2 activates human peripheral blood eosinophils. Cell Immunol *210*, 116-124.

Hogervorst,E., Combrinck,M., and Smith,A.D. (2003). Testosterone and gonadotropin levels in men with dementia. Neuro Endocrinol. Lett. *24*, 203-208.

Honer,W.G., Hu,L., and Davies,P. (1993). Human synaptic proteins with a heterogeneous distribution in cerebellum and visual cortex. Brain Res. *609*, 9-20.

Hopkins,S.J. and Rothwell,N.J. (1995b). Cytokines and the nervous system. I: Expression and recognition. Trends Neurosci. *18* , 83-88.

Hopkins,S.J. and Rothwell,N.J. (1995a). Cytokines and the nervous system. I: Expression and recognition. Trends Neurosci. *18* , 83-88.

Hornberg,M., Arolt,V., Wilke,I., Kruse,A., and Kirchner,H. (1995). Production of interferons and lymphokines in leukocyte cultures of patients with schizophrenia. Schizophr Res. *15*, 237-242.

Hoshino,T., Winkler-Pickett,R.T., Mason,A.T., Ortaldo,J.R., and Young,H.A. (1999). IL-13 production by NK cells: IL-13-producing NK and T cells are present in vivo in the absence of IFN-gamma. J Immunol *162*, 51-59.

Hoskin,E.K., Tang,M.X., Manly,J.J., and Mayeux,R. (2004). Elevated sex-hormone binding globulin in elderly women with Alzheimer's disease. Neurobiol. Aging *25*, 141-147.

Hou,J., Schindler,U., Henzel,W.J., Ho,T.C., Brasseur,M., and McKnight,S.L. (1994). An interleukin-4-induced transcription factor: IL-4 Stat. Science *265*, 1701-1706.

Howard,M., O'Garra,A., Ishida,H., de Waal,M.R., and de Vries,J. (1992). Biological properties of interleukin 10. J Clin Immunol *12*, 239-247.

Huber,S.A., Kupperman,J., and Newell,M.K. (1999). Estradiol prevents and testosterone promotes Fas-dependent apoptosis in CD4+ Th2 cells by altering Bcl 2 expression. Lupus *8*, 384-387.

Hyde,G.N., Seale,A.P., Grau,E.G., and Borski,R.J. (2004). Cortisol rapidly suppresses intracellular calcium and voltage-gated calcium channel activity in prolactin cells of the tilapia (Oreochromis mossambicus). Am J Physiol Endocrinol. Metab *286*, E626-E633.

Inglis,V.I., Jones,M.P., Tse,A.D., and Easton,A.S. (2004). Neutrophils both reduce and increase permeability in a cell culture model of the blood-brain barrier. Brain Res. *998*, 218-229.

Inglot,A.D., Leszek,J., Piasecki,E., and Sypula,A. (1994). Interferon responses in schizophrenia and major depressive disorders. Biol Psychiatry *35*, 464-473.

Inoue,K. (2002). Microglial activation by purines and pyrimidines. Glia *40*, 156-163.

Itoh,K. and Hirohata,S. (1995). The role of IL-10 in human B cell activation, proliferation, and differentiation. J Immunol *154*, 4341-4350.

Iwata,M., Eshima,Y., Kagechika,H., and Miyaura,H. (2004). The endocrine disruptors nonylphenol and octylphenol exert direct effects on T cells to suppress Th1 development and enhance Th2 development. Immunol Lett. *94*, 135-139.

Izuhara,K. and Arima,K. (2004). Signal transduction of IL-13 and its role in the pathogenesis of bronchial asthma. Drug News Perspect. *17*, 91-98.

Jankovic,D., Kullberg,M.C., Noben-Trauth,N., Caspar,P., Paul,W.E., and Sher,A. (2000). Single cell analysis reveals that IL-4 receptor/Stat6 signaling is not required for the in vivo or in vitro development of CD4+ lymphocytes with a Th2 cytokine profile. J Immunol *164*, 3047-3055.

Jaworowicz,D.J., Jr., Korytko,P.J., Singh,L.S., and Boje,K.M. (1998). Nitric oxide and prostaglandin E2 formation parallels blood-brain barrier disruption in an experimental rat model of bacterial meningitis. Brain Res. Bull *46*, 541-546.

Jeannin,P., Lecoanet,S., Delneste,Y., Gauchat,J.F., and Bonnefoy,J.Y. (1998). IgE versus IgG4 production can be differentially regulated by IL-10. J Immunol *160*, 3555-3561.

Jiang,H.K. and Wang,J.Y. (1998). Diurnal melatonin and cortisol secretion profiles in medicated schizophrenic patients. J Formos. Med Assoc. *97*, 830-837.

Jiao,L., Gan,D., and Fang,Y. (1998). [Effects of smoking on interleukin-2 and its receptor system in human peripheral blood]. Wei Sheng Yan. Jiu. *27*, 385-388.

Johnson,D.A. (1977). Treatment of chronic schizophrenia. Drugs *14*, 291-299.

Kaminska,T., Wysocka,A., Marmurowska-Michalowska,H., Dubas-Slemp,H., and Kandefer-Szerszen,M. (2001). Investigation of serum cytokine levels and cytokine production in whole blood cultures of paranoid schizophrenic patients. Arch Immunol Ther Exp (Warsz) *49*, 439-445.

Kaplan,M.H. and Grusby,M.J. (1998). Regulation of T helper cell differentiation by STAT molecules. J Leukoc. Biol *64*, 2-5.

Karanfilov,C.I., Liu,B., Fox,C.C., Lakshmanan,R.R., and Whisler,R.L. (1999). Age-related defects in Th1 and Th2 cytokine production by human T cells can be dissociated from altered frequencies of CD45RA+ and CD45RO+ T cell subsets. Mech. Ageing Dev. *109*, 97-112.

Katila,H., Cantell,K., Hirvonen,S., and Rimon,R. (1989). Production of interferon-alpha and gamma by leukocytes from patients with schizophrenia. Schizophr Res. *2*, 361-365.

Katsikis,P.D., Cohen,S.B., Londei,M., and Feldmann,M. (1995). Are CD4+ Th1 cells pro-inflammatory or anti-inflammatory? The ratio of IL-10 to IFN-gamma or IL-2 determines their function. Int Immunol *7*, 1287-1294.

Kelly,J. and Murray,R.M. (2000). What risk factors tell us about the causes of schizophrenia and related psychoses. Curr Psychiatry Rep. *2*, 378-385.

Kielian,T. (2004). Immunopathogenesis of brain abscess. J Neuroinflammation. *1*, 16.

Kim,D.J., Kim,W., Yoon,S.J., Go,H.J., Choi,B.M., Jun,T.Y., and Kim,Y.K. (2001). Effect of risperidone on serum cytokines. Int J Neurosci. *111*, 11-19.

Kim,Y.K., Kim,L., and Lee,M.S. (2000). Relationships between interleukins, neurotransmitters and psychopathology in drug-free male schizophrenics. Schizophr Res. *44*, 165-175.

Kim,Y.K., Lee,M.S., and Suh,K.Y. (1998). Decreased interleukin-2 production in Korean schizophrenic patients. Biol Psychiatry *43*, 701-704.

Kim,Y.K., Suh,I.B., Kim,H., Han,C.S., Lim,C.S., Choi,S.H., and Licinio,J. (2002). The plasma levels of interleukin-12 in schizophrenia, major depression, and bipolar mania: effects of psychotropic drugs. Mol Psychiatry *7*, 1107-1114.

Kinney,D.K. and Matthysse,S. (1978). Genetic transmission of schizophrenia. Annu. Rev Med *29*, 459-473.

Kinouchi,K., Brown,G., Pasternak,G., and Donner,D.B. (1991). Identification and characterization of receptors for tumor necrosis factor-alpha in the brain. Biochem. Biophys. Res. Commun. *181*, 1532-1538.

Kirch,D.G. (1993). Infection and autoimmunity as etiologic factors in schizophrenia: a review and reappraisal. Schizophr Bull *19*, 355-370.

Koponen,H., Rantakallio,P., Veijola,J., Jones,P., Jokelainen,J., and Isohanni,M. (2004). Childhood central nervous system infections and risk for schizophrenia. Eur Arch Psychiatry Clin Neurosci. *254*, 9-13.

Kossmann,T., Hans,V., Imhof,H.G., Trentz,O., and Morganti-Kossmann,M.C. (1996). Interleukin-6 released in human cerebrospinal fluid following traumatic brain injury may trigger nerve growth factor production in astrocytes. Brain Res. *713*, 143-152.

Kotenko,S.V., Krause,C.D., Izotova,L.S., Pollack,B.P., Wu,W., and Pestka,S. (1997). Identification and functional characterization of a second chain of the interleukin-10 receptor complex. EMBO J *16*, 5894-5903.

Kotowicz,K., Callard,R.E., Friedrich,K., Matthews,D.J., and Klein,N. (1996). Biological activity of IL-4 and IL-13 on human endothelial cells: functional evidence that both cytokines act through the same receptor. Int Immunol *8*, 1915-1925.

Kowalski,J., Blada,P., Kucia,K., Lawniczek,T., Madej,A., Belowski,D., and Herman,Z.S. (2000). In-vitro immunomodulatory effects of haloperidol and perazine in schizophrenia. World J Biol Psychiatry *1*, 190-196.

Kowalski,J., Blada,P., Kucia,K., Madej,A., and Herman,Z.S. (2001). Neuroleptics normalize increased release of interleukin- 1 beta and tumor necrosis factor-alpha from monocytes in schizophrenia. Schizophr Res. *50*, 169-175.

Kreutzberg,G.W. (1996). Microglia: a sensor for pathological events in the CNS. Trends Neurosci. *19*, 312-318.

Kroemer,G., Hirsch,F., Gonzalez-Garcia,A., and Martinez,C. (1996). Differential involvement of Th1 and Th2 cytokines in autoimmune diseases. Autoimmunity *24*, 25-33.

Kuchroo,V.K., Byrne,M.C., Greenfield,E., Whitters,M.J., Nalefsky,E.A., Rao,A., Collins,M., and Dorf,M.E. (1995). Transfection of TCR alpha-chains into suppressor and T helper cell hybridomas. Production of suppressor factors with predicted antigen specificity. J Immunol *154*, 5030-5038.

Kuhlwein,E. and Irwin,M. (2001). Melatonin modulation of lymphocyte proliferation and Th1/Th2 cytokine expression. J Neuroimmunol. *117*, 51-57.

Kuhnle,G., Collins,R.A., Scott,J.E., and Keil,G.M. (1996). Bovine interleukins 2 and 4 expressed in recombinant bovine herpesvirus 1 are biologically active secreted glycoproteins. J Gen Virol. *77 (Pt 9)* , 2231-2240.

Kushima,Y. and Hatanaka,H. (1992). Culture of neuronal cells from postnatal rat brain: application to the study of neurotrophic factors. Prog Neuropsychopharmacol Biol Psychiatry *16*, 617-633.

Lalani,I., Bhol,K., and Ahmed,A.R. (1997). Interleukin-10: biology, role in inflammation and autoimmunity. Ann Allergy Asthma Immunol *79*, 469-483.

Lambert,K.C., Curran,E.M., Judy,B.M., Lubahn,D.B., and Estes,D.M. (2004). Estrogen receptor-{alpha} deficiency promotes increased TNF-{alpha} secretion and bacterial killing by murine macrophages in response to microbial stimuli in vitro. J Leukoc. Biol.

Lawn,S.D., Rudolph,D., Ackah,A., Coulibaly,D., Wiktor,S., and Lal,R.B. (2001). Lack of induction of interleukin-2-receptor-alpha in patients with tuberculosis and human immunodeficiency virus co-infection: implications for pathogenesis. Trans. R. Soc. Trop. Med Hyg *95*, 449-452.

Lee,M.A. and Meltzer,H.Y. (2001). 5-HT(1A) receptor dysfunction in female patients with schizophrenia. Biol Psychiatry *50*, 758-766.

Lee,S.C., Dickson,D.W., Liu,W., and Brosnan,C.F. (1993). Induction of nitric oxide synthase activity in human astrocytes by interleukin-1 beta and interferon-gamma. J Neuroimmunol. *46*, 19-24.

Lee,Y.B., Nagai,A., and Kim,S.U. (2002). Cytokines, chemokines, and cytokine receptors in human microglia. J Neurosci. Res. *69*, 94-103.

Leff,J., Kuipers,L., Berkowitz,R., Vaughn,C., and Sturgeon,D. (1983). Life events, relatives' expressed emotion and maintenance neuroleptics in schizophrenic relapse. Psychol. Med *13*, 799-806.

Leff,J.P. (1981). Prevention of relapse of schizophrenia by social and pharmacological treatments. Bibl. Psychiatr 15-21.

Lertmemongkolchai,G., Cai,G., Hunter,C.A., and Bancroft,G.J. (2001). Bystander activation of CD8+ T cells contributes to the rapid production of IFN-gamma in response to bacterial pathogens. J Immunol *166* , 1097-1105.

Levison,S.W., Jiang,F.J., Stoltzfus,O.K., and Ducceschi,M.H. (2000). IL-6-type cytokines enhance epidermal growth factor-stimulated astrocyte proliferation. Glia *32*, 328-337.

Li-Weber,M., Giaisi,M., Baumann,S., Palfi,K., and Krammer,P.H. (2004). NF-kappa B synergizes with NF-AT and NF-IL6 in activation of the IL-4 gene in T cells. Eur J Immunol *34*, 1111-1118.

Li,H., Sim,T.C., and Alam,R. (1996a). IL-13 released by and localized in human basophils. J Immunol *156*, 4833-4838.

Li,L., Young,D., Wolf,S.F., and Choi,Y.S. (1996b). Interleukin-12 stimulates B cell growth by inducing IFN-gamma. Cell Immunol *168*, 133-140.

Li,L., Zhou,Z.G., Zeng,K., Liang,L.P., Zhou,X.Y., and Yi,Z.S. (2003). Changes in peripheral blood Th1/Th2 cell balance in patients with condyloma acuminatum. Di Yi. Jun Yi. Da. Xue. Xue. Bao. *23*, 737-739.

Liao,D.L., Chen,H., Lee,S.M., and Tsai,S.J. (2002). Estrogen supplementation for female schizophrenics treated with atypical antipsychotics. Gen Hosp Psychiatry *24*, 357-359.

Limosin,F., Rouillon,F., Payan,C., Cohen,J.M., and Strub,N. (2003). Prenatal exposure to influenza as a risk factor for adult schizophrenia. Acta Psychiatr Scand. *107*, 331-335.

Lin,A., Kenis,G., Bignotti,S., Tura,G.J., De Jong,R., Bosmans,E., Pioli,R., Altamura,C., Scharpe,S., and Maes,M. (1998). The inflammatory response system in treatment-resistant schizophrenia: increased serum interleukin-6. Schizophr Res. *32*, 9-15.

Lindamer,L.A., Buse,D.C., Lohr,J.B., and Jeste,D.V. (2001). Hormone replacement therapy in postmenopausal women with schizophrenia: positive effect on negative symptoms? Biol Psychiatry *49*, 47-51.

Liu,J., Cao,S., Herman,L.M., and Ma,X. (2003). Differential regulation of interleukin (IL)-12 p35 and p40 gene expression and interferon (IFN)-gamma-primed IL-12 production by IFN regulatory factor 1. J Exp Med *198*, 1265-1276.

