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HLA class I- and class II-restricted T cell receptors for immunotherapy of human cytomegalovirus disease

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Erklärung

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Summary

Human cytomegalovirus (HCMV) frequently reactivates after hematopoietic stem cell transplantation (HSCT). Antiviral medication is not universally effective and has side effects that prohibit prophylaxis and limit pre-emptive use. Adoptive transfer of HCMV-specific T lymphocytes can protect patients at risk of HCMV disease, but such T cells are difficult to obtain if the HSC donor is HCMV-negative. Transferring the T cell receptor (TCR) genes of HCMV-specific T cell clones to donor T cells will convey HCMV specificity to those cells. But if αβ T cells are engineered to co-express a second αβ TCR, mixed αβ TCR heterodimers of unknown and potentially hazardous specificity may form. This problem can be solved by expressing αβ TCRs in γδ T cells from the donor.

HCMV-specific $CD8^+$ T cells limit HCMV infection, but presentation of their antigenic peptides is effectively impaired by HCMV's immunoevasins, especially in case of human leukocyte antigen (HLA)-A and -B allotypes. In contrast, $CD8^+$ T cells restricted by HLA-C*07:02 resist the immunoevasins. In this study, the genes of eight HLA-C*07:02-restricted TCRs specific for immediate-early protein 1 (IE-1) or UL28 protein were derived from $CD8^+$ T cell clones and retrovirally transferred into T cells from HCMV-negative donors. While all of those TCRs mediated strong recognition of an HCMV strain that is devoid of the four immunoevasins, their response to target cells infected with wild-type HCMV strains was weaker. Transduction experiments with different variations of the TCRs pointed to the existence of additional T cell-endogenous factors that contribute to recognition of infected cells.

HCMV-specific $CD4^+$ T cells are known to play a critical role in controlling HCMV and maintaining immunity. Nonetheless, possibilities of their transgenic generation have not been explored. In this study, HCMV-specific TCRs from $CD4^+$ T cell clones were expressed in T cells from HCMV-negative donors. A panel of ten HCMV-specific, HLA class II-restricted TCRs was established that target seven distinct epitopes of phosphoprotein 65 (pp65) and IE-1 presented by a wide range of HLA class II allotypes. These TCR genes were retrovirally transferred into αβ T cells and γδ T cells from HCMV-negative donors. The resulting T cells specifically recognised HLA-matched, HCMV-infected dendritic cells. TCR-transduced αβ and γδ T cells were similarly effective, but backgrounds due to alloreactivity were even lower in γδ T cells than in αβ T cells. Experiments with inactivated virus showed that pp65-specific, HLA class II-restricted TCRs conveyed similar sensitivity towards HCMV-infected target cells and target cells that had acquired viral proteins through ingestion of extracellular material. In contrast, IE-1-specific, HLA class II-restricted TCRs mediated selective recognition of *bona fide* infected target cells. These results identify γδ T cells that transgenically express HLA class II-restricted TCRs as particularly promising immunotherapeutic agents against HCMV infection.

1 Introduction

Humans are constantly exposed to infectious agents as a result of their interactions with the environment and uptake of material from it. Considering the multitude and omnipresence of different pathogens – bacteria, fungi, viruses, protozoa and helminths – it is fascinating that people rarely suffer from disease. This is due to the human immune system, which has evolved to defend its host against pathogens. It is comprised of various molecules and cells, organised in a complex network involving different tissues and spread throughout the human body. In the mid 20th century, Macfarlane Burnet hypothesised that the immune system is able to recognise pathogens while generally maintaining the integrity of innocent cells or tissues by distinguishing between agents originating from the host ("self") and agents being foreign to the human body ("non-self", Macfarlane Burnet, 1954). However, several observations, such as reactivity of the immune system directed against "self" ("autoimmunity"), could not be explained by the "self/non-self" model. In contrast, Matzinger (1994) proposed that the immune system is far less concerned with things that are foreign, but with the detection of damage and danger signals that are usually accompanied by pathogens or injured host tissue. Since then, the "danger model" has been adapted to accommodate the immunobiology of phenomena, such as transplantation, allergy, autoimmunity and cancer, but still "explains most of what the immune system seems to do right, as well as most of what it appears to do wrong" (Matzinger, 2012).

Once a pathogen has bypassed the chemical and physical barriers of the human body, such as the skin, it will immediately encounter molecules and cells of the innate immune system that serve as the first line of defence. These molecules and cells will counteract the pathogen by attacking it directly or indirectly, trapping it and recruiting more immune effectors to the site of infection. While the actions of the innate immune system are very quick and intense, they are rather unspecific and similarly applied to a broad range of pathogens. In order to efficiently fight and potentially clear a certain pathogen from the human body, however, a specific immune response is often required. This second line of defence is provided by the adaptive immune system, which is initiated by innate molecules and/or cells. Adaptive immune cells need several days to mount a counter-attack, but develop an immunologic memory, which will be re-activated by a second encounter of the same pathogen and will result in an increased and quicker response. The defence mechanisms of both the innate and the adaptive immune system encompass the secretion of molecules ("humoral response"), as well as direct cellular interactions ("cellular response").

1.1 Cellular immune responses

All immune cells arise from pluripotent hematopoietic stem cells (HSCs) that differentiate into common myeloid and common lymphoid progenitor cells in the bone marrow. Granulocytes, monocytes and dendritic cells (DCs), but also megakaryocytes and erythroblasts, are derived from common myeloid progenitor cells. In contrast, the common lymphoid progenitor cells give rise to B cells, T cells, natural killer (NK) cells and also DCs. The innate immune cells, namely granulocytes, monocytes/macrophages, DCs and NK cells, rely on pattern recognition receptors (PRRs) to recognise invading microbes. These PRRs are invariant receptors that bind to conserved microbial molecules or patterns of molecular structures generalised under the term of

pathogen-associated molecular patterns (PAMPs).

Upon recognition of a PAMP or an alarm signal such as tissue damage, tissue-resident macrophages and immature DCs will release cytokines and chemokines that convey crucial informations to other cells and guide other immune cells towards the site of infection. As a result, an inflammation is triggered that aims to immobilise and destroy the pathogens, to hamper their replication, to recruit more immune molecules and cells, as well as to promote repair of injured tissue. The first cells that are attracted to a site of inflammation are neutrophils – a type of granulocyte – and monocytes. During their migration, monocytes can differentiate into macrophages if triggered by macrophage colony-stimulating factor (M-CSF), or into immature DCs if interleukin (IL)-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) are present.
Phagocytic cells, such as DCs, macrophages and neutrophils, do not only drive inflammation after encountering pathogens or associated alarm signals, but also possess a variety of different cell surface receptors to bind and engulf microbes, their metabolites and cellular debris from the environment. Their phagocytic activity, however, depends on their activation status. Immature DCs, for example, capture antigens in the periphery, but gradually lose their ability to take up antigens by phagocytosis during maturation (Moutaftsi et al., 2002). The obtained material is processed and presented to T cells that will be activated if they can recognise the antigen with their own T cell receptors and if the presenting cell simultaneously provides co-stimulation. The expression of co-stimulatory molecules, such as CD86 (B7.2), is triggered by PAMPs and supports the activation of T cells. Co-stimulation is essential for effective T cell activation because if absent, the T cell will die, become anergic – i.e. "non-responsive" to its specific antigen – or induce tolerance concerning the specific antigen.

B and T lymphocytes are the key players of the adaptive immune system. Their specificity is determined by the B cell receptor (BCR) or T cell receptor (TCR), respectively. While the BCR can recognise a conformational polypeptide substructure, the TCR binds to linear peptides only if presented by major histocompatibility complex (MHC) proteins. Importantly, B and T cells differ greatly in function: upon activation, B cells can differentiate into plasma cells and strongly enhance their capacity to produce antibodies, which are a soluble form of their specific BCR. Thus, B cells contribute to the humoral immune response by the secretion of antibodies that can specifically bind to a certain structure of a pathogen. Moreover, B cells can also serve as antigen-presenting cells (APCs) after binding to antigenic material via the BCR, engulfing the material and digesting it.
In contrast to B cells, T cells exert their functions by direct or indirect interactions with host cells. They can be grouped into the two major categories of cytotoxic T lymphocytes (CTLs) and T helper (T_H) cells, most of which express either the TCR co-receptor CD8 or CD4, respectively. CTLs eliminate pathogen-infected host cells thereby preventing further replication and dissemination of the pathogen. In contrast, T_H cells control immune responses by orchestrating and regulating various immune cells, such as B cells, CTLs and macrophages. Thus, CD4-positive ($CD4^+$) T_H cells are of vital importance for the generation of appropriate and effective immune responses. B cells, for example, present fragments of processed antigens to T_H cells of corresponding specificity, which in turn stimulate the B cells if the T_H cells recognised the presented

antigen with their TCRs – a process that is required for establishing effective humoral immunity. During an infection, a small number of antigen-specific T cells expands by several orders of magnitude in order to combat the infection. After the acute immune response has subsided and the infection is resolved, most of the antigen-specific T cells die, but a small proportion persists. These T cells will react more quickly and strongly if they encounter their antigen again in case of re-infection with the same pathogen. This kind of T cells is called memory T cells and their formation depends on T_H cells. After primary infection with certain persistent pathogens, such as HCMV, has been resolved, the number of T cells that are maintained is relatively high. Also, there is a debate whether such T cells should be considered "memory" T cells because there is periodic or continuous challenge with at least low-level antigen in such infections (reviewed in Virgin et al., 2009).

1.1.1 Composition and diversity of the T cell receptor

Every T cell expresses a unique T cell receptor (TCR) that defines its specificity and enables it to survey a small part of the molecular universe within the human body. A TCR is a heterodimer composed either of a TCRα and a TCRβ chain or a TCRγ and a TCRδ chain, which are linked by a disulfide bond. Each TCR chain consists of a variable (V) and a constant (C) domain, both of which are extracellular and immunoglobulin-like, a short hinge, a transmembrane domain and a cytoplasmic tail. The specificity of a TCR is defined by the combined protein surface formed by the extracellular V domains of both TCR chains. Each of these V domains has three protruding loops that mediate most of the binding to the antigen and the molecule presenting it. Accordingly, the loops are called complementarity determining regions (CDRs).
While the CDRs 1 and 2 are encoded within the V gene segment of a given TCR chain, the CDR3 is located at the junctions of V(D)J segments (fig. 1.1). Due to the diverse, partially random events during V(D)J recombination, the CDR3 is highly variable (fig. 1.1). In contrast, the germline-encoded CDR1 and CDR2 are less variable, because there are at most as many different versions of them as there are corresponding V gene segments to choose from in the genome (e.g. ≈60 for TCRβ), and their genome-encoded sequences are not altered. This fits the binding properties of these loops well, since CDR1 and CDR2 bind the less variable MHC molecule(s) and CDR3 binds to the highly variable peptide antigen that is being presented on MHC molecule(s) (see fig. 1.4 & 1.5).

In the cell membrane, the TCR heterodimer associates with and is stabilised by a ζζ homodimer and the CD3 complex that consists of two heterodimers, namely εγ and εδ. Like the TCR chains, these proteins are membrane-anchored by transmembrane domains. Their intracellular tails contain immunoreceptor tyrosine-based activation motifs (ITAMs) that are essential for TCR signal transmission.
The interaction of an αβ T cell with a peptide antigen presented on MHC class I or class II molecules (peptide/MHC complex) is mediated by its TCR and can be stabilised by the co-receptor CD8 or CD4, respectively. Both co-receptors are transmembrane proteins and directly bind to MHC molecules thus increasing the overall strength of the TCR-MHC binding. CD8 can either be expressed as αα homodimer or as αβ heterodimer. The latter is pre-dominant on

CTLs and binds to the α2 and α3 domain of MHC class I molecules, while it does not interfere with TCR recognition. CD4 is a single chain protein mainly found on T_H cells and binds to a crevice between MHC class II α2 and β2 domains. The interaction of a single TCR with a peptide/MHC complex is not sufficient to fully activate the T cell even if supported by CD4 or CD8 co-receptor and co-stimulatory molecule binding. In order to activate the T cell, a multitude of TCR-peptide/MHC interactions has to occur, usually in an arrangement of molecules termed the immunological synapse. The affinity of each interaction contributes to the overall avidity of the interaction between the T cell and an APC. This synapse is further stabilised by interactions of adhesion molecules (e.g. integrins) expressed by the APC and the T cell.

TCRs have a tremendous variability, which is estimated to encompass more than 10^8 unique TCRβ sequences in naïve CD4 and CD8 T cell repertoires of young adults (Qi et al., 2014). This is a result of (I) many different gene segments of one TCR chain that can be combined in various ways (combinatorial diversity), (II) imprecise processing of DNA ends during the rearrangement (junctional diversity) and (III) different combinations of TCRα and TCRβ or TCRγ and TCRδ chains. Each of the TCR chains is created separately by rearrangement of TCR genes in the T cell precursor (somatic recombination) during its maturation in the thymus.
In order to understand the combinatorial diversity, we must take a look at the composition of the TCR chains and their genetic loci (fig. 1.2): Human TCRα chains are comprised of a variable (V), a joining (J) and a constant (C) region each encoded by respective gene segments. While there is only one Cα gene segment, there are 59 Vα and 61 Jα gene segments. Similarly, human TCRβ chains are also composed of one V, J and C region, each, but additionally contain a diversity (D) region between Vβ and Jβ. Like Vα, many different Vβ gene segments exist (68), but

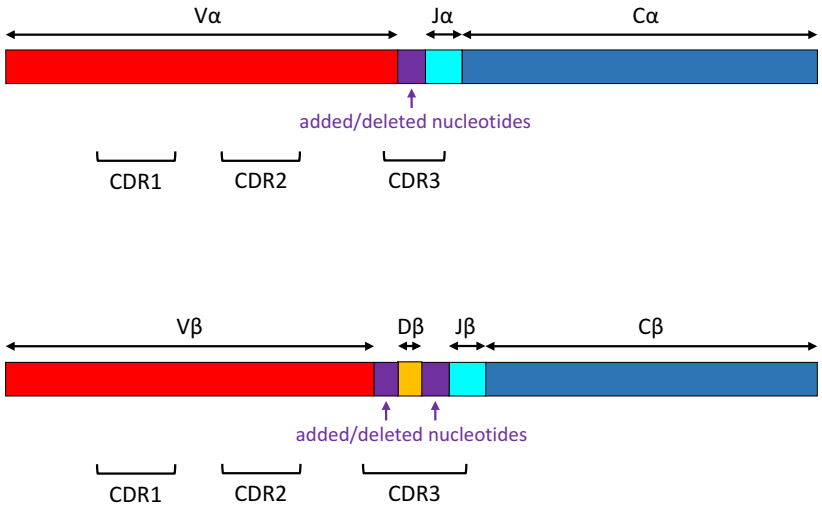

Figure 1.1. mRNA structure of TCRα (top) **and TCRβ chain** (bottom)**.** The complementarity determining regions (CDRs) 1 and 2 are germline-encoded within the respective V gene segment. However, the CDR3 spans across the junction of the rearranged V (red) and J (cyan) gene segments (TCRα) or V, D (yellow) and J gene segments (TCRβ). At the junctions between V, D (if available) and J segments, nucleotides are randomly added or deleted (purple) by the terminal deoxynucleotidyl transferase (TdT) or exonucleases, respectively. This greatly contributes to CDR3 diversity. C gene segments are displayed in blue. Modified from Attaf et al. (2015).

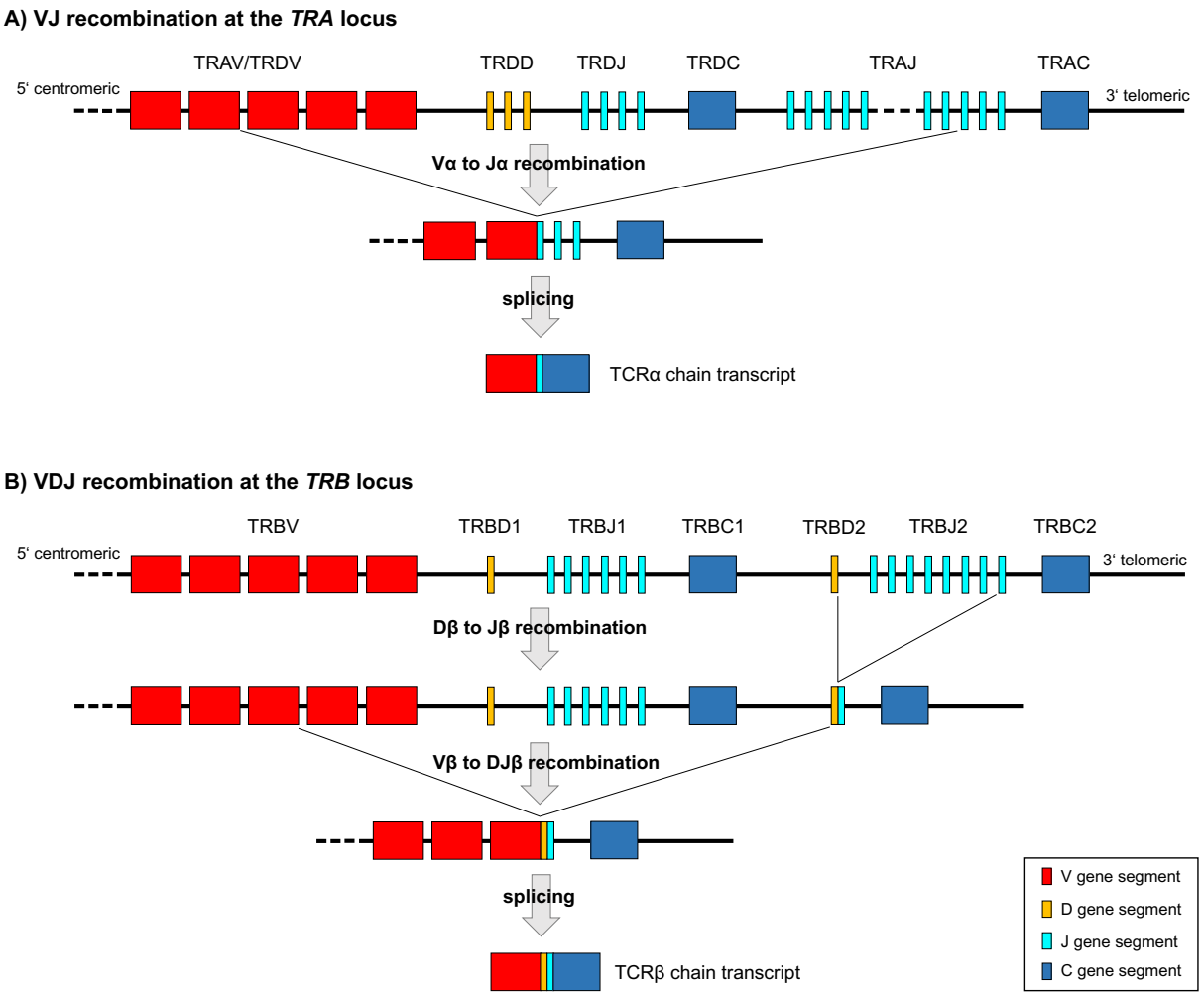

Figure 1.2. TCR gene rearrangement at the TR loci. A: The *TRA* locus contains 59 Vα, 61 Jα and 1 Cα gene segment and includes the whole *TRD* locus (3 Vδ, 3 Dδ, 4 Jδ and 1 Cδ). V to J recombination joins one Vα gene segment with one Jα gene segment, while intervening gene segments are excised. The final TCRα transcript is generated by splicing. **B:** The *TRB* locus contains 68 Vβ, 2 Dβ, 14 Jα and 2 Cβ gene segments, with two separate DJC clusters. Firstly, one Dβ gene segment is fused to one Jβ gene segment and intervening gene segments are excised (D to J recombination). Secondly, one Vβ gene segment is joined with the rearranged DJβ, excluding intervening gene segments again (V to DJ recombination). The final TCRβ transcript is generated by splicing. Modified from Attaf et al. (2015).

in contrast to the high number of Jα gene segments, fewer Jβ gene segments exist (14). While the TCRα locus contains no D and only one C gene segment, the *TRB* locus offers two, each. Human TCRγ and TCRδ chains are composed similarly to human TCRα (V-J-C) and TCRβ chains (V-D-J-C), respectively, but the number of germline encoded Vγ and Vδ genes is a lot lower than that of Vα and Vβ: 13 Vγ, 5 Jγ, 2 Cγ, 3 Vδ, 3 Dδ, 4 Jδ and 1 Cδ gene segment exist. However, T cells expressing a γδ TCR (γδ T cells) may compensate for this by junctional diversity (reviewed in Carding and Egan, 2002). The *TRD* locus is part of the *TRA* locus with Vδ gene segments being interspersed with Vα gene segments and Dδ, Jδ and Cδ gene segments being located between Vα/δ and Jα gene segments.