Liu,Y., Wei,S.H., Ho,A.S., de Waal,M.R., and Moore,K.W. (1994). Expression cloning and characterization of a human IL-10 receptor. J Immunol *152*, 1821-1829.

Livni,E., Munitz,H., Tyano,S., Englander,T., Kuritzky,A., Wysenbeek,H., and Joshua,H. (1979). Further studies on cell-mediated immunity to myelin basic protein in schizophrenic patients. J Neurol. Sci *42*, 437-440.

Lorentz,A., Schwengberg,S., Sellge,G., Manns,M.P., and Bischoff,S.C. (2000). Human intestinal mast cells are capable of producing different cytokine profiles: role of IgE receptor cross-linking and IL-4. J Immunol *164*, 43-48.

Lou,J., Ythier,A., Burger,D., Zheng,L., Juillard,P., Lucas,R., Dayer,J.M., and Grau,G.E. (1997). Modulation of soluble and membrane-bound TNF-induced phenotypic and functional changes of human brain microvascular endothelial cells by recombinant TNF binding protein I. J Neuroimmunol. *77*, 107-115.

Loza,B., Kucharska-Pietura,K., Kopacz,G., and Debowska,G. (2003). Factor structure of paranoid schizophrenia: a prospective study. Psychopathology *36*, 132-141.

Lucas,R., Juillard,P., Decoster,E., Redard,M., Burger,D., Donati,Y., Giroud,C., Monso-Hinard,C., De Kesel,T., Buurman,W.A., Moore,M.W., Dayer,J.M., Fiers,W., Bluethmann,H., and Grau,G.E. (1997). Crucial role of tumor necrosis factor (TNF) receptor 2 and membrane-bound TNF in experimental cerebral malaria. Eur J Immunol *27*, 1719-1725.

Mach,F. (2002). Immunosuppressive effects of statins. Atheroscler. Suppl *3*, 17-20.

Maes,M., Bocchio,C.L., Bignotti,S., Battisa Tura,G.J., Pioli,R., Boin,F., Kenis,G., Bosmans,E., de Jongh,R., and Altamura,C.A. (2002). Increased serum interleukin-8 and interleukin-10 in schizophrenic patients resistant to treatment with neuroleptics and the stimulatory effects of clozapine on serum leukemia inhibitory factor receptor. Schizophr Res. *54*, 281-291.

Maes,M., Bocchio,C.L., Bignotti,S., Battisa,T.G., Pioli,R., Boin,F., Kenis,G., Bosmans,E., de Jongh,R., Lin,A., Racagni,G., and Altamura,C.A. (2000). Effects of atypical antipsychotics on the inflammatory response system in schizophrenic patients resistant to treatment with typical neuroleptics. Eur Neuropsychopharmacol *10*, 119-124.

Maes,M., Bosmans,E., Calabrese,J., Smith,R., and Meltzer,H.Y. (1995a). Interleukin-2 and interleukin-6 in schizophrenia and mania: effects of neuroleptics and mood stabilizers. J Psychiatr Res. *29*, 141-152.

Maes,M., Bosmans,E., Kenis,G., De Jong,R., Smith,R.S., and Meltzer,H.Y. (1997). In vivo immunomodulatory effects of clozapine in schizophrenia. Schizophr Res. *26*, 221-225.

Maes,M., De,M., I, Scharpe,S., Desnyder,R., Ranjan,R., and Meltzer,H.Y. (1996). Alterations in plasma dipeptidyl peptidase IV enzyme activity in depression and schizophrenia: effects of antidepressants and antipsychotic drugs. Acta Psychiatr Scand. *93*, 1-8.

Maes,M., Meltzer,H.Y., and Bosmans,E. (1994). Immune-inflammatory markers in schizophrenia: comparison to normal controls and effects of clozapine. Acta Psychiatr Scand. *89*, 346-351.

Maes,M., Meltzer,H.Y., Buckley,P., and Bosmans,E. (1995b). Plasma-soluble interleukin-2 and transferrin receptor in schizophrenia and major depression. Eur Arch Psychiatry Clin Neurosci. *244*, 325-329.

Magram,J., Sfarra,J., Connaughton,S., Faherty,D., Warrier,R., Carvajal,D., Wu,C.Y., Stewart,C., Sarmiento,U., and Gately,M.K. (1996). IL-12-deficient mice are defective but not devoid of type 1 cytokine responses. Ann N Y Acad Sci *795*, 60-70.

Malek,T.R. (2003). The main function of IL-2 is to promote the development of T regulatory cells. J Leukoc. Biol *74*, 961-965.

Marklund,N., Peltonen,M., Nilsson,T.K., and Olsson,T. (2004). Low and high circulating cortisol levels predict mortality and cognitive dysfunction early after stroke. J Intern. Med *256*, 15-21.

Marz,P., Heese,K., Dimitriades-Schmutz,B., Rose-John,S., and Otten,U. (1999). Role of interleukin-6 and soluble IL-6 receptor in region-specific induction of astrocytic differentiation and neurotrophin expression. Glia *26*, 191-200.

Matalka,K.Z. (2003a). Prolactin enhances production of interferon-gamma, interleukin-12, and interleukin-10, but not of tumor necrosis factor-alpha, in a stimulus-specific manner. Cytokine *21*, 187-194.

Matalka,K.Z. (2003b). The effect of estradiol, but not progesterone, on the production of cytokines in stimulated whole blood, is concentration-dependent. Neuro Endocrinol. Lett. *24*, 185-191.

Matera,L. (1997). Action of pituitary and lymphocyte prolactin. Neuroimmunomodulation. *4*, 171-180.

Matera,L. and Mori,M. (2000). Cooperation of pituitary hormone prolactin with interleukin-2 and interleukin-12 on production of interferon-gamma by natural killer and T cells. Ann N Y Acad Sci *917*, 505-513.

Mathers,A.R. and Cuff,C.F. (2004). Role of interleukin-4 (IL-4) and IL-10 in serum immunoglobulin G antibody responses following mucosal or systemic reovirus infection. J Virol. *78*, 3352-3360.

McCarron,R.M., Wang,L., Racke,M.K., McFarlin,D.E., and Spatz,M. (1993). Cytokine-regulated adhesion between encephalitogenic T lymphocytes and cerebrovascular endothelial cells. J Neuroimmunol. *43*, 23-30.

McDonald,P.P., Cassatella,M.A., Bald,A., Maggi,E., Romagnani,S., Gruss,H.J., and Pizzolo,G. (1995). CD30 ligation induces nuclear factor-kappa B activation in human T cell lines. Eur J Immunol *25*, 2870-2876.

McElhaney,J.E., Herre,J.M., Lawson,M.L., Cole,S.K., Burke,B.L., and Hooton,J.W. (2004). Effect of congestive heart failure on humoral and ex vivo cellular immune responses to influenza vaccination in older adults. Vaccine *22*, 681-688.

McGeer,P.L., Kawamata,T., Walker,D.G., Akiyama,H., Tooyama,I., and McGeer,E.G. (1993). Microglia in degenerative neurological disease. Glia *7*, 84-92.

McGuirk,P. and Mills,K.H. (2002). Pathogen-specific regulatory T cells provoke a shift in the Th1/Th2 paradigm in immunity to infectious diseases. Trends Immunol *23*, 450-455.

McKenzie,A.N., Li,X., Largaespada,D.A., Sato,A., Kaneda,A., Zurawski,S.M., Doyle,E.L., Milatovich,A., Francke,U., Copeland,N.G., and . (1993). Structural comparison and chromosomal localization of the human and mouse IL-13 genes. J Immunol *150*, 5436-5444.

McKinley,M.J., McAllen,R.M., Davern,P., Giles,M.E., Penschow,J., Sunn,N., Uschakov,A., and Oldfield,B.J. (2003). The sensory circumventricular organs of the mammalian brain. Adv. Anat. Embryol. Cell Biol *172*, III-122, back.

McMurray,R.W., Ndebele,K., Hardy,K.J., and Jenkins,J.K. (2001). 17-beta-estradiol suppresses IL-2 and IL-2 receptor. Cytokine *14*, 324-333.

Meaney,A.M., Smith,S., Howes,O.D., O'Brien,M., Murray,R.M., and O'Keane,V. (2004). Effects of long-term prolactin-raising antipsychotic medication on bone mineral density in patients with schizophrenia. Br J Psychiatry *184*, 503-508.

Mehler,M.F. and Kessler,J.A. (1997). Hematolymphopoietic and inflammatory cytokines in neural development. Trends Neurosci. *20*, 357-365.

Meltzer,H.Y., Koenig,J.I., Nash,J.F., and Gudelsky,G.A. (1989). Melperone and clozapine: neuroendocrine effects of atypical neuroleptic drugs. Acta Psychiatr Scand. Suppl *352*, 24-29.

Meltzer,H.Y., Lee,M.A., and Jayathilake,K. (2001). The blunted plasma cortisol response to apomorphine and its relationship to treatment response in patients with schizophrenia. Neuropsychopharmacology *24* , 278-290.

Meltzer,H.Y. and Stahl,S.M. (1976). The dopamine hypothesis of schizophrenia: a review. Schizophr Bull *2*, 19-76.

Merrill,J.E. and Benveniste,E.N. (1996a). Cytokines in inflammatory brain lesions: helpful and harmful. Trends Neurosci. *19*, 331-338.

Merrill,J.E. and Benveniste,E.N. (1996b). Cytokines in inflammatory brain lesions: helpful and harmful. Trends Neurosci. *19*, 331-338.

Metzger,D.W., Buchanan,J.M., Collins,J.T., Lester,T.L., Murray,K.S., Van Cleave,V.H., Vogel,L.A., and Dunnick,W.A. (1996). Enhancement of humoral immunity by interleukin-12. Ann N Y Acad Sci *795*, 100-115.

Micallef,M.J., Tanimoto,T., Torigoe,K., Nishida,Y., Kohno,K., Ikegami,H., and Kurimoto,M. (1999). Simultaneous exposure to interleukin-18 and interleukin-10 in vitro synergistically augments murine spleen natural killer cell activity. Cancer Immunol Immunother. *48*, 109-117.

Minami,Y., Kono,T., Miyazaki,T., and Taniguchi,T. (1993). The IL-2 receptor complex: its structure, function, and target genes. Annu. Rev Immunol *11*, 245-268.

Miska,W. and Mahmoud,M. (1993). Determination of soluble interleukin-2 receptor in human seminal plasma. Arch Androl *30*, 23-28.

Mittleman,B.B., Castellanos,F.X., Jacobsen,L.K., Rapoport,J.L., Swedo,S.E., and Shearer,G.M. (1997). Cerebrospinal fluid cytokines in pediatric neuropsychiatric disease. J Immunol *159*, 2994-2999.

Miyamoto,S., LaMantia,A.S., Duncan,G.E., Sullivan,P., Gilmore,J.H., and Lieberman,J.A. (2003). Recent advances in the neurobiology of schizophrenia. Mol Interv. *3*, 27-39.

Miyaura,H. and Iwata,M. (2002). Direct and indirect inhibition of Th1 development by progesterone and glucocorticoids. J Immunol *168*, 1087-1094.

Mizoguchi,K., Kunishita,T., Chui,D.H., and Tabira,T. (1992). Stress induces neuronal death in the hippocampus of castrated rats. Neurosci. Lett. *138*, 157-160.

Mocchegiani,E., Giacconi,R., Cipriano,C., Gasparini,N., Bernardini,G., Malavolta,M., Menegazzi,M., Cavalieri,E., Muzzioli,M., Ciampa,A.R., and Suzuki,H. (2004). The variations during the circadian cycle of liver CD1d-unrestricted NK1.1+TCR gamma/delta+ cells lead to successful ageing. Role of metallothionein/IL-6/gp130/PARP-1 interplay in very old mice. Exp Gerontol. *39*, 775-788.

Moises,H.W., Schindler,L., Leroux,M., and Kirchner,H. (1985). Decreased production of interferon alpha and interferon gamma in leucocyte cultures of schizophrenic patients. Acta Psychiatr Scand. *72*, 45-50.

Moller,H.J. (2003). Management of the negative symptoms of schizophrenia: new treatment options. CNS. Drugs *17*, 793-823.

Monteleone,P., Fabrazzo,M., Tortorella,A., and Maj,M. (1997). Plasma levels of interleukin-6 and tumor necrosis factor alpha in chronic schizophrenia: effects of clozapine treatment. Psychiatry Res. *71*, 11-17.

Monteleone,P., Tortorella,A., Borriello,R., Cassandro,P., and Maj,M. (1999). Prolactin hyperresponsiveness to D-fenfluramine in drug-free schizophrenic patients: a placebo-controlled study. Biol Psychiatry *45*, 1606-1611.

Moreno,J., Varas,A., Vicente,A., and Zapata,A.G. (1998). Role of prolactin in the recovered T-cell development of early partially decapitated chicken embryo. Dev. Immunol *5*, 183-195.

Morris,C.S. and Esiri,M.M. (1998). The expression of cytokines and their receptors in normal and mildly reactive human brain. J Neuroimmunol. *92*, 85-97.

Morrison,B.W. and Leder,P. (1992). A receptor binding domain of mouse interleukin-4 defined by a solid-phase binding assay and in vitro mutagenesis. J Biol Chem. *267*, 11957-11963.

Morton,S.M., Bocaccio,I., Depetris,D., Mattei,M., and Dessein,A. (1997). Assignment of IL12RB2 to human chromosome 1p31.3-->p31.2 between D1S230 and D1S198. Cytogenet. Cell Genet. *79*, 282-283.

Mosmann,T.R. and Coffman,R.L. (1989). TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu. Rev Immunol *7*, 145-173.

Mosmann,T.R. and Sad,S. (1996). The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today *17*, 138-146.

Muller,N., Empl,M., Riedel,M., Schwarz,M., and Ackenheil,M. (1997). Neuroleptic treatment increases soluble IL-2 receptors and decreases soluble IL-6 receptors in schizophrenia. Eur Arch Psychiatry Clin Neurosci. *247*, 308-313.

Muller,N., Riedel,M., Gruber,R., Ackenheil,M., and Schwarz,M.J. (2000). The immune system and schizophrenia. An integrative view. Ann N Y Acad Sci *917*, 456-467.

Muller,N., Ulmschneider,M., Scheppach,C., Schwarz,M.J., Ackenheil,M., Moller,H.J., Gruber,R., and Riedel,M. (2004). COX-2 inhibition as a treatment approach in schizophrenia: immunological considerations and clinical effects of celecoxib add-on therapy. Eur Arch Psychiatry Clin Neurosci. *254*, 14-22.

Munn,N.A. (2000). Microglia dysfunction in schizophrenia: an integrative theory. Med Hypotheses *54*, 198-202.

Murata,T., Obiri,N.I., and Puri,R.K. (1998). Structure of and signal transduction through interleukin-4 and interleukin-13 receptors (review). Int J Mol Med *1*, 551-557.

Murphy,K.M., Ouyang,W., Farrar,J.D., Yang,J., Ranganath,S., Asnagli,H., Afkarian,M., and Murphy,T.L. (2000). Signaling and transcription in T helper development. Annu. Rev Immunol *18*, 451-494.

Murphy,K.M., Ouyang,W., Szabo,S.J., Jacobson,N.G., Guler,M.L., Gorham,J.D., Gubler,U., and Murphy,T.L. (1999). T helper differentiation proceeds through Stat1-dependent, Stat4-dependent and Stat4-independent phases. Curr Top. Microbiol Immunol *238*, 13-26.

Nadeau,S. and Rivest,S. (2000). Role of microglial-derived tumor necrosis factor in mediating CD14 transcription and nuclear factor kappa B activity in the brain during endotoxemia. J Neurosci. *20*, 3456-3468.

Nakajima,H., Kobayashi,M., Pollard,R.B., and Suzuki,F. (2000). A pathogenic role of Th2 responses on the severity of encephalomyelitis induced in mice by herpes simplex virus type 2 infection. J Neuroimmunol. *110*, 106-113.

Nakayama,T., Hieshima,K., Nagakubo,D., Sato,E., Nakayama,M., Kawa,K., and Yoshie,O. (2004). Selective induction of Th2-attracting chemokines CCL17 and CCL22 in human B cells by latent membrane protein 1 of Epstein-Barr virus. J Virol. *78*, 1665-1674.

Naudin,J., Capo,C., Giusano,B., Mege,J.L., and Azorin,J.M. (1997). A differential role for interleukin-6 and tumor necrosis factor-alpha in schizophrenia? Schizophr Res. *26*, 227-233.

Naudin,J., Mege,J.L., Azorin,J.M., and Dassa,D. (1996). Elevated circulating levels of IL-6 in schizophrenia. Schizophr Res. *20*, 269-273.

Naume,B., Gately,M., and Espevik,T. (1992). A comparative study of IL-12 (cytotoxic lymphocyte maturation factor)-, IL-2-, and IL-7-induced effects on immunomagnetically purified CD56+ NK cells. J Immunol *148*, 2429-2436.

Naylor,S.L., Sakaguchi,A.Y., Shows,T.B., Law,M.L., Goeddel,D.V., and Gray,P.W. (1983). Human immune interferon gene is located on chromosome 12. J Exp Med *157*, 1020-1027.

Neumann,H., Boucraut,J., Hahnel,C., Misgeld,T., and Wekerle,H. (1996). Neuronal control of MHC class II inducibility in rat astrocytes and microglia. Eur J Neurosci. *8*, 2582-2590.

Neumann,H., Schmidt,H., Cavalie,A., Jenne,D., and Wekerle,H. (1997). Major histocompatibility complex (MHC) class I gene expression in single neurons of the central nervous system: differential regulation by interferon (IFN)-gamma and tumor necrosis factor (TNF)-alpha. J Exp Med *185*, 305-316.

Newcomer,J.W., Craft,S., Askins,K., Hershey,T., Bardgett,M.E., Csernansky,J.G., Gagliardi,A.E., and Vogler,G. (1998). Glucocorticoid interactions with memory function in schizophrenia. Psychoneuroendocrinology *23*, 65-72.

Ng,E.K., Panesar,N., Longo,W.E., Shapiro,M.J., Kaminski,D.L., Tolman,K.C., and Mazuski,J.E. (2003). Human intestinal epithelial and smooth muscle cells are potent producers of IL-6. Mediators Inflamm. *12*, 3-8.

Niiro,H., Otsuka,T., Kuga,S., Nemoto,Y., Abe,M., Hara,N., Nakano,T., Ogo,T., and Niho,Y. (1994). IL-10 inhibits prostaglandin E2 production by lipopolysaccharide-stimulated monocytes. Int Immunol *6*, 661-664.

Nikcevich,K.M., Gordon,K.B., Tan,L., Hurst,S.D., Kroepfl,J.F., Gardinier,M., Barrett,T.A., and Miller,S.D. (1997). IFN-gamma-activated primary murine astrocytes express B7 costimulatory molecules and prime naive antigen-specific T cells. J Immunol *158*, 614-621.

Nikkila,H.V., Ahokas,A., Wahlbeck,K., Rimon,R., and Andersson,L.C. (2002). Neopterin and macrophage inflammatory protein-1alpha in the cerebrospinal fluid of schizophrenic patients: no evidence of intrathecal inflammation. Neuropsychobiology *46*, 169-172.

Nikkila,H.V., Muller,K., Ahokas,A., Rimon,R., and Andersson,L.C. (2001). Increased frequency of activated lymphocytes in the cerebrospinal fluid of patients with acute schizophrenia. Schizophr Res. *49*, 99-105.

O'Donnell,M.C., Catts,S.V., Ward,P.B., Liebert,B., Lloyd,A., Wakefield,D., and McConaghy,N. (1996). Increased production of interleukin-2 (IL-2) but not soluble interleukin-2 receptors (sIL-2R) in unmedicated patients with schizophrenia and schizophreniform disorder. Psychiatry Res. *65*, 171-178.

O'Garra,A. and Arai,N. (2000). The molecular basis of T helper 1 and T helper 2 cell differentiation. Trends Cell Biol *10*, 542-550.

Obiri,N.I., Debinski,W., Leonard,W.J., and Puri,R.K. (1995). Receptor for interleukin 13. Interaction with interleukin 4 by a mechanism that does not involve the common gamma chain shared by receptors for interleukins 2, 4, 7, 9, and 15. J Biol Chem. *270*, 8797-8804.

Obiri,N.I., Murata,T., Debinski,W., and Puri,R.K. (1997). Modulation of interleukin (IL)-13 binding and signaling by the gammac chain of the IL-2 receptor. J Biol Chem. *272*, 20251-20258.

Oh,S. and Eichelberger,M.C. (2000). Polarization of allogeneic T-cell responses by influenza virus-infected dendritic cells. J Virol. *74*, 7738-7744.

Ohteki,T., Fukao,T., Suzue,K., Maki,C., Ito,M., Nakamura,M., and Koyasu,S. (1999). Interleukin 12-dependent interferon gamma production by CD8alpha+ lymphoid dendritic cells. J Exp Med *189*, 1981-1986.

Okumura,M., Hidaka,Y., Matsuzuka,F., Takeoka,K., Tada,H., Kuma,K., and Amino,N. (1999). CD30 expression and interleukin-4 and interferon-gamma production of intrathyroidal lymphocytes in Graves' disease. Thyroid *9*, 333-339.

Omari,K.M. and Dorovini-Zis,K. (2003a). CD40 expressed by human brain endothelial cells regulates CD4+ T cell adhesion to endothelium. J Neuroimmunol. *134*, 166-178.

Omari,K.M. and Dorovini-Zis,K. (2003b). CD40 expressed by human brain endothelial cells regulates CD4+ T cell adhesion to endothelium. J Neuroimmunol. *134*, 166-178.

Opal,S.M., Wherry,J.C., and Grint,P. (1998). Interleukin-10: potential benefits and possible risks in clinical infectious diseases. Clin Infect. Dis. *27*, 1497-1507.

Otero,G.C. and Merrill,J.E. (1994). Cytokine receptors on glial cells. Glia *11*, 117-128.

Otto,V.I., Heinzel-Pleines,U.E., Gloor,S.M., Trentz,O., Kossmann,T., and Morganti-Kossmann,M.C. (2000). sICAM-1 and TNF-alpha induce MIP-2 with distinct kinetics in astrocytes and brain microvascular endothelial cells. J Neurosci. Res. *60*, 733-742.

Pandita,R., Pocsik,E., and Aggarwal,B.B. (1992). Interferon-gamma induces cell surface expression for both types of tumor necrosis factor receptors. FEBS Lett. *312*, 87-90.

Path,G., Scherbaum,W.A., and Bornstein,S.R. (2000). The role of interleukin-6 in the human adrenal gland. Eur J Clin Invest *30 Suppl 3*, 91-95.

Pellegrini,P., Berghella,A.M., Contasta,I., and Adorno,D. (2003). CD30 antigen: not a physiological marker for TH2 cells but an important costimulator molecule in the regulation of the balance between TH1/TH2 response. Transpl. Immunol *12*, 49-61.

Pelonero,A.L., Pandurangi,A.K., and Calabrese,V.P. (1990). Serum IgG antibody to herpes viruses in schizophrenia. Psychiatry Res. *33*, 11-17.

Perez,C., Albert,I., DeFay,K., Zachariades,N., Gooding,L., and Kriegler,M. (1990). A nonsecretable cell surface mutant of tumor necrosis factor (TNF) kills by cell-to-cell contact. Cell *63*, 251-258.

Peritt,D., Robertson,S., Gri,G., Showe,L., Aste-Amezaga,M., and Trinchieri,G. (1998). Differentiation of human NK cells into NK1 and NK2 subsets. J Immunol *161*, 5821-5824.

Petitto,J.M., McNamara,R.K., Gendreau,P.L., Huang,Z., and Jackson,A.J. (1999). Impaired learning and memory and altered hippocampal neurodevelopment resulting from interleukin-2 gene deletion. J Neurosci. Res. *56*, 441-446.

Petrovsky,N. and Harrison,L.C. (1998). The chronobiology of human cytokine production. Int Rev Immunol *16*, 635-649.

Pfizenmaier,K., Wiegmann,K., Scheurich,P., Kronke,M., Merlin,G., Aguet,M., Knowles,B.B., and Ucer,U. (1988). High affinity human IFN-gamma-binding capacity is encoded by a single receptor gene located in proximity to c-ros on human chromosome region 6q16 to 6q22. J Immunol *141*, 856-860.

Piccinni,M.P., Scaletti,C., Maggi,E., and Romagnani,S. (2000). Role of hormone-controlled Th1- and Th2-type cytokines in successful pregnancy. J Neuroimmunol. *109*, 30-33.

Piccinni,M.P., Scaletti,C., Vultaggio,A., Maggi,E., and Romagnani,S. (2001). Defective production of LIF, M-CSF and Th2-type cytokines by T cells at fetomaternal interface is associated with pregnancy loss. J Reprod. Immunol *52*, 35-43.

Pizzolo,G., Vincenzi,C., Vinante,F., Rigo,A., Veneri,D., Chilosi,M., Dusi,S., Poli,G., Zambello,R., Semenzato,G., and . (1992). Highly concentrated urine-purified Tac peptide fails to inhibit IL-2-dependent cell proliferation in vitro. Cell Immunol *141*, 253-259.

Plackett,T.P., Schilling,E.M., Faunce,D.E., Choudhry,M.A., Witte,P.L., and Kovacs,E.J. (2003). Aging enhances lymphocyte cytokine defects after injury. FASEB J *17*, 688-689.

Plaut,M., Pierce,J.H., Watson,C.J., Hanley-Hyde,J., Nordan,R.P., and Paul,W.E. (1989). Mast cell lines produce lymphokines in response to cross-linkage of Fc epsilon RI or to calcium ionophores. Nature *339*, 64-67.

Pollmacher,T., Hinze-Selch,D., Fenzel,T., Kraus,T., Schuld,A., and Mullington,J. (1997). Plasma levels of cytokines and soluble cytokine receptors during treatment with haloperidol. Am J Psychiatry *154*, 1763-1765.

Pollmacher,T., Hinze-Selch,D., Mullington,J., and Holsboer,F. (1995). Clozapine-induced increase in plasma levels of soluble interleukin-2 receptors. Arch Gen Psychiatry *52*, 877-878.

Portielje,J.E., Lamers,C.H., Kruit,W.H., Sparreboom,A., Bolhuis,R.L., Stoter,G., Huber,C., and Gratama,J.W. (2003). Repeated administrations of interleukin (IL)-12 are associated with persistently elevated plasma levels of IL-10 and declining IFN-gamma, tumor necrosis factor-alpha, IL-6, and IL-8 responses. Clin Cancer Res. *9*, 76-83.

Powell,M.J., Thompson,S.A., Tone,Y., Waldmann,H., and Tone,M. (2000). Posttranscriptional regulation of IL-10 gene expression through sequences in the 3'-untranslated region. J Immunol *165*, 292-296.

Prabhu Das,M.R., Zamvil,S.S., Borriello,F., Weiner,H.L., Sharpe,A.H., and Kuchroo,V.K. (1995). Reciprocal expression of co-stimulatory molecules, B7-1 and B7-2, on murine T cells following activation. Eur J Immunol *25*, 207-211.

Prescott,C.A. and Gottesman,I.I. (1993). Genetically mediated vulnerability to schizophrenia. Psychiatr Clin North Am *16*, 245-267.

Presky,D.H., Minetti,L.J., Gillessen,S., Gubler,U., Chizzonite,R., Stern,A.S., and Gately,M.K. (1996a). Evidence for multiple sites of interaction between IL-12 and its receptor. Ann N Y Acad Sci *795*, 390-393.

Presky,D.H., Yang,H., Minetti,L.J., Chua,A.O., Nabavi,N., Wu,C.Y., Gately,M.K., and Gubler,U. (1996b). A functional interleukin 12 receptor complex is composed of two beta-type cytokine receptor subunits. Proc. Natl. Acad Sci U. S A *93*, 14002-14007.

Protonotariou,E., Malamitsi-Puchner,A., Rizos,D., Papagianni,B., Moira,E., Sarandakou,C.A., and Botsis,D. (2004). Age-related differentiations of Th1/Th2 cytokines in newborn infants. Mediators Inflamm. *13*, 89-92.

Punnonen,J., Aversa,G., Cocks,B.G., McKenzie,A.N., Menon,S., Zurawski,G., de Waal,M.R., and de Vries,J.E. (1993). Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. Proc. Natl. Acad Sci U. S A *90*, 3730-3734.

Purnell,J.Q., Brandon,D.D., Isabelle,L.M., Loriaux,D.L., and Samuels,M.H. (2004). Association of 24-hour cortisol production rates, cortisol-binding globulin, and plasma-free cortisol levels with body composition, leptin levels, and aging in adult men and women. J Clin Endocrinol. Metab *89*, 281-287.

Qiu,Y.H., Peng,Y.P., and Wang,J.J. (2003). [Cellular and molecular mechanisms of regulation of immune functions by catecholamines]. Sheng Li Ke. Xue. Jin. Zhan. *34*, 303-308.

Quelle,F.W., Shimoda,K., Thierfelder,W., Fischer,C., Kim,A., Ruben,S.M., Cleveland,J.L., Pierce,J.H., Keegan,A.D., Nelms,K., and . (1995). Cloning of murine Stat6 and human Stat6, Stat proteins that are tyrosine phosphorylated in responses to IL-4 and IL-3 but are not required for mitogenesis. Mol Cell Biol *15*, 3336-3343.

Racke,M.K., Bonomo,A., Scott,D.E., Cannella,B., Levine,A., Raine,C.S., Shevach,E.M., and Rocken,M. (1994). Cytokine-induced immune deviation as a therapy for inflammatory autoimmune disease. J Exp Med *180* , 1961-1966.

Raghavendra,V., Singh,V., Kulkarni,S.K., and Agrewala,J.N. (2001). Melatonin enhances Th2 cell mediated immune responses: lack of sensitivity to reversal by naltrexone or benzodiazepine receptor antagonists. Mol Cell Biochem. *221*, 57-62.

Raison,C.L. and Miller,A.H. (2003). When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. Am J Psychiatry *160*, 1554-1565.

Rao,A. and Avni,O. (2000). Molecular aspects of T-cell differentiation. Br Med Bull *56*, 969-984.

Rapaport,M.H. and Lohr,J.B. (1994). Serum-soluble interleukin-2 receptors in neuroleptic-naive schizophrenic subjects and in medicated schizophrenic subjects with and without tardive dyskinesia. Acta Psychiatr Scand. *90*, 311-315.