During TCR gene rearrangement, one V, one D (only for TCRβ and TCRδ) and one J gene segment are linked together and subsequently connected to one C gene segment, while all other

intervening V and J gene segments between the chosen V and J gene segments are excised (see fig. 1.2). Any TCRα rearrangement results in the loss of the complete *TRD* locus. While gene rearrangement is taking place, junctional diversity comes into play, too: When V, (D,) and J gene segments are joined, the enzyme terminal deoxynucleotidyl transferase (TdT) adds short, random stretches of nucleotides to the coding ends and exonucleases may delete nucleotides at gene segment junctions (fig. 1.1). Both processes greatly contribute to CDR3 diversity of both TCR chains, but come at the cost of many non-productive rearrangements due to random DNA sequence alterations that can induce a frame shift or stop codon. However, the development of a T cell does not necessarily fail in case of a non-productive TCR gene rearrangement, since at most loci remaining gene segments allow for successive rearrangements on the same chromosome (see fig. 1.2). Also, if TCR gene rearrangement on one chromosome is unsuccessful, the process may be repeated on the other chromosome in order to create a functional TCR chain gene.

After successful rearrangements of TCRα and TCRβ genes that are able to successfully pair with each other and create a TCR signal, a developing αβ T cell will simultaneously express both CD4 and CD8 co-receptors and undergo a TCR-driven selection process in the thymus, during which its capability to recognise self-peptides on self-MHC proteins is tested. CD4- and CD8-double-positive (DP) T cells bind to self-peptide/self-MHC complexes presented by cortical epithelial cells in the thymic cortex, which triggers their proliferation (a process called "positive selection"). Thus, a DP T cell that is able to bind to any of the offered MHC class I or class II molecules survives and will subsequently become CD4- or CD8-single-positive (SP). If the developing T cell binds too strongly to self-peptide/self-MHC complexes, however, it may possess a TCR that is specific for a self-peptide and thus has to be deleted in order to avoid autoimmune reactivity. This is a process known as "negative selection", which can happen during both DP and SP stages of T cell development. On the contrary, if the T cell binds too weakly or not at all to self-peptide/self-MHC complexes, it may not be able to bind to any peptide-presenting self-MHC at all and thus will be of no immunologic use. Indeed, the vast majority of αβ T cells succumbs to these selection processes and dies or becomes anergic. Having successfully passed thymic selection, a T cell emerging from the thymus will express either CD4 or CD8, depending on the MHC class its TCR binds to.
Whether and to which extent thymic selection shapes the γδ T cell repertoire is less well understood. γδ T cells do not necessarily undergo thymic selection the way that αβ T cells do. At least in the mouse, normal γδ T cell development requires proper intrathymic localisation (Reinhardt et al., 2014) and thymic selection was shown to determine the effector fate of γδ T cells rather than constrain their antigen specificities (Jensen et al., 2008).

1.1.2 γδ T cells

On average, about 4% of T cells in peripheral blood of healthy adults are γδ T cells (Groh et al., 1989; Pitard et al., 2008). They constitute a phenotypically diverse T cell population and most of them do not express CD4 or CD8 (Groh et al., 1989), so they are not sub-grouped like αβ T cells, but according to their Vδ expression. Vδ1 T cells and Vδ3 T cells make up about 1% and

less than 0.5% of peripheral blood T cells, respectively, but are enriched in mucosal epithelia and the spleen (Pitard et al., 2008). The majority of γδ T cells found in peripheral blood are Vδ2 T cells (3% among peripheral blood T cells). In the 1990s, Vδ2 T cells were identified to be activated by non-peptidic mycobacterial antigens (Constant et al., 1994; Davodeau et al., 1993) that were characterised as prenyl pyrophosphates and included isopentenyl pyrophosphate (IPP Tanaka et al., 1995). Later, (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP) of the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, which is not present in eukaryotes, was found to be $10^4\times$ more potent than IPP (Hintz et al., 2001). IPP is produced by the MEP pathway, but also by the mevalonate pathway of eukaryotes. In a macaque model, Vδ2 T cells were shown to respond to infection with *Mycobacterium tuberculosis in vivo*, to mount memory responses on re-infection and to confer protective immunity to macaques that had been vaccinated with *Mycobacterium bovis* Bacille Calmette-Guérin (Shen et al., 2002). In humans, numerous pathogenic bacteria and protozoan parasites were reported to drive immune responses of Vδ2 T cells (reviewed in Morita et al., 2007).

In vitro and *in vivo*, aminobisphosphonates, like pamidronate or zoledronate, have been shown to trigger human Vδ2 T cell activation and proliferation (Gober et al., 2003; Khan et al., 2014; Wilhelm et al., 2003) as a result of the inhibition of farnesyl pyrophosphate synthesis in the mevalonate pathway. This inhibition causes the accumulation of IPP (Gober et al., 2003). Miyagawa et al. (2001) reported that stimulation of Vδ2 T cells by pamidronate strictly depended on the presence of cells from the monocyte lineage *in vitro*. The sensitivity for phosphoantigens can be conferred to other T cells by transfer of Vγ9Vδ2 TCR genes (Bukowski et al., 1995; Das et al., 2001). The membrane-expressed butyrophilin (BTN) 3A1 molecule has been identified to play a key role in target cells that activate Vδ2 T cells (Harly et al., 2012). More precisely, phosphoantigens directly bind to BTN3A1's intracellular B30.2 domain, causing immobilisation of BTN3A1 in the plasma membrane. Subsequently, BTN3A1 either recruits an additional factor and/or changes the conformation of its extracellular domain thereby generating a structure that can directly be detected by the Vγ9Vδ2 TCR (Sandstrom et al., 2014; Vavassori et al., 2013). Sebestyen et al. (2016) support both notions adding the small GTPase RhoB to the model: RhoB, which is activated and relocated for example by dysregulation of the mevalonate pathway, induces immobilisation of BTN3A1, but ultimately dissociates from BTN3A1 most likely after phosphoantigen binding and the conformational change of the extracellular domain of BTN3A1.

Vδ2 T cells can be activated by certain cancer cells (Gober et al., 2003; Morita et al., 2007), probably due to dysregulation of metabolism in cancer cells leading to the accumulation of phosphoantigens, and thus can be used in cancer therapy (reviewed in Scheper et al., 2014). Recently, Vδ2 T cells and Vγ9Vδ2 TCRs (Hoeres et al., 2018; Straetemans et al., 2018) were developed for cancer therapy and their function was assessed in clinical trials. In general, the administration of γδ T cells was safe regardless of *in vitro* or *in vivo* stimulation, but clinical responses varied and showed limited success (reviewed in Scheper et al., 2014).

1.1.3 Antigen presentation and the major histocompatibility complex

An αβ T cell will be activated if its TCR binds to a peptidic antigen that is presented on major histocompatibility complex (MHC) class I or class II molecules. In humans, these proteins are also called human leukocyte antigens (HLAs). The human MHC locus on chromosome number 6 contains three MHC class I α genes, one HLA-A, -B and -C gene each, and three pairs of MHC class II α and β genes, one pair of HLA-DR, -DQ and -DP genes each. Additionally, the MHC locus can contain an extra HLA-DRβ chain gene (fig. 1.3). The particular set of HLA alleles that is found on a single chromosome is called the HLA haplotype. This polygeny and the polymorphism of most of those HLA genes have evolved to at least partially match the enormous diversity of peptides. Thus, being heterozygous for the genes encoding HLA class I and II molecules is likely advantageous and since HLA alleles are expressed co-dominantly, an individual can express 3 to 6 and 3 to 12 different HLA class I and class II molecules, respectively.
While HLA class I molecules are expressed on almost every cell of the human body, HLA class II molecules are mainly present on the surface of immune cells, such as DCs, monocytes/macrophages, B and T cells. In addition, HLA class II molecules can also be expressed by non-hematopoietic cells, like fibroblasts and endothelial cells, if induced by interferon (IFN)-γ (Collins et al., 1984; Pober et al., 1983; Stevanovic et al., 2013).

HLA class I molecules consist of an α chain (also termed "heavy chain") that includes a transmembrane domain and forms a heterodimer with the invariant β2-microglobulin (β2-m). Peptides bound to the extracellular domains of an HLA class I molecule are typically 8 to 10 amino acids

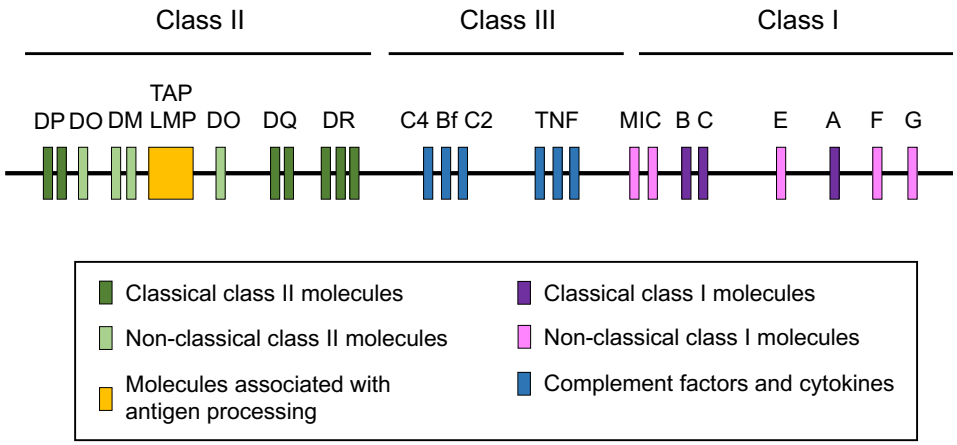

Figure 1.3. Organisation of the human *major histocompatibility complex (MHC)* locus on chromosome 6. The human *MHC* (also called *HLA*) locus is divided in three regions: class I, class II and class III. Class I encodes the genes of HLA-A, -B and -C (the classical HLA class I molecules), as well as the genes of HLA-E, -F, -G and MHC class I chain-related (MIC) proteins (the non-classical HLA class I molecules). Class II encodes the genes for α and β chains of HLA-DR, -DQ and -DP (the classical HLA class II molecules), as well as the genes of transporter associated with antigen-processing (TAP), HLA-DO and -DM (the non-classical HLA class II molecules). Class III encodes the genes of tumour necrosis factor (TNF) and the complement proteins Bf, C2 and C4. Only some of the genes located at the *MHC* locus are shown. Distances between genes are not in scale. According to Trowsdale (2002).