Rapaport,M.H., McAllister,C.G., Pickar,D., Nelson,D.L., and Paul,S.M. (1989). Elevated levels of soluble interleukin 2 receptors in schizophrenia. Arch Gen Psychiatry *46*, 291-292.

Rapaport,M.H., McAllister,C.G., Pickar,D., Tamarkin,L., Kirch,D.G., and Paul,S.M. (1997). CSF IL-1 and IL-2 in medicated schizophrenic patients and normal volunteers. Schizophr Res. *25*, 123-129.

Rapaport,M.H. and Stein,M.B. (1994). Serum interleukin-2 and soluble interleukin-2 receptor levels in generalized social phobia. Anxiety. *1*, 50-53.

Rapaport,M.H., Torrey,E.F., McAllister,C.G., Nelson,D.L., Pickar,D., and Paul,S.M. (1993). Increased serum soluble interleukin-2 receptors in schizophrenic monozygotic twins. Eur Arch Psychiatry Clin Neurosci. *243*, 7-10.

Reboul,J., Gardiner,K., Monneron,D., Uze,G., and Lutfalla,G. (1999). Comparative genomic analysis of the interferon/interleukin-10 receptor gene cluster. Genome Res. *9*, 242-250.

Rempel,J.D., Murray,S.J., Meisner,J., and Buchmeier,M.J. (2004). Differential regulation of innate and adaptive immune responses in viral encephalitis. Virology *318*, 381-392.

Rey,E.R., Bailer,J., Brauer,W., Handel,M., Laubenstein,D., and Stein,A. (1994). Stability trends and longitudinal correlations of negative and positive syndromes within a three-year follow-up of initially hospitalized schizophrenics. Acta Psychiatr Scand. *90*, 405-412.

Riley,B. (2004). Linkage studies of schizophrenia. Neurotox. Res. *6*, 17-34.

Rogge,L., Barberis-Maino,L., Biffi,M., Passini,N., Presky,D.H., Gubler,U., and Sinigaglia,F. (1997). Selective expression of an interleukin-12 receptor component by human T helper 1 cells. J Exp Med *185*, 825-831.

Romagnani,S. (1999). Th1/Th2 cells. Inflamm. Bowel. Dis. *5*, 285-294.

Romanowicz,K., Misztal,T., and Barcikowski,B. (2004). The effects of intracerebroventricular infusion of prolactin in luteinizing hormone, testosterone and growth hormone secretion in male sheep. Anim Reprod. Sci *81*, 261-271.

Roper,R.L., Conrad,D.H., Brown,D.M., Warner,G.L., and Phipps,R.P. (1990). Prostaglandin E2 promotes IL-4-induced IgE and IgG1 synthesis. J Immunol *145*, 2644-2651.

Rothermundt,M., Arolt,V., Leadbeater,J., Peters,M., Rudolf,S., and Kirchner,H. (2000). Cytokine production in unmedicated and treated schizophrenic patients. Neuroreport *11*, 3385-3388.

Rothermundt,M., Arolt,V., Weitzsch,C., Eckhoff,D., and Kirchner,H. (1998). Immunological dysfunction in schizophrenia: a systematic approach. Neuropsychobiology *37*, 186-193.

Rothoeft,T., Gonschorek,A., Bartz,H., Anhenn,O., and Schauer,U. (2003). Antigen dose, type of antigen-presenting cell and time of differentiation contribute to the T helper 1/T helper 2 polarization of naive T cells. Immunology *110*, 430-439.

Rousset,F., Garcia,E., Defrance,T., Peronne,C., Vezzio,N., Hsu,D.H., Kastelein,R., Moore,K.W., and Banchereau,J. (1992). Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc. Natl. Acad Sci U. S A *89*, 1890-1893.

Rovensky,J., Lackovic,V., Veselkova,Z., Horvathova,M., Koska,J., Blazickova,S., and Vigas,M. (1999). Plasma cytokine concentration and the cytokine producing ability of whole blood cell cultures from healthy females with pharmacologically induced hyperprolactinemia. Int J Tissue React. *21*, 43-49.

Roy,B., Bhattacharjee,A., Xu,B., Ford,D., Maizel,A.L., and Cathcart,M.K. (2002a). IL-13 signal transduction in human monocytes: phosphorylation of receptor components, association with Jaks, and phosphorylation/activation of Stats. J Leukoc. Biol *72*, 580-589.

Roy,M.P., Kim,C.H., and Butcher,E.C. (2002b). Cytokine control of memory B cell homing machinery. J Immunol *169*, 1676-1682.

Rudolf,S., Peters,M., Rothermundt,M., Arolt,V., and Kirchner,H. (2002). The influence of typical and atypical neuroleptic drugs in the production of interleukin-2 and interferon-gamma in vitro. Neuropsychobiology *46*, 180-185.

Ruedig,C. and Dringen,R. (2004). TNF alpha increases activity of gamma-glutamyl transpeptidase in cultured rat astroglial cells. J Neurosci. Res. *75*, 536-543.

Ryan,J.J., McReynolds,L.J., Keegan,A., Wang,L.H., Garfein,E., Rothman,P., Nelms,K., and Paul,W.E. (1996). Growth and gene expression are predominantly controlled by distinct regions of the human IL-4 receptor. Immunity. *4*, 123-132.

Saija,A., Princi,P., Lanza,M., Scalese,M., Aramnejad,E., and De Sarro,A. (1995). Systemic cytokine administration can affect blood-brain barrier permeability in the rat. Life Sci *56*, 775-784.

Saint-Vis,B., Fugier-Vivier,I., Massacrier,C., Gaillard,C., Vanbervliet,B., Ait-Yahia,S., Banchereau,J., Liu,Y.J., Lebecque,S., and Caux,C. (1998). The cytokine profile expressed by human dendritic cells is dependent on cell subtype and mode of activation. J Immunol *160*, 1666-1676.

Sakami,S., Ishikawa,T., Kawakami,N., Haratani,T., Fukui,A., Kobayashi,F., Fujita,O., Araki,S., and Kawamura,N. (2002). Coemergence of insomnia and a shift in the Th1/Th2 balance toward Th2 dominance. Neuroimmunomodulation. *10*, 337-343.

Salem,M.L., Matsuzaki,G., Madkour,G.A., and Nomoto,K. (1999). Beta-estradiol-induced decrease in IL-12 and TNF-alpha expression suppresses macrophage functions in the course of Listeria monocytogenes infection in mice. Int J Immunopharmacol. *21*, 481-497.

Salokangas,R.K. (2004). Gender and the use of neuroleptics in schizophrenia. Schizophr Res. *66*, 41-49.

Sandmand,M., Bruunsgaard,H., Kemp,K., Andersen-Ranberg,K., Pedersen,A.N., Skinhoj,P., and Pedersen,B.K. (2002). Is ageing associated with a shift in the balance between Type 1 and Type 2 cytokines in humans? Clin Exp Immunol *127*, 107-114.

Sandyk,R. and Kay,S.R. (1991). Tobacco addiction as a marker of age at onset of schizophrenia. Int J Neurosci. *57*, 259-262.

Santana,M.A. and Rosenstein,Y. (2003). What it takes to become an effector T cell: the process, the cells involved, and the mechanisms. J Cell Physiol *195*, 392-401.

Satoh,J., Kastrukoff,L.F., and Kim,S.U. (1991). Cytokine-induced expression of intercellular adhesion molecule-1 (ICAM-1) in cultured human oligodendrocytes and astrocytes. J Neuropathol. Exp Neurol. *50*, 215-226.

Sauro,M.D., Jorgensen,R.S., and Pedlow,C.T. (2003). Stress, glucocorticoids, and memory: a meta-analytic review. Stress. *6*, 235-245.

Schafer,K.H., Mestres,P., Marz,P., and Rose-John,S. (1999). The IL-6/sIL-6R fusion protein hyper-IL-6 promotes neurite outgrowth and neuron survival in cultured enteric neurons. J Interferon Cytokine Res. *19*, 527-532.

Schmand,B., Kuipers,T., Van der,G.M., Bosveld,J., Bulthuis,F., and Jellema,M. (1994). Cognitive disorders and negative symptoms as correlates of motivational deficits in psychotic patients. Psychol. Med *24*, 869-884.

Schobitz,B., de Kloet,E.R., Sutanto,W., and Holsboer,F. (1993). Cellular localization of interleukin 6 mRNA and interleukin 6 receptor mRNA in rat brain. Eur J Neurosci. *5*, 1426-1435.

Schobitz,B., Pezeshki,G., Pohl,T., Hemmann,U., Heinrich,P.C., Holsboer,F., and Reul,J.M. (1995). Soluble interleukin-6 (IL-6) receptor augments central effects of IL-6 in vivo. FASEB J *9*, 659-664.

Schobitz,B., Voorhuis,D.A., and de Kloet,E.R. (1992). Localization of interleukin 6 mRNA and interleukin 6 receptor mRNA in rat brain. Neurosci. Lett. *136*, 189-192.

Schottelius,A.J., Moldawer,L.L., Dinarello,C.A., Asadullah,K., Sterry,W., and Edwards,C.K., III (2004). Biology of tumor necrosis factor-alpha- implications for psoriasis. Exp Dermatol. *13*, 193-222.

Schroder,K., Hertzog,P.J., Ravasi,T., and Hume,D.A. (2004). Interferon-gamma: an overview of signals, mechanisms and functions. J Leukoc. Biol *75*, 163-189.

Schultze,J.L., Michalak,S., Lowne,J., Wong,A., Gilleece,M.H., Gribben,J.G., and Nadler,L.M. (1999). Human non-germinal center B cell interleukin (IL)-12 production is primarily regulated by T cell signals CD40 ligand, interferon gamma, and IL-10: role of B cells in the maintenance of T cell responses. J Exp Med *189*, 1-12.

Schwab,S.G., Mondabon,S., Knapp,M., Albus,M., Hallmayer,J., Borrmann-Hassenbach,M., Trixler,M., Gross,M., Schulze,T.G., Rietschel,M., Lerer,B., Maier,W., and Wildenauer,D.B. (2003). Association of tumor necrosis factor alpha gene -G308A polymorphism with schizophrenia. Schizophr Res. *65*, 19-25.

Schwarz,M.J., Chiang,S., Muller,N., and Ackenheil,M. (2001a). T-helper-1 and T-helper-2 responses in psychiatric disorders. Brain Behav Immun *15*, 340-370.

Schwarz,M.J., Muller,N., Riedel,M., and Ackenheil,M. (2001b). The Th2-hypothesis of schizophrenia: a strategy to identify a subgroup of schizophrenia caused by immune mechanisms. Med Hypotheses *56*, 483-486.

Segal,M., Avital,A., Rojas,M., Hausvater,N., Sandbank,S., Liba,D., Moguillansky,L., Tal,I., and Weizman,A. (2004). Serum prolactin levels in unmedicated first-episode and recurrent schizophrenia patients: a possible marker for the disease's subtypes. Psychiatry Res. *127*, 227-235.

Seguin,R., Biernacki,K., Rotondo,R.L., Prat,A., and Antel,J.P. (2003). Regulation and functional effects of monocyte migration across human brain-derived endothelial cells. J Neuropathol. Exp Neurol. *62*, 412-419.

Shibata,Y., Foster,L.A., Kurimoto,M., Okamura,H., Nakamura,R.M., Kawajiri,K., Justice,J.P., Van Scott,M.R., Myrvik,Q.N., and Metzger,W.J. (1998). Immunoregulatory roles of IL-10 in innate immunity: IL-10 inhibits macrophage production of IFN-gamma-inducing factors but enhances NK cell production of IFN-gamma. J Immunol *161*, 4283-4288.

Shintani,F., Kanba,S., Maruo,N., Nakaki,T., Nibuya,M., Suzuki,E., Kinoshita,N., and Yagi,G. (1991). Serum interleukin-6 in schizophrenic patients. Life Sci *49*, 661-664.

Shrikant,P., Chung,I.Y., Ballestas,M.E., and Benveniste,E.N. (1994b). Regulation of intercellular adhesion molecule-1 gene expression by tumor necrosis factor-alpha, interleukin-1 beta, and interferon-gamma in astrocytes. J Neuroimmunol. *51*, 209-220.

Shrikant,P., Chung,I.Y., Ballestas,M.E., and Benveniste,E.N. (1994a). Regulation of intercellular adhesion molecule-1 gene expression by tumor necrosis factor-alpha, interleukin-1 beta, and interferon-gamma in astrocytes. J Neuroimmunol. *51*, 209-220.

Sibson,N.R., Blamire,A.M., Bernades-Silva,M., Laurent,S., Boutry,S., Muller,R.N., Styles,P., and Anthony,D.C. (2004). MRI detection of early endothelial activation in brain inflammation. Magn Reson. Med *51*, 248-252.

Sieburth,D., Jabs,E.W., Warrington,J.A., Li,X., Lasota,J., LaForgia,S., Kelleher,K., Huebner,K., Wasmuth,J.J., and Wolf,S.F. (1992). Assignment of genes encoding a unique cytokine (IL12) composed of two unrelated subunits to chromosomes 3 and 5. Genomics *14*, 59-62.

Siewert,E., Muller-Esterl,W., Starr,R., Heinrich,P.C., and Schaper,F. (1999). Different protein turnover of interleukin-6-type cytokine signalling components. Eur J Biochem. *265*, 251-257.

Skjolaas,K.A., Grieger,D.M., Hill,C.M., and Minton,J.E. (2002). Glucocorticoid regulation of type 1 and type 2 cytokines in cultured porcine splenocytes. Vet. Immunol Immunopathol. *87*, 79-87.

Smirnov,D.V., Smirnova,M.G., Korobko,V.G., and Frolova,E.I. (1995). Tandem arrangement of human genes for interleukin-4 and interleukin-13: resemblance in their organization. Gene *155*, 277-281.

Smith,R.S. (1992). A comprehensive macrophage-T-lymphocyte theory of schizophrenia. Med Hypotheses *39*, 248-257.

Smith,R.S. and Maes,M. (1995). The macrophage-T-lymphocyte theory of schizophrenia: additional evidence. Med Hypotheses *45*, 135-141.

Smith,S. (2003). Effects of antipsychotics on sexual and endocrine function in women: implications for clinical practice. J Clin Psychopharmacol *23*, S27-S32.

Snapper,C.M. and Paul,W.E. (1987). Interferon-gamma and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. Science *236*, 944-947.

Sobel,R.A., Mitchell,M.E., and Fondren,G. (1990). Intercellular adhesion molecule-1 (ICAM-1) in cellular immune reactions in the human central nervous system. Am J Pathol. *136*, 1309-1316.

Soderquist,B., Jones,I., Fredlund,H., and Vikerfors,T. (1998). Bacterial or crystal-associated arthritis? Discriminating ability of serum inflammatory markers. Scand. J Infect. Dis. *30*, 591-596.

Sofuni,T. and Yoshida,M.C. (1992). Combined use of several mitogens for mitotic stimulation to human lymphocytes. J Radiat. Res. (Tokyo) *33 Suppl*, 222-230.

Song,C., Lin,A., Kenis,G., Bosmans,E., and Maes,M. (2000). Immunosuppressive effects of clozapine and haloperidol: enhanced production of the interleukin-1 receptor antagonist. Schizophr Res. *42*, 157-164.