in length and binding is almost always stabilised by an anchor residue at the carboxy (C) terminus of the peptide. Additional anchor residues can be in other positions of the peptide, this depends on the HLA in question. The binding cleft of the HLA class I α chain provides limited space for the accommodation of a peptide, so long or bulky peptides protrude from the cleft in order to fit (fig. 1.4). The loading of HLA class I molecules with antigenic peptides takes place in the endoplasmic reticulum (ER). During translation of HLA class I chains, they are translocated to the ER lumen, where they fold, form a heterodimer together with β2-m, and associate with proteins that stabilise them and support peptide loading. The majority of peptides loaded onto HLA class I molecules is produced by the proteasome by degradation of proteins translated in the same cell. These peptides are continually transported from the cytosol into the ER by transporters associated with antigen processing (TAP), which are proteins of the ATP binding cassette family that prefer peptides of 8 to 16 amino acids harbouring hydrophobic or basic residues at the C terminus (van Endert, 1999; Peters et al., 2003). However, peptides translocated by TAP are often too long at the amino (N) terminus to bind to HLA class I molecules. The N terminus can be shortened by the ER aminopeptidases ERAP1 and ERAP2 which act in concert in order to produce peptides that can fit into the HLA molecule's binding cleft (Saveanu et al., 2005).
HLA class II molecules are heterodimers consisting of an α and a β chain, both of which encompass a transmembrane domain. Together the two chains form a peptide binding cleft, which is not closed at the ends, like that of HLA class I α chain, and can thus accommodate longer peptides that protrude from the binding cleft (fig. 1.5). Peptides bound to HLA class II molecules are usually 13 to 17 amino acids long. Most of those peptides are produced by intravesicular proteases that degrade extracellular pathogens and proteins after their internalisation by endocytosis. Newly synthesised HLA class II heterodimers are bound by the invariant chain ("Ii"), which blocks the peptide-binding cleft to prevent premature binding of peptides. Ii targets the delivery of HLA class II heterodimers from the ER to endosomes, where Ii is degraded and HLA class II heterodimers are loaded with peptides, which is mediated by HLA-DM. After peptide loading, peptide/HLA class II complexes are transported to the cell surface (the whole process is reviewed in Kropshofer et al., 1999; Schulze and Wucherpfennig, 2012).
In the absence of peptides, HLA molecules that are expressed on the cell surface are unstable resulting in their internalisation and degradation. The presentation of peptides from extracellular sources on HLA class I molecules is also possible in some cell types and is of special importance in professional antigen-presenting cells. This process is called cross-presentation (Burgdorf and Kurts, 2008; Mantegazza et al., 2013). On the other hand, peptides derived from cytosolic proteins may be presented on HLA class II molecules if they have been degraded by autophagy (Nimmerjahn et al., 2003).

Individuals express different sets of HLA molecules. If "foreign" tissues are transplanted into a recipient, the immune system can exert strong alloreactivity due to recognition of non-self HLA molecules and other antigens. After solid organ transplantation (SOT), the immune system of the recipient can cause graft rejection, which is usually inhibited by immunosuppressive medication. However, immunosuppression always needs to be balanced with the accompanied increase in risk of infection. After hematopoietic stem cell transplantation (HSCT), T lymphocytes that are contained in the graft may recognise HLA molecules from the recipient as alloantigens and

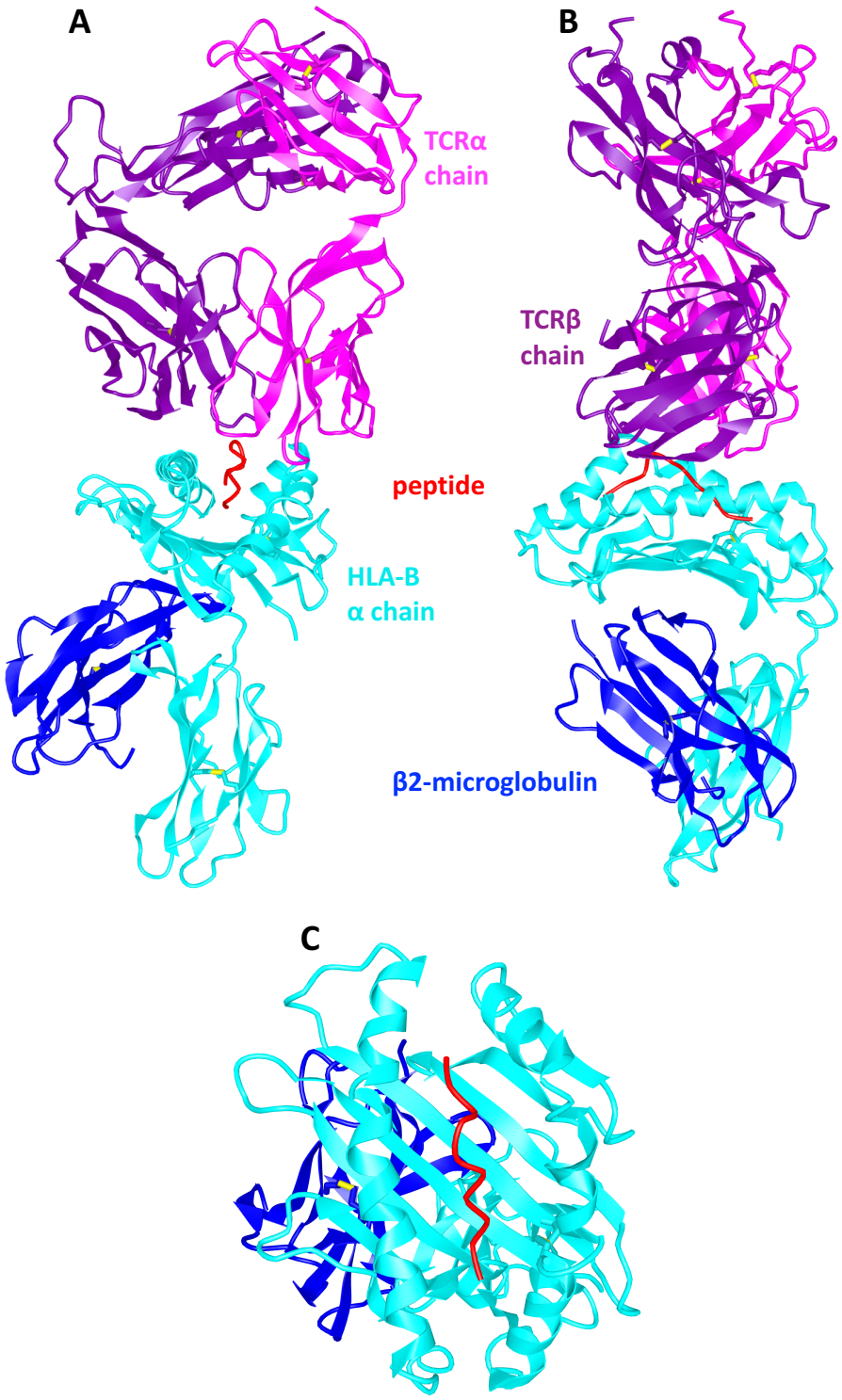

Figure 1.4. Structure of the extracellular domains of αβ TCR and HLA class I complex. A TCR consists of an α chain (pink) and a β chain (purple) and interacts with a peptide (red) that is bound to the HLA class I complex, which is composed of the HLA class I α chain (cyan) and the invariant β2-microglobulin (blue). Typically, HLA class I complexes accommodate peptides of 8 to 10 amino acids length. In this case, the peptide is 10 amino acids long and bound to an HLA-B*44:05. Figures **A** and **B** show the same structure of TCR and HLA class I complex, in B rotated by 90°. The CDR3 forms a loop that mediates most of the contact to the peptide (nicely visible in **A**). Figure **C** only shows the peptide/HLA class I complex viewed from the top. Disulfide bonds are shown in yellow. Modified from Archbold et al. (2009, NCBI Protein Data Bank ID: 3DXA, accessed on 21.05.2019).

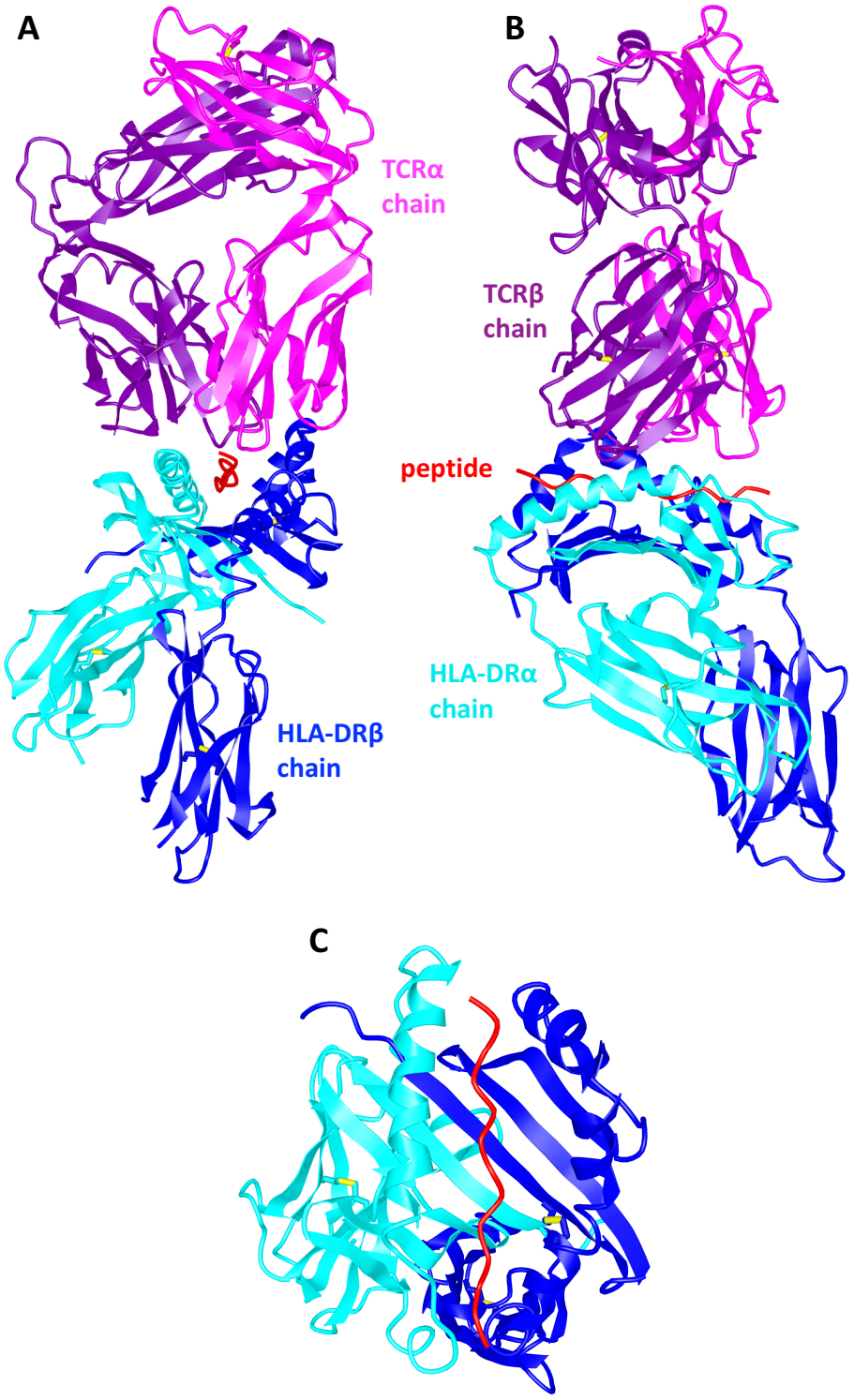

Figure 1.5. Structure of the extracellular domains of αβ TCR and HLA class II complex. A TCR consists of an α chain (pink) and a β chain (purple) and interacts with a peptide (red) that is bound to the HLA class II complex, which is composed of the HLA class II α chain (cyan) and β chain (blue). Typically, HLA class II complexes accommodate peptides of 13 to 17 amino acids length. In this case, the peptide is 13 amino acids long and bound to an HLA-DRA1*01:01/DRB1*04:01 complex. Figures **A** and **B** show the same structure of TCR and HLA class II complex, in B rotated by 90°. The CDR3 forms a loop that mediates most of the contact to the peptide. Figure **C** only shows the peptide/HLA class II complex viewed from the top. Disulfide bonds are shown in yellow. Modified from Hennecke and Wiley (2002, NCBI Protein Data Bank ID: 1J8H, accessed on 21.05.2019).

trigger an immune reaction towards them. This graft-versus-host disease (GvHD) is a major complication after allogeneic transplantation of hematopoietic stem cells (HSCs) that can be reduced in incidence by matching the recipient's HLA type with the donor. This alloreactivity can even be employed to kill remaining tumour cells (graft-versus-leukemia, GvL), which is part of the therapeutic principle of allogeneic HSCT. Apart from HLA molecules, genetically different proteins that are called minor histocompatibility antigens may also trigger GvHD. If the reaction cannot be controlled by immunosuppression, the recipient suffers from tissue damage that may result in fever, pain, diarrhea, erythema and even death.

1.2 Human cytomegalovirus

Human cytomegalovirus (HCMV), also known as human herpesvirus 5 (HHV-5), is an opportunistic and widespread pathogen with a seroprevalence ranging from 50–100 % depending on socio-economic status, geography and ethnicity (reviewed in Cannon et al., 2010). The other human herpesviruses are herpes simplex virus type 1 and 2, varicella zoster virus, Epstein-Barr virus (EBV), HHV-6A and -6B, HHV-7 and Kaposi's sarcoma-associated herpesvirus. HCMV carries a linear, double-stranded, ≈235 kb DNA genome that was estimated to harbour 164 to 167 protein-coding open reading frames (ORFs, Davison et al., 2003). Later, wild-type (WT) HCMV was reported to harbour about 170 genes (Murphy and Shenk, 2008; Sijmons et al., 2014). In comparison to clinical isolates, the genome of laboratory HCMV strains, such as AD169, contains mutations, deletions and rearrangements (Bradley et al., 2009), which likely are the result of prolonged *in vitro* passaging. HCMV's genome consists of a unique long (UL) and a unique short (US) region that are both flanked by a pair of inverted repeats (Sijmons et al., 2014). Viral genes are labelled with the region they are located in and a sequential number (e.g. UL55).

The viral genome is contained in an icosahedral capsid structure that is surrounded by a matrix of proteins (the tegument) and a lipid bilayer envelope (Chen et al., 1999) with membrane-anchored glycoproteins. The tegument is comprised of many viral proteins, of which the 65 kDa phosphoprotein (pp65, gene product of UL83) is the most abundant protein of virions, at least in some HCMV strains such as AD169 (Reyda et al., 2014; Varnum et al., 2004). pp65 plays an important structural role and participates in the assembly of new virions. Glycoprotein B (gB), gH, gL, gM, gN and gO are also part of the viral proteome (Varnum et al., 2004) and form gH/gL complexes that are involved in cell attachment and penetration (Kinzler and Compton, 2005). In contrast, other proteins such as the 72 kDa immediate-early protein 1 (IE-1), which is the gene product of UL123, play a largely intracellular role in HCMV replication. IE-1 was not identified as a component of virions (Büscher et al., 2015; Varnum et al., 2004) or was contained at very low levels only (Reyda et al., 2014).
Except for the viral DNA, HCMV particles also contain various RNAs that allow for the expression of viral genes in an infected cell immediately after virus entry and independent of the viral genome (Bresnahan and Shenk, 2000).

1.2.1 Human cytomegalovirus infection and disease

HCMV can be transmitted via bodily fluids (e.g. saliva, breast milk, urine, blood) of infected persons with active virus replication and can also be transmitted from mother to fetus (congenital infection) or through solid organ transplantation (SOT) and hematopoietic stem cell transplantation (HSCT). Primary HCMV infection of immunocompetent individuals is usually asymptomatic and rarely causes severe complications (reviewed in Gandhi and Khanna, 2004), since the virus is controlled by the host's immune system. In contrast, the immature immune system of perinatal and postnatal infants often cannot cope with the infection on its own. HCMV is one of the most frequent congenital infections and can lead to severe developmental abnormalities and fetal death (reviewed in Gandhi and Khanna, 2004; Manicklal et al., 2013; Ross and Boppana, 2005). Also, patients that are immunosuppressed after SOT or HSCT and patients that are infected with human immunodeficiency virus (HIV) and suffer from the acquired immunodeficiency syndrome (AIDS) are at risk because their immune system is compromised and can therefore not sufficiently control HCMV infection. Since HCMV has a broad cell tropism, it infects and replicates in cells of many different tissues (reviewed in Sinzger et al., 2008a) causing inflammation and dysfunction of various organs (e.g. lung, gastro-intestinal tract, liver, retina and central nervous system) if infection is not held in check by the immune system.
In allogeneic HSCT (allo-HSCT), human cytomegalovirus is one of the most significant infectious pathogens causing substantial morbidity and mortality after transplantation (Boeckh et al., 2003; Boeckh and Nichols, 2004; Walker et al., 2007). If both donor and recipient are HCMV-seronegative ($HCMV^-$) before transplantation, the risk of viral reactivation can be excluded, but primary infection with HCMV occurs in 30% of HSCT recipients after transplantation (D^- R^-, reviewed in Ljungman, 2007). In contrast, viral reactivation occurs in 80% of cases if the HSCT recipient was HCMV-seropositive ($HCMV^+$) before transplantation.
In SOT, $HCMV^-$ patients who received an organ from an $HCMV^+$ donor (D^+ R^-) are at high risk of HCMV infection by viral reactivation from the graft (reviewed in Rubin, 2007) because they do not possess a pre-established HCMV-specific immunity that may prevent dissemination of the virus. Infection with HCMV also increases the risk of chronic graft rejection (Evans et al., 2000).

HCMV enters a host cell by direct fusion with its cell membrane (Compton et al., 1992), which is promoted by gH/gL complexes on the virion surface (Kinzler and Compton, 2005). Virus entry is mediated by the cellular receptor neuropilin-2 (Martinez-Martin et al., 2018). Two types of viral gH/gL complexes are known: gH/gL/gO and gH/gL/pUL(128,130,131A) (Scrivano et al., 2011). Many laboratory HCMV strains, such as AD169 (Ryckman et al., 2010), only express gH/gL/gO complexes which restricts virus entry to a few cell types including fibroblasts (Hahn et al., 2004; Scrivano et al., 2011; Wang and Shenk, 2005). Clinical HCMV isolates, however, also express gH/gL/pUL(128,130,131A) complexes (Baldanti et al., 2006; Grazia Revello et al., 2001; Ryckman et al., 2010) and are therefore able to infect leukocytes, DCs, and endothelial cells, too (Gerna et al., 2005; Hahn et al., 2004). Interestingly, HCMV virions display different selectivity for host cells depending on the cell type in which virus replication took place (Scrivano et al., 2011).

After fusion of the HCMV virus envelope with the cell membrane, the tegument and capsid enter the cytoplasm. Tegument proteins deliver the capsid to the nucleus and initiate viral gene expression (reviewed in Kalejta, 2008). In the cell nucleus, the viral genome is released from the capsid and viral genes are transcribed, whose products orchestrate the translation of other viral proteins and the replication of viral DNA. Viral gene products can be grouped according to the time of synthesis after productive infection, however, these phases overlap with each other: immediate-early (IE, 0–2 h), early (E, 0–15 h), late (L, >15 h, Stinski, 1978). The immediate-early proteins 1 (IE-1, product of UL123) and 2 (IE-2, product of UL122) function as transcriptional regulators and trigger the expression of viral early proteins, which drive viral DNA replication (Greaves and Mocarski, 1998; Marchini et al., 2001). Viral late proteins participate in virus assembly or represent structural components of the virion. The viral capsid is formed in the nucleus and translocated to the cytosol, where it associates with tegument proteins. Subsequently, the viral envelope and its glycoproteins are acquired by budding into intracellular vesicles of the ER, Golgi apparatus and endosomal machinery. Finally, enveloped infectious virus particles are released into the extracellular space (the whole viral "life" cycle is reviewed in Jean Beltran and Cristea, 2014) after 72–96 hours (Loewendorf and Benedict, 2010).
In vitro, HCMV-infected fibroblasts release infectious virions but also two types of particles that lack viral DNA and are not infectious: dense bodies and non-infectious enveloped particles (NIEPs, Irmiere and Gibson, 1983). NIEPs contain the full complement of virion proteins in approximately the same relative amounts, but dense bodies lack all of the capsid and most of the tegument proteins of the virion (Gibson and Irmiere, 1984). Dense bodies are being developed as a form of HCMV vaccine (Pepperl et al., 2000; Pepperl-Klindworth et al., 2003).