Sortino,M.A., Chisari,M., Merlo,S., Vancheri,C., Caruso,M., Nicoletti,F., Canonico,P.L., and Copani,A. (2004). Glia mediates the neuroprotective action of estradiol on {beta}-amyloid-induced neuronal death. Endocrinology.

Sperner-Unterweger,B., Barnas,C., Fuchs,D., Kemmler,G., Wachter,H., Hinterhuber,H., and Fleischhacker,W.W. (1992). Neopterin production in acute schizophrenic patients: an indicator of alterations of cell-mediated immunity. Psychiatry Res. *42*, 121-128.

Starkenburg,S., Munroe,M.E., and Waltenbaugh,C. (2001). Early alteration in leukocyte populations and Th1/Th2 function in ethanol-consuming mice. Alcohol Clin Exp Res. *25*, 1221-1230.

Stenzel-Poore,M.P., Bruderer,U., and Rittenberg,M.B. (1988). The adaptive potential of the memory response: clonal recruitment and epitope recognition. Immunol Rev *105*, 113-136.

Stern,A.S., Magram,J., and Presky,D.H. (1996). Interleukin-12 an integral cytokine in the immune response. Life Sci *58*, 639-654.

Stiernberg,J., Low,M.G., Flaherty,L., and Kincade,P.W. (1987). Removal of lymphocyte surface molecules with phosphatidylinositol-specific phospholipase C: effects on mitogen responses and evidence that ThB and certain Qa antigens are membrane-anchored via phosphatidylinositol. J Immunol *138*, 3877-3884.

Sugimoto,N., Nakahira,M., Ahn,H.J., Micallef,M., Hamaoka,T., Kurimoto,M., and Fujiwara,H. (2003). Differential requirements for JAK2 and TYK2 in T cell proliferation and IFN-gamma production induced by IL-12 alone or together with IL-18. Eur J Immunol *33*, 243-251.

Sykora,K.W., Kolitz,J., Szabo,P., Grzeschik,K.H., Moore,M.A., and Mertelsmann,R. (1984). Human interleukin 2 gene is located on chromosome 4. Cancer Invest *2*, 261-265.

Szabo,S.J., Dighe,A.S., Gubler,U., and Murphy,K.M. (1997). Regulation of the interleukin (IL)-12R beta 2 subunit expression in developing T helper 1 (Th1) and Th2 cells. J Exp Med *185*, 817-824.

Tada,M., Diserens,A.C., Desbaillets,I., and de Tribolet,N. (1994). Analysis of cytokine receptor messenger RNA expression in human glioblastoma cells and normal astrocytes by reverse-transcription polymerase chain reaction. J Neurosurg. *80*, 1063-1073.

Taga,K., Cherney,B., and Tosato,G. (1993). IL-10 inhibits apoptotic cell death in human T cells starved of IL-2. Int Immunol *5*, 1599-1608.

Taga,T. and Kishimoto,T. (1997). Gp130 and the interleukin-6 family of cytokines. Annu. Rev Immunol *15*, 797-819.

Taherianfard,M. and Shariaty,M. (2004). Evaluation of serum steroid hormones in schizophrenic patients. Indian J Med Sci *58*, 3-9.

Tanaka,K.F., Shintani,F., Fujii,Y., Yagi,G., and Asai,M. (2000). Serum interleukin-18 levels are elevated in schizophrenia. Psychiatry Res. *96*, 75-80.

Tangye,S.G., Ferguson,A., Avery,D.T., Ma,C.S., and Hodgkin,P.D. (2002). Isotype switching by human B cells is division-associated and regulated by cytokines. J Immunol *169*, 4298-4306.

Tangye,S.G. and Raison,R.L. (1997). Human cytokines suppress apoptosis of leukaemic CD5+ B cells and preserve expression of bcl-2. Immunol Cell Biol *75*, 127-135.

Taniyama,T., Takai,S., Miyazaki,E., Fukumura,R., Sato,J., Kobayashi,Y., Hirakawa,T., Moore,K.W., and Yamada,K. (1995). The human interleukin-10 receptor gene maps to chromosome 11q23.3. Hum Genet. *95*, 99-101.

Tartaglia,L.A., Weber,R.F., Figari,I.S., Reynolds,C., Palladino,M.A., Jr., and Goeddel,D.V. (1991). The two different receptors for tumor necrosis factor mediate distinct cellular responses. Proc. Natl. Acad Sci U. S A *88*, 9292-9296.

Tau,G.Z., von der,W.T., Lu,B., Cowan,S., Kvatyuk,M., Pernis,A., Cattoretti,G., Braunstein,N.S., Coffman,R.L., and Rothman,P.B. (2000). Interferon gamma signaling alters the function of T helper type 1 cells. J Exp Med *192*, 977-986.

te Velde,A.A., Huijbens,R.J., Heije,K., de Vries,J.E., and Figdor,C.G. (1990). Interleukin-4 (IL-4) inhibits secretion of IL-1 beta, tumor necrosis factor alpha, and IL-6 by human monocytes. Blood *76*, 1392-1397.

Terabe,M., Park,J.M., and Berzofsky,J.A. (2004). Role of IL-13 in regulation of anti-tumor immunity and tumor growth. Cancer Immunol Immunother. *53*, 79-85.

Theodoropoulou,S., Spanakos,G., Baxevanis,C.N., Economou,M., Gritzapis,A.D., Papamichail,M.P., and Stefanis,C.N. (2001). Cytokine serum levels, autologous mixed lymphocyte reaction and surface marker analysis in never medicated and chronically medicated schizophrenic patients. Schizophr Res. *47*, 13-25.

Thierfelder,W.E., van Deursen,J.M., Yamamoto,K., Tripp,R.A., Sarawar,S.R., Carson,R.T., Sangster,M.Y., Vignali,D.A., Doherty,P.C., Grosveld,G.C., and Ihle,J.N. (1996). Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. Nature *382*, 171-174.

Thomas,W.E. (1992). Brain macrophages: evaluation of microglia and their functions. Brain Res. Brain Res. Rev *17*, 61-74.

Thyphronitis,G., Tsokos,G.C., June,C.H., Levine,A.D., and Finkelman,F.D. (1989). IgE secretion by Epstein-Barr virus-infected purified human B lymphocytes is stimulated by interleukin 4 and suppressed by interferon gamma. Proc. Natl. Acad Sci U. S A *86*, 5580-5584.

Tobet,S.A., Chickering,T.W., Hanna,I., Crandall,J.E., and Schwarting,G.A. (1994). Can gonadal steroids influence cell position in the developing brain? Horm. Behav *28*, 320-327.

Toda,M. (2003). Immuno-viral therapy as a new approach for the treatment of brain tumors. Drug News Perspect. *16*, 223-229.

Toulmond,S., Vige,X., Fage,D., and Benavides,J. (1992). Local infusion of interleukin-6 attenuates the neurotoxic effects of NMDA on rat striatal cholinergic neurons. Neurosci. Lett. *144*, 49-52.

Trinchieri,G. (1995). Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Annu. Rev Immunol *13*, 251-276.

Tryzmel,J., Miskolci,V., Castro-Alcaraz,S., Vancurova,I., and Davidson,D. (2003). Interleukin-10 inhibits proinflammatory chemokine release by neutrophils of the newborn without suppression of nuclear factor-kappa B. Pediatr. Res. *54*, 382-386.

Uchida,K., Yoshikawa-Ebesu,J.S., Kajimura,S., Yada,T., Hirano,T., and Gordon,G.E. (2004). In vitro effects of cortisol on the release and gene expression of prolactin and growth hormone in the tilapia, Oreochromis mossambicus. Gen Comp Endocrinol. *135*, 116-125.

Unterberg,A., Wahl,M., Hammersen,F., and Baethmann,A. (1987). Permeability and vasomotor response of cerebral vessels during exposure to arachidonic acid. Acta Neuropathol. (Berl) *73*, 209-219.

Van Cauter,E., Linkowski,P., Kerkhofs,M., Hubain,P., L'Hermite-Baleriaux,M., Leclercq,R., Brasseur,M., Copinschi,G., and Mendlewicz,J. (1991). Circadian and sleep-related endocrine rhythms in schizophrenia. Arch Gen Psychiatry *48*, 348-356.

van Kammen,D.P., McAllister-Sistilli,C.G., Kelley,M.E., Gurklis,J.A., and Yao,J.K. (1999). Elevated interleukin-6 in schizophrenia. Psychiatry Res. *87*, 129-136.

van Leeuwen,B.H., Martinson,M.E., Webb,G.C., and Young,I.G. (1989). Molecular organization of the cytokine gene cluster, involving the human IL-3, IL-4, IL-5, and GM-CSF genes, on human chromosome 5. Blood *73*, 1142-1148.

Vannier,E., Miller,L.C., and Dinarello,C.A. (1992). Coordinated antiinflammatory effects of interleukin 4: interleukin 4 suppresses interleukin 1 production but up-regulates gene expression and synthesis of interleukin 1 receptor antagonist. Proc. Natl. Acad Sci U. S A *89*, 4076-4080.

Vera-Lastra,O., Jara,L.J., and Espinoza,L.R. (2002). Prolactin and autoimmunity. Autoimmun. Rev *1*, 360-364.

Vermeulen,A. (2003). Diagnosis of partial androgen deficiency in the aging male. Ann Endocrinol. (Paris) *64*, 109-114.

Verreck,F.A., de Boer,T., Langenberg,D.M., Hoeve,M.A., Kramer,M., Vaisberg,E., Kastelein,R., Kolk,A., Waal-Malefyt,R., and Ottenhoff,T.H. (2004). Human IL-23-producing type 1 macrophages promote but IL-10-producing type 2 macrophages subvert immunity to (myco)bacteria. Proc. Natl. Acad Sci U. S A *101*, 4560-4565.

Vidovic,M., Sparacio,S.M., Elovitz,M., and Benveniste,E.N. (1990). Induction and regulation of class II major histocompatibility complex mRNA expression in astrocytes by interferon-gamma and tumor necrosis factor-alpha. J Neuroimmunol. *30*, 189-200.

Villemain,F., Chatenoud,L., Galinowski,A., Homo-Delarche,F., Ginestet,D., Loo,H., Zarifian,E., and Bach,J.F. (1989). Aberrant T cell-mediated immunity in untreated schizophrenic patients: deficient interleukin-2 production. Am J Psychiatry *146*, 609-616.

Villemain,F., Chatenoud,L., Guillibert,E., Pelicier,Y., and Bach,J.F. (1987). Decreased production of interleukin-2 in schizophrenia. Ann N Y Acad Sci *496*, 669-675.

Visser,J., Boxel-Dezaire,A., Methorst,D., Brunt,T., de Kloet,E.R., and Nagelkerken,L. (1998). Differential regulation of interleukin-10 (IL-10) and IL-12 by glucocorticoids in vitro. Blood *91*, 4255-4264.

Volavka,J., Czobor,P., Cooper,T.B., Sheitman,B., Lindenmayer,J.P., Citrome,L., McEvoy,J.P., and Lieberman,J.A. (2004). Prolactin levels in schizophrenia and schizoaffective disorder patients treated with clozapine, olanzapine, risperidone, or haloperidol. J Clin Psychiatry *65*, 57-61.

Waguespack,P.J., Banks,W.A., and Kastin,A.J. (1994). Interleukin-2 does not cross the blood-brain barrier by a saturable transport system. Brain Res. Bull *34*, 103-109.

Walder,D.J., Walker,E.F., and Lewine,R.J. (2000). Cognitive functioning, cortisol release, and symptom severity in patients with schizophrenia. Biol Psychiatry *48*, 1121-1132.

Wallaschofski,H., Eigenthaler,M., Kiefer,M., Donne,M., Hentschel,B., Gertz,H.J., and Lohmann,T. (2003). Hyperprolactinemia in patients on antipsychotic drugs causes ADP-stimulated platelet activation that might explain the increased risk for venous thromboembolism: pilot study. J Clin Psychopharmacol *23*, 479-483.

Walton,P.E. and Cronin,M.J. (1990). Tumor necrosis factor-alpha and interferon-gamma reduce prolactin release in vitro. Am J Physiol *259*, E672-E676.

Waltrip,R.W., Buchanan,R.W., Carpenter,W.T., Jr., Kirkpatrick,B., Summerfelt,A., Breier,A., Rubin,S.A., and Carbone,K.M. (1997). Borna disease virus antibodies and the deficit syndrome of schizophrenia. Schizophr Res. *23*, 253-257.

Wang,L.F., Wu,J.T., and Sun,C.C. (2002). Local but not systemic administration of IFN-gamma during the sensitization phase of protein antigen immunization suppress Th2 development in a murine model of atopic dermatitis. Cytokine *19*, 147-152.

Weber,F., Meinl,E., Aloisi,F., Nevinny-Stickel,C., Albert,E., Wekerle,H., and Hohlfeld,R. (1994). Human astrocytes are only partially competent antigen presenting cells. Possible implications for lesion development in multiple sclerosis. Brain *117 (Pt 1)*, 59-69.

Weickert,T.W., Goldberg,T.E., Gold,J.M., Bigelow,L.B., Egan,M.F., and Weinberger,D.R. (2000). Cognitive impairments in patients with schizophrenia displaying preserved and compromised intellect. Arch Gen Psychiatry *57*, 907-913.

Weihua,X., Hu,J., Roy,S.K., Mannino,S.B., and Kalvakolanu,D.V. (2000). Interleukin-6 modulates interferon-regulated gene expression by inducing the ISGF3 gamma gene using CCAAT/enhancer binding protein-beta(C/EBP-beta). Biochim. Biophys. Acta *1492*, 163-171.

Weiss,E., Mamelak,A.J., La Morgia,S., Wang,B., Feliciani,C., Tulli,A., and Sauder,D.N. (2004). The role of interleukin 10 in the pathogenesis and potential treatment of skin diseases. J Am Acad Dermatol. *50*, 657-675.

Widner,B., Wirleitner,B., Baier-Bitterlich,G., Weiss,G., and Fuchs,D. (2000). Cellular immune activation, neopterin production, tryptophan degradation and the development of immunodeficiency. Arch Immunol Ther Exp (Warsz) *48*, 251-258.

Wilke,I., Arolt,V., Rothermundt,M., Weitzsch,C., Hornberg,M., and Kirchner,H. (1996). Investigations of cytokine production in whole blood cultures of paranoid and residual schizophrenic patients. Eur Arch Psychiatry Clin Neurosci. *246*, 279-284.

Williams,K., Jr., Ulvestad,E., Cragg,L., Blain,M., and Antel,J.P. (1993). Induction of primary T cell responses by human glial cells. J Neurosci. Res. *36*, 382-390.

Wilt,S.G., Milward,E., Zhou,J.M., Nagasato,K., Patton,H., Rusten,R., Griffin,D.E., O'Connor,M., and Dubois-Dalcq,M. (1995). In vitro evidence for a dual role of tumor necrosis factor-alpha in human immunodeficiency virus type 1 encephalopathy. Ann Neurol. *37*, 381-394.

Winzen,R., Wallach,D., Kemper,O., Resch,K., and Holtmann,H. (1993). Selective up-regulation of the 75-kDa tumor necrosis factor (TNF) receptor and its mRNA by TNF and IL-1. J Immunol *150*, 4346-4353.

Woodland,D.L. (2003). Cell-mediated immunity to respiratory virus infections. Curr Opin Immunol *15*, 430-435.