After initial infection, HCMV, like all other herpesviruses, can establish persistence for a lifetime despite the strong antiviral immunity developed by a healthy host (see section below). During the subsequent phase known as latency, viral gene expression is strongly decreased and viral replication is shut off, which hampers immunological detection of latently infected cells. The latency reservoir of HCMV is formed by CD34-positive ($CD34^+$) hematopoietic progenitor cells and cells of the myeloid lineage (Kondo et al., 1994; Sinclair and Sissons, 2006; Sindre et al., 1996). Periodically HCMV is reactivated from latently infected cells particularly in response to immunosuppression, inflammation or stress (Kutza et al., 1998; Mutimer et al., 1997; Prösch et al., 2000), which causes the transcription of viral IE genes of HCMV and ultimately leads to lytic viral replication (Prösch et al., 1995; Stein et al., 1993). Tumour necrosis factor alpha (TNF-α) is one of the key mediators of HCMV reactivation by induction of NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells) in latently infected cells (Prösch et al., 1995). Prostaglandin E_2 (PGE_2) that acts via the cyclic adenosine monophosphate (cAMP) pathway and IL-1β were also reported to trigger HCMV reactivation (Kline et al., 1998). Reactivation of HCMV often goes unnoticed in immunocompetent individuals, since virus replication is rapidly controlled by effector functions of memory T cells that were previously established in response to primary infection. In contrast, these effective defence mechanisms are impaired in immunocompromised persons, such as AIDS patients and HSCT recipients or immunosuppressed patients after SOT. They cannot be protected from pathogens that reside within their body by isolation in a germ-free environment. Due to their deficient or impaired immune system HCMV replication

may not be controlled, eventually resulting in HCMV disease.

1.2.2 Immune response towards human cytomegalovirus

Innate immune cells can sense and attack HCMV infection early on. The PRR Toll-like receptor 2 (TLR2) can detect the viral surface glycoproteins gB and gH and trigger inflammatory cytokine production in response (Compton et al., 2003) even before the cell is infected. As a result, more effector cells of the innate immune system, like DCs, macrophages and NK cells, are attracted to the site of infection. Recruitment of DCs is very important because they possess the unique ability to prime antigen-inexperienced ("naïve") T cells, but are also able to reactivate memory T cells. However, DCs can be infected by HCMV themselves (Jahn et al., 1999; Sénéchal et al., 2004) and the virus encodes mechanisms that impair DC function, such as stimulation of T cells (Moutaftsi et al., 2002; Raftery et al., 2001, also see section 1.2.3). Recovery of NK cell activity was associated with survival from HCMV disease after HSCT (Quinnan et al., 1982) and patients who lack functional NK cells may suffer from recurrent episodes of HCMV disease (Biron et al., 1989). The importance of NK cell activity is indirectly highlighted by the large array of mechanisms that HCMV employs to prevent activation of NK cells (reviewed in Jackson et al., 2011, also see section 1.2.3).

Infection with HCMV induces a strong humoral and cellular response of the adaptive immune system. But at least in the mouse, antibodies specific for murine cytomegalovirus (MCMV) are not essential for controlling infection but rather limit viral dissemination (Jonjić et al., 1994). In humans, pre-existing anti-HCMV antibodies can prevent congenital infection of the fetus during pregnancy (Fowler et al., 1992). Although numerous antigens of HCMV are targeted by antibodies, those that are specific for the gH/gL/pUL(128,130,131A) complex show superior neutralising activity regarding HCMV infection of endothelial, epithelial, and myeloid cells in comparison to antibodies specific for gB or gH (Macagno et al., 2010). This further illustrates the major role of gH/gL/pUL(128,130,131A) complexes in HCMV infection of leukocytes, DCs, and endothelial cells as described by Gerna et al. (2005) and Hahn et al. (2004). Therefore, some current vaccine approaches aim at generating an antibody response against this pentameric complex (Plotkin and Boppana, 2018).

1.2.2.1 HCMV-specific $CD8^+$ cytotoxic T lymphocytes

The proportion of HCMV-specific T cells among peripheral blood T cells of an $HCMV^+$ person is high: it was described that, on average, 10% of the blood memory T cell population is specific for HCMV in healthy carriers (Sylwester et al., 2005). The frequency of HCMV-specific T cells gradually increases with age ("memory inflation") and this phenomenon may contribute to immune system exhaustion and dysfunction in the elderly (Moss, 2010). This profound expansion of HCMV-specific T cell populations is likely due to continuous expression and presentation, although at low levels, of HCMV antigens during latency.
As patients without detectable HCMV-specific CTL activity are at risk of developing severe HCMV disease, these effector T cells were proposed to mediate recovery from HCMV infection

already more than three decades ago (Quinnan et al., 1982; Reusser et al., 1991). In fact, $CD8^+$ CTLs were identified to be crucial for limiting replication of HCMV (Einsele et al., 2002; Reddehase, 2002) by killing infected target cells and their efficacy was demonstrated by reconstitution of HCMV-specific CTL immunity in immunocompromised patients after HSCT (see section 1.2.4). $CD8^+$ T cell responses were reported against many HCMV antigens regardless of their function: transcription factors, like IE-1 and IE-2, immunomodulatory proteins, like glycoprotein (gp) US3 (see section 1.2.3), and structural proteins, like gB, gH or pp65 (Elkington et al., 2003; Sylwester et al., 2005). Within these studies and others (Khan et al., 2007; Nastke et al., 2005; Slezak et al., 2007) a large number of $CD8^+$ T cell epitopes derived from many different antigens of HCMV were identified.
The T cell responses to the immunodominant antigens pp65 (Kern et al., 2002) and IE-1 (Kern et al., 1999) were studied in greater detail than those to most other HCMV antigens, since $CD8^+$ T cell responses to both antigens were detected in a majority of $HCMV^+$ donors (see Khan et al., 2005; Sylwester et al., 2005). Strikingly, CTLs specific for IE-1, but not CTLs specific for pp65, were found to correlate with protection from HCMV disease (Bunde et al., 2005). Moreover, a delay in reconstituting IE-1-specific $CD8^+$ T cells in the first 3 months after HSCT was shown to correlate with a lack of protection from HCMV disease (Sacre et al., 2008). The protective effect of IE-1-specific $CD8^+$ T cells was confirmed by adoptive transfer of murine IE-1-specific $CD8^+$ T cells into mice challenged with MCMV (Pahl-Seibert et al., 2005). However, protection is not necessarily mediated by immunodominant viral epitopes, as the latter did not correlate with antiviral efficacy of epitope-specific $CD8^+$ T cell immunotherapy in murine models (reviewed in Lemmermann and Reddehase, 2016).
Since IE-1 is expressed very early during infection and reactivation, IE-1-specific T cells may be able to detect productively infected host cells at an early stage of the viral "life" cycle and may thus be able to prevent shedding of virus by killing the infected cell before the release of progeny virus. pp65-specific T cells may also be able to detect primary infection at an early time point because pp65 is contained in HCMV particles in large quantities (Varnum et al., 2004) and thus abundantly available for presentation via HLA class I molecules. In contrast, pp65-specific T cells will not be able to detect HCMV reactivation from cellular latency as early as IE-1-specific T cells because in this case no exogenous source of pp65 or other structural proteins is available, and to prevent new virion synthesis targeting of IE/E regulatory factors will be necessary. The same is likely to apply to T cells specific for other late antigens, too. This makes IE-1 an attractive target in immunotherapy or prophylaxis of HCMV infection and reactivation.

Recently, $CD8^+$ T cell clones restricted by HLA-C*07:02 and specific for the epitope IE-$1_{309\text{-}317}$ (CRV) were shown to strongly recognise AD169-infected fibroblasts despite the presence of the four immunoevasive proteins ("immunoevasins") gpUS2, gpUS3, gpUS6 and gpUS11 (Ameres et al., 2014, 2013). In contrast, IE-1-specific $CD8^+$ T cell clones restricted by several HLA-A and -B allotypes did not elicit a significant IFN-γ secretion in response to AD1619-infected fibroblasts. If fibroblasts were infected with the AD169-derived variant CMV-Δall that lacks immunoevasins US2, US3, US6 and US11 (Besold et al., 2009, originally named RV-KB6), all IE-1-specific $CD8^+$ T cell clones strongly recognised the virus infected target cells irrespective of HLA restriction (Ameres et al., 2013). Since HLA-C*07:02 is less affected by these four im-

munoevasins in comparison to other HLA molecules, it may play an important role in controlling HCMV infection in individuals that possess this HLA allele.

1.2.2.2 HCMV-specific γδ T cells

Vδ2$^-$ γδ T cells are likely involved in the immune response directed against HCMV, too. Vδ2$^-$ γδ T cells, but not Vδ2$^+$ T cells, were significantly expanded due to HCMV reactivation after SOT or HSCT, which suggests that Vδ2$^-$ γδ T cells participate in the anti-HCMV immune response (Déchanet et al., 1999; Knight et al., 2010, respectively). *In vitro*, Vδ2$^-$ γδ T cells, but not Vδ2$^+$ T cells, killed HCMV-infected target cells through a mechanism involving TCR engagement, but not HLA presentation by target cells (Halary et al., 2005). Moreover, higher numbers of Vδ2$^-$ γδ T cells were observed in HCMV$^+$ healthy persons compared with HCMV$^-$ persons in the aforementioned study. Some of these Vδ2$^-$ γδ T cells appear to be specific for endothelial protein C receptor (EPCR), a stress-induced molecule (Khairallah et al., 2017; Willcox et al., 2012). Pitard et al. (2008) showed that HCMV$^+$ persons possess a slightly but significantly increased proportion of Vδ1$^+$ T cells (≈1.2%) in comparison to HCMV$^-$ persons (≈0.9%), while Vδ2$^+$ and Vδ3$^+$ T cells did not differ between the two groups. They also found that Vδ1$^+$ T cells are more likely to express memory markers in HCMV carriers. Thus, Vδ2$^-$ γδ T cells show consistent changes in their phenotype and frequency in HCMV carriers, which indicates that they are part of the adaptive immune response to infection (Gerna and Lilleri, 2019).

1.2.2.3 HCMV-specific CD4$^+$ T helper cells

Functional CD4$^+$ T cells play important roles in the surveillance and control of HCMV infection:

1. The maintenance of HCMV-specific CD8$^+$ T cells depends on recovery of CD4$^+$ T cell function after HSCT (Riddell and Greenberg, 1995; Walter et al., 1995). Immunosuppressed patients after SOT are only protected from HCMV reactivation if HCMV-specific CD4$^+$ cells reconstitute their functions and provide help to CD8$^+$ T cells (Gabanti et al., 2014).
2. Functional CD4$^+$ T cells are required for resolution of symptomatic HCMV disease (Lilleri et al., 2008; Rentenaar et al., 2000) with IFN-γ playing a critical role as antiviral mediator (Gamadia et al., 2003).
3. HCMV-specific CD4$^+$ T cells attack the viral infection through direct effector functions, such as cytotoxicity and secretion of pro-inflammatory molecules (Crompton et al., 2008; Pachnio et al., 2016), and thus contribute to virus clearance and prevent dissemination (Jackson et al., 2017). Although CD4$^+$ T cells are not cytotoxic effectors in many other situations, some HCMV-specific CD4$^+$ T cells were found to have direct cytotoxic function (Crompton et al., 2008; Elkington et al., 2004; Hopkins et al., 1996; Le Roy et al., 2002; Pachnio et al., 2016; Weekes et al., 2004).
4. A lack of HCMV-specific CD4$^+$ T cells was associated with persistent shedding of virus in immunocompetent young children (Tu et al., 2004) and with an increased risk for HCMV disease after HSCT (Boeckh et al., 2003; Krause et al., 1997; Ljungman et al., 1993) and in AIDS patients (Komanduri et al., 1998).

These observations are supported by findings in mouse models: MCMV-specific $CD4^+$ T cells can prevent infection of salivary glands and thus shedding of virus (Lucin et al., 1992; Yunis et al., 2018). Additionally, murine $CD4^+$ T cells were found to successfully control MCMV infection in the absence of $CD8^+$ T cells *in vivo* (Jonjić et al., 1990). In vaccinated HLA-A*02:01-transgenic mice, $pp65_{495\text{-}503}$ (NLV)-specific $CD8^+$ T cells critically depend on $CD4^+$ T cell help (Reiser et al., 2011).

Similar to $CD8^+$ CTLs, human $CD4^+$ T cells are specific for epitopes from various HCMV antigens including the transcription factors IE-1 and IE-2, the immunomodulatory protein gpUS3, and the structural proteins gB, gH or pp65 (Sylwester et al., 2005). Elkington et al. (2004) identified $CD4^+$ T cell epitopes from gB and gH. Several $CD4^+$ T cell epitopes from pp65 were published almost two decades ago (Gallot et al., 2001; Khattab et al., 1997; Li Pira et al., 2004; Wiesner et al., 2005). pp65 apparently is an immunodominant antigen of HCMV in both the $CD8^+$ and the $CD4^+$ T cell repertoire. In contrast, low numbers of IE-1-specific $CD4^+$ T cells were reported (Bunde et al., 2005; Gratama et al., 2008; Kern et al., 2002), although IE-1-specific $CD4^+$ T cell clones and some of their epitopes had been described early, too (Alp et al., 1991; Davignon et al., 1996). Recently, the $CD4^+$ T cell repertoire directed against IE-1 was analysed and many IE-1 epitopes and their HLA restrictions were identified in great detail (Ameres et al., 2015; Braendstrup et al., 2014). The problem of generally low numbers of IE-1-specific $CD4^+$ T cells was overcome by expansion with peptides and subsequent multimer staining (Braendstrup et al., 2014) or by mini-LCL expansion (see sections 2.3.2 & 3.3.5) and single-cell cloning followed by functional testing of the T cell clones (Ameres et al., 2015). In summary, $CD4^+$ T cells are a key component of an HCMV-specific immune response.

1.2.3 Viral immune evasion

Due to their long-term co-evolution with their host species, CMV species are intricately adapted to their host's immune responses and have developed many mechanisms to escape and counteract them. For this purpose, HCMV codes for several micro-RNAs (miRNAs) and immunomodulatory proteins. miRNAs are small non-coding RNAs that can post-transcriptionally regulate gene expression by interfering with 3' untranslated regions of mRNA. HCMV's miRNAs target the expression of both viral and cellular genes and thus regulate viral replication, cell survival, establishment and maintenance of viral latency, but also affect innate and adaptive immunity (reviewed in Hook et al., 2014). For example, the miRNA US4-1 of HCMV specifically down-regulates ERAP1 expression thereby inhibiting the trimming of peptides in the ER and leading to reduced susceptibility of infected cells to HCMV-specific CTLs (Kim et al., 2011b).
HCMV down-regulates expression of HLA class I molecules on the surface of infected cells (Barnes and Grundy, 1992), which is a mechanism to evade detection of viral peptides by T cells. The viral genes US2-US11 are involved in this process (Jones et al., 1995). The relevant glycoproteins encoded by this genomic region are gpUS2, gpUS3, gpUS6, gpUS10 and gpUS11. These glycoproteins are immunoevasins since they help infected cells evade the attention of virus-specific T cells, in particular HLA class I-restricted $CD8^+$ T cells. gpUS2 and gpUS11 are early proteins and promote the dislocation of HLA class I α chains from the ER to the cytosol where they are

degraded (Jones and Sun, 1997; Wiertz et al., 1996). gpUS3 is an IE protein and retains HLA class I complexes in the ER (Jones et al., 1996; Zhao and Biegalke, 2003). gpUS3 interacts with components of the peptide loading complex and prevents loading of susceptible HLA molecules with optimal peptides (Park et al., 2004, 2006). gpUS6 is an early/late protein and inhibits peptide translocation by TAP into the ER, which impairs loading of HLA class I molecules with peptide (Ahn et al., 1997). gpUS10 selectively down-regulates cell surface expression of HLA-G, but not that of classical HLA class I molecules (Park et al., 2010).
Taken together, recognition of HCMV-infected cells by $CD8^+$ CTLs is strongly impaired by the immunoevasins (Ameres et al., 2014, 2013) that decrease the amount of peptide/HLA class I complexes on the surface of an infected cell. Down-regulation of the expression of peptide/HLA class I complexes by US2 and US11 was reported to be allele specific (Barel et al., 2003a,b; Schust et al., 1999, 1998). HLA-C, however, was uniquely resistant to the effects of both immunoevasins. The functional relevance of HLA-C for T cell recognition of HCMV infection was shown by Ameres et al. (2014) who confirmed the resistance of HLA-C*07:02 to US2 and US11 while all HLA-A and -B allotypes that were analysed in this study were heavily affected by the immunoevasins. Thus, HLA-C*07:02 is a promising HLA restriction to target in T cell therapy of HCMV infection.
Down-regulation of HLA class I molecules enables HCMV to evade T cell detection, but is accompanied by an increased susceptibility to recognition by NK cells due to low HLA class I expression levels. Thus, HCMV employs other mechanisms of NK cell inhibition. Viral glycoproteins reduce the expression of ligands for NK cell activating receptors thereby decreasing susceptibility to NK cell recognition. For example, gpUL16 and gpUL142 reduce cell surface levels of MHC class I chain-related protein A (MICA), MICB, UL16-binding protein 1 (ULBP1), and ULBP2 to avoid NK cell recognition via NKG2D (Chalupny et al., 2006; Dunn et al., 2003). In parallel, HCMV also enhances the expression of ligands for inhibitory NK cell receptors to compensate for the lack of HLA class I molecules on the surface of the infected cell. The HCMV-encoded UL18 and UL40 proteins promote surface expression of leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1, Chapman et al., 1999) and HLA-E (Tomasec et al., 2000), respectively, to prevent NK cell activation.

It has been proposed that HCMV immunoevasins affect peptide presentation by HLA class II molecules, too. US2 was reported to degrade HLA-DRα and HLA-DMα chains (Hegde and Johnson, 2003; Tomazin et al., 1999) thereby affecting peptide loading and stability of all HLA-DR allotypes. US3 supposedly binds to HLA class II $\alpha\beta$ complexes in the ER and reduces their association with Ii. As a result, HLA class II complexes were not loaded with peptides efficiently and formation of peptide-loaded HLA class II complexes was reduced (Hegde et al., 2002). However, others presented evidence that interference of HCMV immunoevasins with HLA class II levels was weak or absent (Odeberg and Söderberg-Nauclér, 2001; Rehm et al., 2002). It has remained unclear how this interference would operate, since the recognition sequences of US2 and US11 on HLA class I molecules are precisely known (Barel et al., 2006, 2003a,b), are even greatly variable in HLA class I allotypes, and are unlikely to be broadly conserved in HLA class II molecules. However, it remains possible that such effects operate on certain specific HLA class II allotypes that remain to be defined.

Since DCs can be infected with HCMV and are critical immune cells that initiate and maintain immune responses, it is not surprising that HCMV tampers with the functions of DCs. HCMV infection alters the expression of adhesion molecules, which may contribute to an impairment of DC migration (Beck et al., 2003). Immature DCs that were infected with HCMV showed a decreased expression of HLA molecules and co-stimulatory molecules and their maturation as well as cytokine production was inhibited (Moutaftsi et al., 2002). The same study found reduced proliferation and cytotoxicity of T cells specific to a recall antigen if presented by HCMV-infected DCs. Mature DCs that were infected with HCMV lose surface expression of CD83 while soluble CD83 accumulates in the supernatant and inhibits stimulation of T cell proliferation (Sénéchal et al., 2004). If soluble CD83 was removed, T cell stimulation by DCs could be restored.

1.2.4 Therapy for human cytomegalovirus infection

After HSCT or SOT, the immune system of the recipient is heavily compromised due to the necessary myeloablative treatment in HSCT and the resulting immunosuppression after transplantation. As a result, the recipient is susceptible to infections, especially to those that are caused by pathogens that reside within the body, because the patient cannot be shielded from them.