World Health Orgnisation. International clssification of diseases, 10th. edn. 1994. Geneva, WHO. Ref Type: Report

Wu,A.H. and Low,W.C. (2002). Molecular cloning of the rat IL-13 alpha 2 receptor cDNA and its expression in rat tissues. J Neurooncol. *59*, 99-105.

Wustrow,T.P. (1991). [Interactions and biological mechanisms of action of molecular signal peptides. II. Interleukin 2 (IL-2)]. HNO *39*, 323-331.

Wynn,T.A. (2003). IL-13 effector functions. Annu. Rev Immunol *21*, 425-456.

Xie,H.F., Li,J., and Shi,W. (2002). [Effect of corticosteroids on the balance of Th cytokines in patients with systemic lupus erythematosus]. Hunan. Yi. Ke. Da. Xue. Xue. Bao. *27*, 533-535.

Xu,H.M., Wei,J., and Hemmings,G.P. (1994). Changes of plasma concentrations of interleukin-1 alpha and interleukin-6 with neuroleptic treatment for schizophrenia. Br J Psychiatry *164*, 251-253.

Yachie,A., Takano,N., Yokoi,T., Kato,K., Kasahara,Y., Miyawaki,T., and Taniguchi,N. (1990). The capability of neonatal leukocytes to produce IL-6 on stimulation assessed by whole blood culture. Pediatr. Res. *27*, 227-233.

Yaffe,K., Lui,L.Y., Zmuda,J., and Cauley,J. (2002). Sex hormones and cognitive function in older men. J Am Geriatr. Soc. *50*, 707-712.

Yamaguchi,K., Sawada,T., Naraki,T., Igata-Yi,R., Shiraki,H., Horii,Y., Ishii,T., Ikeda,K., Asou,N., Okabe,H., Mochizuki,M., Takahashi,K., Yamada,S., Kubo,K., Yashiki,S., Waltrip,R.W., and Carbone,K.M. (1999). Detection of borna disease virus-reactive antibodies from patients with psychiatric disorders and from horses by electrochemiluminescence immunoassay. Clin Diagn. Lab Immunol *6*, 696-700.

Yamamoto,K., Kobayashi,H., Arai,A., Miura,O., Hirosawa,S., and Miyasaka,N. (1997). cDNA cloning, expression and chromosome mapping of the human STAT4 gene: both STAT4 and STAT1 genes are mapped to 2q32.2-->q32.3. Cytogenet. Cell Genet. *77*, 207-210.

Yamashita,N., Hashimoto,Y., Honjo,M., and Yamashita,N. (2000). The effect of growth hormone on the proliferation of human Th cell clones. Life Sci *66*, 1929-1935.

Yanagawa,H., Takeuchi,E., Suzuki,Y., Hanibuchi,M., Haku,T., Ohmoto,Y., and Sone,S. (1999). Production of interleukin-10 by alveolar macrophages from lung cancer patients. Respir. Med *93*, 666-671.

Yang,Z.W., Chengappa,K.N., Shurin,G., Brar,J.S., Rabin,B.S., Gubbi,A.V., and Ganguli,R. (1994). An association between anti-hippocampal antibody concentration and lymphocyte production of IL-2 in patients with schizophrenia. Psychol. Med *24*, 449-455.

Ye,D., Xie,X., Lu,W., Chen,H., and Cheng,B. (2001). Growth inhibition of interleukin-2 receptor gene-transduced peripheral blood lymphocytes on human ovarian cancer cells. Chin Med J (Engl.) *114*, 303-307.

Young,R.M., Lawford,B.R., Barnes,M., Burton,S.C., Ritchie,T., Ward,W.K., and Noble,E.P. (2004). Prolactin levels in antipsychotic treatment of patients with schizophrenia carrying the DRD2*A1 allele. Br J Psychiatry *185*, 147-151.

Yssel,H., Aversa,G., Punnonen,J., Cocks,B., and de Vries,J.E. (1993a). Regulation of IgE synthesis by T cells and cytokines. Ann Fr. Anesth. Reanim. *12*, 109-113.

Yssel,H., de Waal,M.R., Roncarolo,M.G., Abrams,J.S., Lahesmaa,R., Spits,H., and de Vries,J.E. (1992). IL-10 is produced by subsets of human CD4+ T cell clones and peripheral blood T cells. J Immunol *149*, 2378-2384.

Yssel,H., Schneider,P., and Spits,H. (1993b). Production of IL4 by human T cells and regulation of differentiation of T-cell subsets by IL4. Res. Immunol *144*, 610-616.

Zhang,Q.H., Cao,J., Hu,Y.Z., Huang,Y.H., Lu,S.Y., Wei,G.Z., and Zhao,Y.F. (2004). Estrogen affects the differentiation and function of splenic monocyte-derived dendritic cells from normal rats. Xi. Bao. Yu Fen. Zi. Mian. Yi. Xue. Za Zhi. *20*, 129-134.

Zhang,S., Lukacs,N.W., Lawless,V.A., Kunkel,S.L., and Kaplan,M.H. (2000). Cutting edge: differential expression of chemokines in Th1 and Th2 cells is dependent on Stat6 but not Stat4. J Immunol *165*, 10-14.

Zhang,X.Y., Zhou,D.F., Cao,L.Y., Zhang,P.Y., and Wu,G.Y. (2002a). Decreased production of interleukin-2 (IL-2), IL-2 secreting cells and CD4+ cells in medication-free patients with schizophrenia. J Psychiatr Res. *36*, 331-336.

Zhang,X.Y., Zhou,D.F., Zhang,P.Y., Wu,G.Y., Cao,L.Y., and Shen,Y.C. (2002b). Elevated interleukin-2, interleukin-6 and interleukin-8 serum levels in neuroleptic-free schizophrenia: association with psychopathology. Schizophr Res. *57*, 247-258.

Zubieta,J.K., Dannals,R.F., and Frost,J.J. (1999). Gender and age influences on human brain mu-opioid receptor binding measured by PET. Am J Psychiatry *156*, 842-848.

Zurawski,G. and de Vries,J.E. (1994). Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. Immunol Today *15*, 19-26.

Zurawski,S.M., Vega,F., Jr., Huyghe,B., and Zurawski,G. (1993). Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction. EMBO J *12*, 2663-2670.

Table index

Figure index

Criteria = IFN-γ: magnitude of importance by individual predictor						
Serum	Whole CON (N = 72)	Whole SCH (N = 40)	♂ CON (N = 38)	♂ SCH (N = 25)	♀ CON (N = 34)	♀ SCH (N = 15)
IL-2	1.80 (.08)	.40 (.69)	3.18 (.004)	.75 (.47)	.86 (.40)	1.48 (.24)
TNF-α	.82 (.42)	1.07 (.29)	.77 (.45)	.86 (.41)	1.08 (.29)	-.18 (.87)
IL-4	-.10 (.92)	.54 (.60)	-.11 (.91)	1.09 (.30)	-.68 (.50)	-1.33 (.28)
IL-10	2.11 (.04)	-.02 (.99)	1.00 (.33)	.60 (.56)	1.72 (.10)	-.16 (.89)
IL-6	2.97 (.004)	-.03 (.98)	2.21 (.04)	-.04 (.97)	.51 (.61)	1.46 (.24)
Cortisol	-.42 (.68)	-2.18 (.04)	.42 (.68)	-1.28 (.22)	.07 (.95)	.72 (.52)
Prolactin	1.38 (.17)	1.76 (.09)	1.11 (.28)	.01 (.99)	.78 (.44)	3.78 (.03)
Estradiol	-.08 (.94)	-1.23 (.23)	-1.30 (.20)	-.84 (.41)	-.40 (.69)	1.55 (.22)
Testosterone	-1.12 (.27)	-.25 (.81)	.66 (.52)	-1.05 (.31)	-1.01 (.32)	-2.11 (.13)
SHBG	-1.13 (.26)	.01 (1.00)	.37 (.72)	-.08 (.93)	-1.30 (.21)	-.35 (.75)
Age	-.96 (.34)	-.93 (.36)	-1.52 (.14)	-.94 (.37)	-.08 (.94)	-1.02 (.38)
Adjusted R², Signif.Test — WB	R^2 = 1.00 F = 18575.53, p < .001	R^2 = .39 F = 1.62, p = .15	R^2 = 1.00 F = 13914.45, p < .001	R^2 = .61 F = 1.85, p = .15	R^2 = .42 F = 1.45, p = .22	R^2 = .44 F = 2.01, p = .31
IL-2	.57 (.57)	.23 (.82)	2.27 (.03)	.05 (.96)	-.38 (.71)	1.30 (.28)
TNF-α	1.50 (.14)	1.38 (.18)	-.90 (.38)	1.05 (.31)	1.71 (.10)	-.24 (.83)
IL-4	-3.05 (.003)	-2.12 (.04)	-1.20 (.24)	-1.35 (.20)	-1.74 (.10)	.21 (.85)
IL-10	5.70 (.000)	2.90 (.007)	3.24 (.003)	1.67 (.12)	3.45 (.002)	.92 (.43)
IL-6	3.09 (.003)	2.02 (.05)	2.30 (.03)	1.42 (.18)	1.99 (.06)	-.03 (.98)
Cortisol	-.74 (.46)	.62 (.54)	.73 (.47)	.28 (.78)	-.28 (.78)	-.02 (.98)
Prolactin	2.07 (.04)	.40 (.69)	1.99 (.06)	-.34 (.74)	.28 (.78)	.50 (.65)
Estradiol	-1.16 (.25)	1.20 (.24)	-1.26 (.22)	1.08 (.30)	-.93 (.36)	.49 (.66)
Testosterone	1.43 (.16)	2.18 (.04)	1.45 (.16)	.72 (.49)	-.17 (.87)	-.24 (.83)
SHBG	1.30 (.20)	-.50 (.62)	1.42 (.17)	.20 (.84)	.55 (.59)	-.07 (.95)
Age	.88 (.38)	-.17 (.97)	-1.06 (.30)	-.84 (.42)	.87 (.39)	.10 (.93)
Adjusted, Signif.Test — Lymph	R^2 = .61 F = 10.98, p < .001	R^2 = .63 F = 4.37, p = .001	R^2 = .58 F = 5.65, p < .001	R^2 = .67 F = 2.35, p = .07	R^2 = .62 F = 5.86, p < .001	R^2 = .92 F = 2.99, p = .20
IL-12	1.00 (.32)	.13 (.89)	2.52 (.02)	.01 (.99)	-.39 (.70)	.48 (.66)
IL-10	3.14 (.003)	.80 (.43)	2.55 (.02)	.17 (.87)	2.09 (.05)	.17 (.87)
IL-4	-.01 (.99)	1.57 (.13)	-.78 (.44)	2.36 (.03)	-.45 (.66)	.89 (.42)
IL-13	3.51 (.001)	-.42 (.68)	1.05 (.30)	-.73 (.48)	3.34 (.003)	-.31 (.77)
Cortisol	-1.72 (.09)	.10 (.92)	-1.12 (.27)	.91 (.38)	.02 (.98)	.86 (.44)
Prolactin	.66 (.51)	.38 (.71)	2.56 (.02)	1.52 (.15)	-.80 (.44)	-.70 (.52)
Estradiol	-1.23 (.22)	.77 (.45)	-1.69 (.10)	-.79 (.44)	-.62 (.54)	1.41 (.23)
Testosterone	-.97 (.34)	.33 (.75)	.18 (.86)	-.22 (.83)	-.51 (.61)	.16 (.88)
SHBG	1.10 (.27)	-.73 (.47)	-.26 (.80)	.08 (.94)	-.41 (.69)	-.96 (.39)
Age	2.07 (.04)	.45 (.66)	.84 (.41)	-.89 (.39)	.87 (.39)	.92 (.41)
Adjusted Signif. test	R^2 = .44 F = 6.49, p < .001	R^2 = .06 F = .79, p = .64	R^2 = .61 F = 6.71, p < .001	R^2 = .18 F = 1.53, p = .23	R^2 = .42 F = 3.39, p = .007	R^2 = .40 F = .60, p = .77
Note	WB = whole blood; Lymph = lymphocyte; Adjusted = adjusted R^2; Signif. = significance.					

Appendix 6-6(2): The resources of lymphocyte IL-12 in schizophrenics and controls (results from the complete model).

Criteria = IL-12: magnitude of importance by individual predictor						
Lymph	Whole CON (N = 72)	Whole SCH (N = 40)	♂ CON (N = 38)	♂ SCH (N = 25)	♀ CON (N = 34)	♀ SCH (N = 15)
IFN-γ	1.00 (.32)	.13 (.89)	2.52 (.02)	.01 (.95)	-.39 (.70)	.48 (.66)
IL-10	3.27 (.002)	.78 (.44)	1.27 (.22)	2.11 (.05)	2.73 (.01)	-1.09 (.34)
IL-4	1.14 (.26)	.67 (.51)	1.15 (.26)	.79 (.44)	-.04 (.97)	-.66 (.54)
IL-13	-.48 (.64)	-.44 (.66)	-.15 (.89)	.16 (.88)	.94 (.36)	.58 (.60)
Cortisol	-.77 (.45)	.15 (.88)	.21 (.84)	-.61 (.55)	-1.12 (.27)	-.53 (.62)
Prolactin	-.39 (.70)	2.80 (.009)	-1.53 (.14)	-.08 (.94)	.91 (.37)	3.17 (.03)
Estradiol	1.07 (.29)	.16 (.87)	.97 (.34)	-.19 (.86)	.33 (.75)	.10 (.93)
Testosterone	1.65 (.10)	1.00 (.33)	-.10 (.92)	1.20 (.25)	.97 (.34)	-1.75 (.16)
SHBG	1.08 (.28)	.24 (.82)	.93 (.36)	.14 (.89)	.02 (.99)	1.12 (.33)
Age	2.33 (.02)	1.11 (.28)	1.56 (.13)	-.63 (.54)	.25 (.81)	-.79 (.48)
Adjusted Signif. test	R^2 = .40 F = 5.64, p < .001	R^2 = .32 F = 1.39, p = .24	R^2 = .54 F = 5.38, p < .001	R^2 = .45 F = 1.14, p = .40	R^2 = .47 F = 2.04, p = .08	R^2 = .89 F = 3.21, p = .14
Note	Lymph = lymphocyte; Adjusted = adjusted R^2; Signif. = significance.					

Appendix 6-6(3): The resources of serum and whole blood assay IL-2 in schizophrenics and controls (results from the complete model).

Criteria = IL-2: magnitude of importance by individual predictor						
Serum	Whole CON (N = 72)	Whole SCH (N = 40)	♂ CON (N = 38)	♂ SCH (N = 25)	♀ CON (N = 34)	♀ SCH (N = 15)
IFN-γ	1.50 (.08)	.40 (.69)	.52 (.61)	.75 (.47)	.86 (.40)	1.48 (.24)
TNF-α	9.88 (.000)	.77 (.45)	10.0 (.000)	-.08 (.94)	1.91 (.07)	.76 (.50)
IL-4	-.08 (.94)	.42 (.68)	.81 (.42)	-.16 (.88)	-.92 (.37)	3.07 (.05)
IL-10	-.61 (.54)	1.13 (.27)	-1.85 (.08)	-.20 (.85)	-.05 (.96)	1.22 (.31)
IL-6	-1.13 (.26)	-.98 (.34)	-.28 (.78)	.02 (.98)	-.37 (.71)	-2.45 (.09)
Cortisol	-.02 (.99)	-2.10 (.05)	1.04 (.31)	-1.32 (.21)	.82 (.42)	-2.98 (.06)
Prolactin	-.50 (.62)	.16 (.87)	-.76 (.46)	-.27 (.79)	.42 (.68)	-1.69 (.19)
Estradiol	-.02 (.99)	-1.92 (.07)	-.64 (.53)	-.32 (.76)	.21 (.84)	-5.80 (.01)
Testosterone	1.23 (.22)	.19 (.85)	.91 (.37)	.83 (.42)	.75 (.46)	2.70 (.07)
SHBG	.72 (.48)	1.26 (.22)	-.65 (.52)	-.39 (.71)	-.32 (.75)	1.53 (.22)
Age	.39 (.70)	-.60 (.55)	.64 (.53)	.32 (.75)	-.53 (.60)	.71 (.53)
Adjusted R Signif.Test / WB	R^2 = 1.00 F = 15375.15, p <.001	R^2 = .32 F = 1.17, p = .35	R^2 = 1.00 F = 16692.27, p <.001	R^2 = .27 F = .44, p = .91	R^2 = .35 F = 1.08, p = .42	R^2 = .81 F = 6.46, p = .08
IFN-γ	.57 (.57)	.23 (.82)	2.27 (.03)	.05 (.96)	-.38 (.71)	1.30 (.28)
TNF-α	7.50 (.000)	4.01 (.000)	7.08 (.000)	3.23 (.007)	4.03 (.001)	.72 (.53)
IL-4	-1.33 (.19)	-.65 (.52)	-.86 (.40)	-.61 (.56)	-.31 (.76)	-.73 (.52)
IL-10	.28 (.78)	-.88 (.39)	-.82 (.42)	.58 (.57)	1.67 (.11)	-.41 (.71)
IL-6	-1.81 (.08)	1.57 (.13)	-2.60 (.02)	1.49 (.16)	-1.09 (.29)	-.15 (.89)
Cortisol	-.21 (.83)	-.74 (.47)	-1.75 (.09)	-.02 (.99)	-.64 (.53)	.23 (.84)
Prolactin	.43 (.67)	.88 (.39)	-.56 (.58)	1.10 (.29)	1.80 (.09)	.19 (.86)
Estradiol	.57 (.57)	.09 (.93)	1.41 (.17)	-.09 (.93)	.09 (.93)	-.20 (.85)
Testosterone	.02 (.99)	-.58 (.57)	-1.85 (.08)	-.96 (.35)	-.74 (.47)	-.33 (.76)
SHBG	-.42 (.68)	-.04 (.97)	-.74 (.47)	.49 (.64)	-.46 (.65)	.76 (.51)
Age	.10 (.92)	1.83 (.08)	1.76 (.09)	1.81 (.09)	-.84 (.41)	-.09 (.93)
Adjusted Signif. Test	R^2 = .57 F = 9.49, p < .001	R^2 = .62 F = 4.09, p = .001	R^2 = .75 F = 11.20, p < .001	R^2 = .72 F = 3.10, p = .03	R^2 = .62 F = 3.22, p = .009	R^2 = .88 F = 2.04, p = .30
Note	WB = whole blood; Adjusted = adjusted R^2; Signif. = significance.					

: The resources of serum and whole blood assay TNF-α in schizophrenics and controls (results from the complete model).

Criteria = TNF-α: magnitude of importance by individual predictor						
Serum	Whole CON (N = 72)	Whole SCH (N = 40)	♂ CON (N = 38)	♂ SCH (N = 25)	♀ CON (N = 34)	♀ SCH (N = 15)
IFN-γ	.82 (.42)	1.07 (.29)	.77 (.45)	.86 (.41)	1.08 (.29)	-.18 (.87)
IL-2	9.88 (.000)	.77 (.45)	10.0 (.000)	-.08 (.94)	1.91 (.07)	.76 (.50)
IL-4	.45 (.66)	3.15 (.004)	-.50 (.62)	2.25 (.04)	2.05 (.05)	-.10 (.93)
IL-10	1.13 (.26)	.24 (.81)	2.54 (.02)	-.62 (.55)	.18 (.86)	-.64 (.57)
IL-6	1.38 (.17)	-.43 (.67)	-.04 (.97)	.62 (.55)	1.04 (.31)	.43 (.69)
Cortisol	-.75 (.46)	1.59 (.12)	-2.15 (.04)	-.35 (.73)	.45 (.66)	1.15 (.34)
Prolactin	.47 (.64)	-1.20 (.24)	.78 (.45)	-1.68 (.12)	-.80 (.43)	.21 (.85)
Estradiol	-.21 (.83)	.93 (.36)	1.16 (.26)	-1.99 (.07)	.48 (.64)	.86 (.45)
Testosterone	-1.36 (.18)	-2.69 (.01)	-1.49 (.15)	.38 (.71)	-.43 (.67)	-.76 (.50)
SHBG	-.17 (.86)	-2.44 (.02)	1.02 (.32)	-1.02 (.33)	-.56 (.58)	-1.89 (.16)
Age	.05 (.96)	-.13 (.90)	-.46 (.65)	.41 (.69)	-.06 (.95)	-.42 (.70)
Adjusted R / Signif.Test / WB	R^2 = 1.00 F = 25598.08, p < .001	R^2 = .47 F = 2.24, p = .04	R^2 = 1.00 F = 38493.99, p < .001	R^2 = .54 F = 1.40, p = .28	R^2 = .44 F = 1.57, p = .18	R^2 = .75 F = .82, p = .65
IFN-γ	1.50 (.14)	1.38 (.18)	-.91 (.38)	1.05 (.31)	1.71 (.10)	-.24 (.83)
TNF-α	7.50 (.000)	4.01 (.000)	7.08 (.000)	3.23 (.007)	4.03 (.001)	.72 (.53)
IL-4	3.30 (.002)	1.57 (.13)	1.68 (.11)	1.07 (.30)	1.30 (.21)	3.77 (.03)
IL-10	-.41 (.68)	.36 (.72)	.71 (.49)	-1.08 (.30)	-1.44 (.16)	-.43 (.70)
IL-6	2.45 (.02)	-.16 (.88)	2.62 (.01)	-.46 (.66)	1.56 (.13)	2.19 (.12)
Cortisol	1.22 (.23)	-.12 (.90)	2.07 (.05)	.04 (.97)	1.28 (.21)	-.72 (.52)
Prolactin	-1.54 (.13)	-.55 (.59)	.04 (.97)	-.57 (.58)	-1.68 (.11)	.77 (.50)
Estradiol	.54 (.59)	.05 (.96)	-1.32 (.20)	.04 (.97)	1.03 (.31)	2.07 (.13)
Testosterone	.82 (.42)	.80 (.43)	1.35 (.19)	.20 (.85)	1.05 (.30)	-.75 (.51)
SHBG	.39 (.70)	.42 (.68)	1.03 (.31)	.07 (.94)	.28 (.71)	.29 (.79)
Age	.84 (.40)	-2.05 (.05)	-.75 (.46)	-1.58 (.14)	.64 (.53)	-1.21 (.31)
Adjusted Signif. test	R^2 = .72 F = 17.39, p < .001	R^2 = .70 F = 5.84, p < .001	R^2 = .80 F = 14.69, p < .001	R^2 = .68 F = 2.56, p = .06	R^2 = .73 F = 5.31, p < .001	R^2 = .98 F = 12.92, p = .03
Note	WB = whole blood; Adjusted = adjusted R^2; Signif. = significance.					

Appendix 6-6(5): The resources of serum, whole blood assay, and lymphocyte IL-4 in schizophrenics and controls (results from the complete model).

Criteria = IL-4: magnitude of importance by individual predictor					
Serum	Whole CON (N = 72)	Whole SCH (N = 40)	♂ CON (N = 38)	♂ SCH (N = 25)	♀ CON (N = 34)	♀ SCH (N = 15)
IFN-γ	-.10 (.92)	.54 (.60)	-.11 (.91)	1.09 (.30)	-.68 (.50)	-1.33 (.28)
IL-2	-.08 (.94)	.42 (.68)	.82 (.42)	-.16 (.88)	-.93 (.37)	3.07 (.05)
TNF-α	.45 (.66)	3.15 (.004)	-.50 (.62)	2.25 (.04)	2.05 (.05)	-.10 (.93)
IL-10	.03 (.98)	.31 (.76)	-.42 (.68)	.41 (.69)	-.75 (.46)	-.78 (.49)
IL-6	.35 (.73)	.03 (.98)	.65 (.52)	-.52 (.61)	-.08 (.94)	1.67 (.19)
Cortisol	.44 (.66)	.05 (.96)	1.35 (.19)	.74 (.48)	-.87 (.40)	1.92 (.15)
Prolactin	.01 (1.00)	.98 (.34)	-.32 (.75)	1.78 (.10)	1.19 (.25)	1.47 (.24)
Estradiol	-.50 (.62)	.53 (.60)	-.92 (.36)	2.27 (.04)	-.89 (.38)	2.69 (.07)
Testosterone	1.05 (.30)	2.85 (.008)	.54 (.59)	.63 (.54)	-.44 (.66)	-1.70 (.19)
SHBG	-.55 (.58)	1.73 (.09)	-.36 (.72)	.98 (.35)	.52 (.61)	-.84 (.46)
Age	.09 (.93)	.81 (.43)	.49 (.63)	.41 (.69)	-.43 (.67)	-.50 (.65)
Adjusted Signif.Test **WB**	R^2 = .98 F = 258.05, p < .001	R^2 = ~~.42~~ F = 1.86, p = .09	R^2 = .98 F = 192.14, p < .001	R^2 = ~~.58~~ F = 1.64, p = .20	R^2 = ~~.27~~ F = .76, p = .68	R^2 = ~~.89~~ F = 2.25, p = .27
IFN-γ	-3.05 (.003)	-2.12 (.04)	-1.20 (.24)	-1.35 (.20)	-1.74 (.10)	.21 (.85)
IL-2	-1.33 (.19)	-.65 (.52)	-.86 (.40)	-.61 (.56)	-.31 (.76)	-.73 (.52)
TNF-α	3.30 (.002)	1.57 (.13)	1.68 (.11)	1.07 (.30)	1.30 (.21)	3.77 (.03)
IL-10	6.54 (.000)	3.80 (.001)	5.51 (.000)	2.64 (.02)	1.24 (.23)	1.03 (.38)
IL-6	-.79 (.43)	1.72 (.10)	-1.29 (.21)	1.26 (.23)	.53 (.60)	-2.08 (.13)
Cortisol	-.67 (.51)	.54 (.59)	-1.83 (.08)	-.45 (.66)	1.39 (.18)	1.01 (.39)
Prolactin	1.05 (.30)	.26 (.80)	.81 (.42)	.01 (.99)	-1.32 (.20)	-.89 (.44)
Estradiol	.04 (.97)	.53 (.60)	1.50 (.15)	-.10 (.92)	.85 (.41)	-1.42 (.25)
Testosterone	.32 (.75)	.84 (.41)	-1.22 (.23)	.67 (.51)	-.44 (.67)	.84 (.46)
SHBG	-.13 (.90)	-.34 (.74)	.73 (.47)	-.53 (.60)	-.67 (.51)	-.36 (.75)
Age	1.18 (.24)	.76 (.46)	1.29 (.21)	.87 (.40)	.86 (.40)	1.24 (.30)
Adjusted Signif. Test **Lymph**	R^2 = .55 F = 8.94, p < .001	R^2 = .58 F = 3.56, p = .003	R^2 = .72 F = 9.57, p < .001	R^2 = ~~.50~~ F = 1.17, p = .39	R^2 = ~~.42~~ F = 1.47, p = .21	R^2 = .98 F = 11.38, p = .04
IFN-γ	-.01 (.99)	1.57 (.13)	-.78 (.44)	2.36 (.03)	-.45 (.66)	.89 (.42)
IL-12	1.14 (.26)	.67 (.51)	1.15 (.26)	.79 (.44)	-.04 (.97)	-.66 (.54)
IL-10	-.75 (.45)	.06 (.95)	-.53 (.60)	.12 (.91)	.59 (.56)	-.11 (.92)
IL-13	3.40 (.001)	4.55 (.000)	1.29 (.21)	1.37 (.19)	3.02 (.006)	4.19 (.01)
Cortisol	-1.78 (.08)	-.04 (.97)	-1.08 (.29)	.24 (.82)	-1.03 (.31)	-1.17 (.31)
Prolactin	2.17 (.03)	.44 (.66)	2.17 (.04)	-1.03 (.32)	.76 (.46)	1.79 (.15)
Estradiol	-.89 (.38)	-.81 (.43)	.08 (.94)	.27 (.79)	-.63 (.54)	-1.41 (.23)
Testosterone	-.78 (.44)	.92 (.36)	-1.11 (.28)	.10 (.92)	-.05 (.96)	-1.47 (.22)
SHBG	.82 (.42)	.57 (.57)	-.23 (.82)	.85 (.41)	.33 (.75)	.98 (.39)
Age	-.93 (.35)	1.08 (.29)	.44 (.66)	3.00 (.01)	-1.51 (.15)	-2.98 (.04)
Adjusted Signif. test	R^2 = .22 F = 2.93, p = .005	R^2 = .57 F = 3.84, p = .002	R^2 = ~~.06~~ F = 1.23, p = .31	R^2 = .72 F = 3.60, p =.02	R^2 = .52 F = 2.45, p = .04	R^2 = .96 F = 10.14, p = .02
Note	WB = whole blood; Lymph = lymphocyte; Adjusted = adjusted R^2; Signif. = significance.					

235

Appendix 6-6(6): The resources of serum, whole blood assay, and lymphocyte IL-10 in schizophrenics and controls (results from the complete model).

Criteria = IL-10: magnitude of importance by individual predictor						
Serum	Whole CON (N = 72)	Whole SCH (N = 40)	♂ CON (N = 38)	♂ SCH (N = 25)	♀ CON (N = 34)	♀ SCH (N = 15)
IFN-γ	2.11 (.04)	-.02 (.99)	1.00 (.33)	.60 (.56)	1.72 (.10)	-.16 (.89)
IL-2	-.61 (.54)	1.13 (.27)	-.72 (.48)	-.20 (.85)	-.05 (.96)	1.22 (.31)
TNF-α	1.13 (.26)	.24 (.81)	2.54 (.02)	-.62 (.55)	.18 (.86)	-.64 (.57)
IL-4	.03 (.98)	.31 (.76)	-.42 (.68)	.41 (.69)	-.75 (.46)	-.78 (.49)
IL-6	6.27 (.000)	1.64 (.11)	6.16 (.000)	.89 (.39)	1.82 (.08)	1.04 (.38)
Cortisol	-.31 (.74)	.29 (.78)	1.06 (.30)	-.65 (.53)	-2.01 (.06)	1.08 (.36)
Prolactin	-.10 (.93)	-.41 (.68)	-.33 (.74)	-1.88 (.08)	.47 (.64)	.43 (.70)
Estradiol	.45 (.65)	-.12 (.91)	.43 (.67)	-.30 (.77)	-.15 (.88)	.96 (.41)
Testosterone	.81 (.42)	-.87 (.40)	-.81 (.43)	1.29 (.22)	.75 (.46)	.97 (.40)
SHBG	.16 (.88)	-1.11 (.28)	-.81 (.43)	-1.49 (.16)	2.64 (.02)	-1.30 (.29)
Age	1.44 (.15)	-.27 (.79)	2.03 (.05)	.22 (.83)	.57 (.57)	-.67 (.55)
Adjusted Signif. Test — WB	R^2 = 1.00 F = 20654.58, p < .001	R^2 = .22 F = .71, p = .72	R^2 = 1.00 F = 20553.89, p < .001	R^2 = .49 F = 1.11, p = .42	R^2 = .45 F = 1.65, p = .15	R^2 = .71 F = .66, p = .74
IFN-γ	5.70 (.000)	2.90 (.007)	3.25 (.003)	1.67 (.12)	3.45 (.002)	.92 (.43)
IL-2	.28 (.78)	-.88 (.39)	-.82 (.42)	.58 (.57)	1.67 (.11)	-.41 (.71)
TNF-α	-.41 (.68)	.36 (.72)	.71 (.49)	-1.08 (.30)	-1.44 (.16)	-.43 (.70)
IL-4	6.54 (.000)	3.80 (.001)	5.51 (.000)	2.64 (.02)	1.24 (.23)	1.03 (.38)
IL-6	-.58 (.57)	-.67 (.51)	-.17 (.86)	-.58 (.57)	.29 (.77)	.91 (.43)
Cortisol	.60 (.55)	-.38 (.71)	.39 (.70)	.63 (.54)	1.19 (.25)	-1.19 (.32)
Prolactin	-1.57 (.12)	.23 (.82)	-1.12 (.27)	-.20 (.84)	-1.80 (.09)	.75 (.51)
Estradiol	.44 (.66)	-1.16 (.26)	-.16 (.88)	.02 (.99)	1.18 (.25)	-.002 (1.00)
Testosterone	-.76 (.45)	-1.53 (.14)	.16 (.88)	-.72 (.49)	.74 (.47)	-.80 (.49)
SHBG	.04 (.97)	1.99 (.06)	-1.37 (.18)	.49 (.63)	.71 (.49)	.92 (.43)
Age	-1.06 (.29)	.72 (.48)	-.67 (.51)	-.35 (.73)	.74 (.47)	-.94 (.42)
Adjusted Signif. Test — Lymph	R^2 = .65, F = 12.99, p < .001	R^2 = .68 F = 5.48, p < .001	R^2 = .74, F = 10.56, p < .001	R^2 = .45 F = .98, p = .51	R^2 = .68 F = 4.21, p = .002	R^2 = .95 F = 5.03, p = .11
IFN-γ	3.14 (.003)	.80 (.43)	2.54 (.02)	.17 (.87)	2.09 (.05)	.17 (.87)
IL-12	3.27 (.002)	.78 (.44)	1.27 (.22)	2.11 (.05)	2.73 (.01)	-1.09 (.34)
IL-4	-.75 (.45)	.06 (.95)	-.53 (.60)	.12 (.91)	.59 (.56)	-.11 (.92)
IL-13	-1.54 (.13)	.66 (.51)	-.65 (.52)	.41 (.69)	-1.99 (.06)	.21 (.84)
Cortisol	.25 (.80)	1.27 (.22)	-.14 (.89)	.77 (.45)	.17 (.87)	.21 (.85)
Prolactin	-.18 (.86)	-.76 (.45)	-1.01 (.32)	-.24 (.81)	.23 (.82)	.82 (.46)
Estradiol	-.51 (.62)	1.32 (.20)	.61 (.55)	-.08 (.94)	-.38 (.71)	.67 (.54)
Testosterone	-.83 (.41)	.22 (.83)	.16 (.88)	-.60 (.56)	-1.23 (.23)	-.78 (.48)
SHBG	-1.12 (.27)	1.72 (.10)	-.55 (.59)	.39 (.70)	.23 (.82)	.80 (.47)
Age	.34 (.74)	.19 (.85)	-.20 (.85)	.15 (.88)	1.16 (.26)	-.21 (.84)
Adjusted Signif. test	R^2 = .38 F = 5.44, p < .001	R^2 = .34 F = 1.52, p = .18	R^2 = .39 F = 3.36, p = .006	R^2 = .42 F = 1.00, p = .49	R^2 = .57 F = 3.04, p = .01	R^2 = .57 F = .52, p = .82
Note	WB = whole blood; Lymph = lymphocyte; Adjusted = adjusted R^2; Signif. = significance.					

Appendix 6-6(7): The resources of lymphocyte IL-13 in schizophrenics and controls (results from the complete model).

Lymph	Whole CON (N = 72)	Whole SCH (N = 40)	♂ CON (N = 38)	♂ SCH (N = 25)	♀ CON (N = 34)	♀ SCH (N = 15)
Criteria = IL-13: magnitude of importance by individual predictor						
IFN-γ	3.51 (.001)	-.42 (.68)	1.05 (.30)	-.73 (.48)	3.34 (.003)	-.31 (.77)
IL-12	-.48 (.64)	-.44 (.66)	-.15 (.89)	.16 (.88)	.94 (.36)	.58 (.60)
IL-10	-1.54 (.13)	.66 (.51)	-.65 (.52)	.41 (.69)	-1.99 (.06)	.21 (.84)
IL-4	3.40 (.001)	4.55 (.000)	1.29 (.21)	1.37 (.19)	3.02 (.006)	4.19 (.01)
Cortisol	.51 (.61)	-.21 (.84)	1.35 (.19)	1.01 (.33)	-.57 (.58)	.48 (.66)
Prolactin	-.40 (.69)	.37 (.72)	-.11 (.91)	.61 (.56)	.05 (.96)	-1.43 (.23)
Estradiol	.59 (.56)	.31 (.76)	-.79 (.44)	1.06 (.31)	.57 (.58)	.63 (.57)
Testosterone	.88 (.38)	-.57 (.57)	.43 (.67)	-1.80 (.09)	-.22 (.83)	2.02 (.11)
SHBG	-.39 (.70)	-.39 (.70)	-.43 (.67)	.74 (.47)	1.47 (.16)	-.41 (.70)
Age	-.99 (.33)	-.57 (.58)	-.83 (.41)	-.55 (.60)	.40 (.69)	2.52 (.07)
Adjusted Signif. test	R^2 = .26 F = 3.45, p = .001	R^2 = .48 F = 2.63, p = .02	R^2 = -.04 F = .86, p = .58	R^2 = .43 F = 1.03, p = .47	R^2 = .69 F = 5.22, p = .001	R^2 = .95 F = 7.60, p = .03
Note	Lymph = lymphocyte; Adjusted = adjusted R^2; Signif. = significance.					

Criteria = IL-6: magnitude of importance by individual predictor					
Serum	Whole CON (N = 72)	Whole SCH (N = 40)	♂ CON (N = 38)	♂ SCH (N = 25)	♀ CON (N = 34)	♀ SCH (N = 15)
IFN-γ	2.97 (.004)	-.03 (.98)	2.21 (.04)	-.04 (.97)	.51 (.61)	1.46 (.24)
IL-2	-1.13 (.26)	-.98 (.34)	-.73 (.47)	.02 (.98)	-.37 (.71)	-2.45 (.09)
TNF-α	1.38 (.17)	-.43 (.67)	-.04 (.97)	.62 (.55)	1.04 (.31)	.43 (.69)
IL-4	.35 (.73)	.03 (.98)	.65 (.52)	-.52 (.61)	-.08 (.94)	1.67 (.19)
IL-10	6.27 (.000)	1.64 (.11)	6.16 (.000)	.89 (.39)	1.82 (.08)	1.04 (.38)
Cortisol	1.44 (.16)	.49 (.63)	-.69 (.50)	.35 (.73)	1.59 (.13)	-1.45 (.24)
Prolactin	-1.19 (.24)	-.99 (.33)	-.42 (.68)	.37 (.72)	-.94 (.36)	-1.85 (.16)
Estradiol	-.10 (.93)	-1.23 (.23)	.31 (.76)	1.67 (.12)	-.35 (.73)	-2.37 (.10)
Testosterone	.56 (.58)	-.95 (.35)	.59 (.56)	-1.51 (.16)	-.14 (.89)	2.09 (.13)
SHBG	.53 (.60)	.44 (.67)	.27 (.79)	.73 (.48)	-.49 (.63)	.88 (.44)
Age	-.79 (.43)	.50 (.62)	-.95 (.35)	.76 (.46)	-.76 (.45)	.88 (.44)
WB — Adjusted Signif. Test	R² = 1.00 F = 22507.37, p < .001	R² = 26 F = .91, p = .55	R² = 1.00 F = 22131.61, p < .001	R² = 42 F = .85, p = .60	R² = 42 F = 1.47, p = .21	R² = 77 F = .93, p = .60
IFN-γ	3.09 (.003)	2.02 (.05)	2.30 (.03)	1.42 (.18)	1.99 (.06)	-.03 (.98)
IL-2	-1.81 (.08)	1.57 (.13)	-2.60 (.02)	1.49 (.16)	-1.09 (.29)	-.15 (.89)
TNF-α	2.45 (.02)	-.16 (.88)	2.62 (.01)	-.46 (.66)	1.56 (.13)	2.19 (.12)
IL-4	-.79 (.43)	1.72 (.10)	-1.29 (.21)	1.26 (.23)	.53 (.60)	-2.08 (.13)
IL-10	-.58 (.57)	-.67 (.51)	-.17 (.86)	-.58 (.57)	.29 (.77)	.91 (.43)
Cortisol	-1.23 (.22)	-1.38 (.18)	-1.97 (.06)	-1.46 (.17)	-1.66 (.11)	.71 (.53)
Prolactin	.98 (.33)	-.66 (.52)	-.56 (.58)	-.13 (.90)	1.43 (.17)	-.92 (.43)
Estradiol	-.73 (.47)	-1.33 (.20)	1.59 (.12)	-.19 (.85)	-1.36 (.19)	-1.54 (.22)
Testosterone	-2.59 (.01)	.33 (.75)	-1.36 (.19)	1.18 (.26)	-1.40 (.18)	.83 (.47)
SHBG	-.90 (.37)	.69 (.50)	-1.12 (.27)	-1.24 (.24)	-1.27 (.22)	-.39 (.73)
Age	-1.12 (.27)	-.04 (.97)	1.57 (.13)	-.31 (.76)	-2.46 (.02)	1.33 (.28)
Adjusted Signif. test	R² = .30, F = 3.71, p < .001	R² = .54 F = 2.98, p = .01	R² = 22, F = 1.97, p = .08	R² = 63 F = 2.02, p = .11	R² = .60 F = 2.99, p = .01	R² = 86 F = 1.65, p = .37
Note	WB = whole blood; Adjusted = adjusted R²; Signif. = significance.					

238

Acknowledgment

Curriculum vitae

Name: Sonnig Sue Whei <u>Chiang</u>
Address: #323, San-Fong Road, Fong-Yuan City, Taichung Hsien, Taiwan, R.O.C.
Date of birth: 30.03.1961, Taichung Hsien, Taiwan, R.O.C.

1967-1973 Primary School, Taichung Hsien, Taiwan
1973-1976 Middle School, Taichung Hsien, Taiwan
1976-1979 Taichung Girl High School, Taichung City, Taiwan
1979-1983 National Chengchi University, Taipei City, Taiwan
1983-1985 Graduate School of the National Chenghi University, Taipei City, Taiwan

1985-1986 Editor of the Science Education Center of the Taichung Library, Taichung City,
          Taiwan
1986-1989 Chinese Art and Craft Teacher of the Taipei American School, Taipei City,
          Taiwan
          College lecturer of the Taipei Teacher College, Taipei City, Taiwan

1989-1990 German Course, Munich, Germany
1990-1991 London & USA

1991-1992 Education Science (Ph.D.) in the Munich University, Munich, Germany
1992-1999 Psychology Faculty of the Munich University, Munich, Germany

1995-1998 Academic assistant of the Institute for Therapy (IFT), Munich, Germany

2000-2004 Medicine Faculty of the Munich University, Munich, Germany

Publications

Paper:
1. Sonnig Sue Whei Chiang, Christian G. Schuetz, Michael Soyka
Role of Aggressivity on Reactivity and Craving before and after Cue Exposure in Recently
detoxified Alcoholics: Results from an Experimental Study.
Eur Addic Res 2001; 7: 184-192.

2. Sonnig Sue Whei Chiang, Christian G. Schuetz, Michael Soyka
Effects of Irritability on Craving before and after Cue exposure in Abstinent Alcoholic
Inpatients: Experimental Data on Subjective Response and Heart rate.
Neuropsychobiology 2002; 46: 150-160.

3. M.J. Schwarz, S. Chiang, N. Müller, M. Ackenheil
T-helper-1 and T-helper-2 responses in psychiatric disorders.
Brain Behav Immun 2001; 15: 340-370.

4. S. Chiang, M. Ackenheil, R. Gruber, N. Müller, M. Riedel, M. Schwarz
Th2-shift in schizophrenia: primary findings from whole blood in vitro production. Psychiatry
On Line 2004; http://www.priory.com/psycont.htm.

5. Sonnig Sue Whei Chiang, Christian Schuetz, Michael Soyka (submitted).
Effects of cue exposure on the subjective perception of alcohol dependents with different types of cue reactivity. Journal of Neural Transmission.

Poster:
1. Sonnig S.W. Chiang, Michael Soyka, Christian G. Schuetz. The role of anxiety in induced alcohol craving during the earlier abstinence phase – results from a cue exposure experiment. The 13th Academic Congress of the German Association of Addiction Research and Addiction Therapy [13. Wissenschaftliche Tagung der Deutschen Gesellschaft für Suchtforschung und Suchtthereapie e.V.] (2000).

2. Sonnig Chiang, Markus Schwarz, Michael Riedel, Norbert Mueller, Manfred Ackenheil Imbalance between Th1 and Th2 in Schizophrenia ? An investigation on cytokine expression by means of ELISPOT-technique. Research Festival of the Psychiatric Hospital of Munich University (2000).

3. Sonnig Chiang, Markus Schwarz, Michael Riedel, Norbert Mueller, Manfred Ackenheil Imbalance between Th1 and Th2 in Schizophrenia ? Part I: ELISPOT- Method Establishment. Research Festival of the Psychiatric Hospital of Munich University (2001).

4. Sonnig Chiang, Markus Schwarz, Michael Riedel, Manfred Ackenheil, Norbert Mueller A Th2-shift in a subgroup of schizophrenia: primary results of an immunological exploration. GErman Brain-Endocrine-Immune-Network (GEBIN, 2003).

Thesis:
1. The Relationship between the Parental Rearing Attitudes and the Magnitude of Anxiety in Preschool Children (1985: Chinese thesis). Graduate School of the National Chengchi University, Taipei, Taiwan, R.O.C.

2. The role of impulsivity and anxiety in induced craving in abstinent alcoholics [Die Rolle von Impulsivität und Ängstlichkeit auf induziertes „Craving" bei abstinenten Alkoholikern] (1999: Deutsche Diplomarbeit). Psychology Faculty of Munich University, Munich, Germany.

Dissertation:
Th1/Th2 imbalance in schizophrenia: An immunological exploration of etiology (2005: English). Faculty of Medicine of Munich University, Munich, Germany.

München, den   Sonnig Sue Whei Chiang _____

Ende