1.2.4.1 Antivirals

Standard antiviral therapy using inhibitors, such as foscarnet, ganciclovir or cidofovir, target viral DNA polymerases to impair viral replication. However, these inhibitors also affect human DNA polymerases, but to a lesser degree. HCMV disease in the immunocompromised host can be successfully prevented by antiviral treatment with these compounds, but their administration is often accompanied by serious side effects, such as myelotoxicity, neutropenia and nephrotoxicity (Broers et al., 2000; Sharland and Khare, 2001). Moreover, antiviral drug resistance has been reported for each of the three DNA polymerase inhibitors (Lurain and Chou, 2010).
Recently, the novel anti-HCMV drug letermovir was reported to successfully reduce the risk of HCMV infection after HSCT if applied as a prophylactic treatment in a phase III clinical trial (Marty et al., 2017). Letermovir has a different mechanism of action than established anti-HCMV drugs: it targets the viral terminase complex, which is involved in processing of the concatenated HCMV genome and transfer of a single-unit genome into procapsids (reviewed in Foolad et al., 2018). Importantly, the frequency and severity of adverse events (including myelotoxicity and nephrotoxicity) as a result of letermovir treatment were reported to be similar to treatment with placebo. Whether letermovir will be less susceptible to resistance mutations by HCMV than foscarnet, ganciclovir or cidofovir and whether letermovir will uphold its superior safety profile remains to be evaluated.
While the risk of HCMV infections can be reduced by administration of antivirals early after transplantation (typically 3 months), late-onset HCMV disease (>100 days after transplantation) remains a clinical problem (Boeckh et al., 2003; Zaia et al., 1997).

1.2.4.2 Vaccines

Vaccination against HCMV should provide efficient prevention of HCMV infection or reactivation, especially in immunocompromised patients, without causing side effects. According to developers, a protective HCMV vaccine should induce both humoral and cellular immunity that is not restricted only to a single structural antigen, such as gB or pp65, because those antigens may not confer protection from HCMV infection or reactivation (Bunde et al., 2005). Although numerous clinical trials are ongoing (reviewed in Diamond et al., 2018), no vaccine has yet been licensed (Schleiss, 2016).

Before recombinant vaccines were developed, live attenuated vaccines based on fibroblast-passaged HCMV laboratory strains, such as AD169 or Towne, were evaluated, but they could not provide satisfactory results in clinical trials or animal models (reviewed in Gerna and Lilleri, 2019). A potential reason is their loss of genes encoding elements of the pentameric glycoprotein complex. As a result, these virus strains may not have been able to infect natural HCMV host cells such as endothelial or myeloid cells, resulting in premature abortion of infection and non-immunogenicity.

A recombinant MF59-adjuvanted vaccine that contained only gB as HCMV antigen protected $HCMV^-$ pregnant women from primary infection with an intermediate efficacy of 50% (Pass et al., 2009). The same gB-MF59 vaccine was able to boost antibody responses and IFN-γ secretion by $CD4^+$ T cells in $HCMV^+$ women (Sabbaj et al., 2011). However, the clinical evaluation of this vaccine was halted and its efficacy was not evaluated in a phase III study (Diamond et al., 2018) after it was discovered that antibodies specific for the gH/gL/pUL(128,130,131A) complex show superior neutralising activity in comparison to antibodies specific for gB (Freed et al., 2013; Macagno et al., 2010).

A number of different HCMV vaccines is now in clinical trials. Candidate vaccines for patients after allo-HSCT include recombinant modified vaccinia Ankara (MVA) encoding pp65, IE-1, and IE-2, pp65/HLA-A2 (NLV) peptide vaccines, and the gB subunit vaccine (reviewed in Diamond et al., 2018). Vaccines in development for prevention of infection for different patient groups are based on dense bodies, virus-like particles, heterologous viral vectors (such as vesicular stomatitis virus, MVA and canarypox), protein vaccines with gB or the pentameric complex, and new attenuated forms of HCMV (reviewed in Plotkin and Boppana, 2018). Thus, there is a great diversity of concepts for a HCMV vaccine, but it cannot be predicted at the moment which of these will be successful.

1.2.4.3 Antibodies

Earlier studies described that adoptively transferred immunoglobulin preparations which contain HCMV-specific antibodies from $HCMV^+$ donors can prevent congenital infection of the fetus during pregnancy (Nigro et al., 2005), but a more recent study has contested the efficacy of this treatment (Revello et al., 2014). Some evidence supports the administration of anti-HCMV antibodies to prevent HCMV reactivation and disease after SOT (Falagas et al., 1997; Snydman et al., 1987) and HSCT (Messori et al., 1994). Other studies, however, found no beneficial effect of HCMV immunoglobulins in patients at risk of developing HCMV infection after HSCT

(Muñoz et al., 2001; Ringdén et al., 1987).
Antibodies that are specific for the gH/gL/pUL(128,130,131A) complex showed superior neutralising activity regarding HCMV infection of endothelial, epithelial, and myeloid cells in comparison to antibodies specific for gB or gH (Macagno et al., 2010). Thus, the efficacy of treatment with HCMV-specific antibodies likely depends on antibodies that are specific for antigens which are critical for HCMV infection, such as gH/gL/pUL(128,130,131A) complexes (as described in section 1.2.2).

1.2.4.4 Adoptive T cell transfer

The standard therapy to fight HCMV reactivation is antiviral medication, which is of limited efficacy, shows severe side effects and is counteracted by increasing viral resistance (see section 1.2.4). Adoptive T cell transfer offers an alternative that provides HCMV-specific T cells for the patient at risk, which has been shown to prevent disease and death.

Several studies have shown that adoptive transfer of HCMV-specific $CD8^+$ T cells restores cellular immunity against HCMV after HSCT (Cobbold et al., 2005; Einsele et al., 2002; Riddell et al., 1992; Schmitt et al., 2011; Walter et al., 1995, also see section 1.2.2). However, the *ex vivo* expansion of T cell clones from an $HCMV^+$ HSCT donor (as done by Riddell et al., 1992; Walter et al., 1995) is laborious, time-consuming and thus expensive. Consequently, this method is not suited well to provide a large number of patients with individual HCMV-specific CTLs and was not established as clinical routine. Moreover, adoptive T cell transfer of HCMV-specific T cells should include $CD4^+$ and $CD8^+$ T cells (Einsele et al., 2002; Sellar and Peggs, 2012) to ensure T cell help and maintenance of T cell memory (see section 1.2.2).
An approach that targets multiple epitopes simultaneously is considered an advantage in the field of adoptive T cell transfer against viral disease (Papadopoulou et al., 2014; Peggs et al., 2003) because a broad immune response may provide a better chance of controlling viral infection (Braendstrup et al., 2014) and minimise the risk of viral escape. Earlier protocols used DCs pulsed with HCMV antigens (Peggs et al., 2001) or PBMCs pulsed with libraries of overlapping peptides that spanned the entire sequence of viral proteins (pp65 & IE-1, Papadopoulou et al., 2014) to generate polyclonal T cell lines specific for multiple HCMV epitopes. In 2010, Moosmann et al. generated EBV-specific T cells for adoptive transfer to patients after allo-HSCT by stimulating leukapheresis products from the HSC donor with a peptide mix that consisted of 23 defined peptides with various HLA class I and class II restrictions. After 12 hours of stimulation, IFN-γ-secreting cells were isolated by IFN-γ capture and magnetic separation. The multi-epitope EBV-specific T cells were administered to the patients as a single transfusion and caused a rapid and sustained reconstitution of protective EBV-specific T cell memory.
Gary et al. (2018) recently published a good manufacturing practice (GMP) protocol to generate HCMV-/EBV-peptide-stimulated T cells from leukapheresis products of HSC donors using a pool of 34 HCMV and EBV peptides that represent well-defined dominant T cell epitopes with various HLA restrictions. Virus-specific $CD8^+$ and $CD4^+$ T cells are expanded over 9 days and can be administered early after HSCT (>day 30) as a preventive approach. This protocol allows

for rapid and cost-efficient production of HCMV- and EBV-specific T cells and is currently evaluated in a phase I/IIa clinical trial (https://clinicaltrials.gov, identifier: NCT02227641, accessed on 07.05.19). Other clinical trials (NCT01325636 and NCT02985775, accessed on 07.05.19) also intend to provide the patient with HCMV-specific T cells from HSCT donors by stimulating donor-derived PBMCs with HCMV peptides. However, in all of these clinical trials, there is the prerequisite that the donor is $HCMV^+$ and therefore has an established repertoire of HCMV-specific T cells.

Donor immunity may be transferred from an $HCMV^+$ HSC donor to an $HCMV^-$ recipient (D^+ R^-), but an $HCMV^-$ HSC donor cannot provide HCMV-specific T cells. As a surrogate, HCMV-specific T cells derived from a third-party donor that is partially HLA-matched with the recipient may be administered, but in a study from Neuenhahn et al. (2017) these cells failed to persist and proliferate in 7 out of 8 cases after their adoptive transfer to an HSCT patient. Instead, virus-specific T cells can be produced and subsequently administered to the patient by transferring the genes of HCMV-specific TCRs into T cells from the $HCMV^-$ HSC donor. This strategy was originally developed for tumour immunotherapy, but was adapted to HCMV (Schub et al., 2009). Still, applications in HCMV disease have been very limited. There is only one registered clinical trial (NCT02988258, accessed on 07.05.19) at present that aims to introduce a pp65-specific, HLA-A*02:01-restricted TCR into donor T cells via *ex vivo* GMP-compliant retroviral transduction. However, endowing an αβ T cell with a transgenic αβ TCR harbours two major disadvantages: (I) After TCR transfer, the T cell will express two TCRα and two TCRβ chains, which can potentially form four different TCR heterodimers. One will be the original TCR of the T cell, another TCR will be the transgenic one, but the two remaining mis-paired TCR dimers are of unknown specificity and could thus be harmful. This threat is not just hypothetical, as well-defined neo-reactivity due to TCR chain mis-pairing has been reported *in vitro* (van Loenen et al., 2010). (II) TCR transfer into bulk T cells will generate a multitude of different T cells with at least two specificities per T cell. Such T cells could lead to toxicity in the patient if the endogenous TCR is autoreactive. Since an αβ TCR cannot exchange chains with a γδ TCR (Koning et al., 1987; Saito et al., 1988), γδ T cells may be a safer alternative to αβ T cells in adoptive T cell therapy if effector cells are equipped with a transgenic αβ TCR. This concept was successfully employed to generate anti-leukemic γδ T cells that were modified to express an αβ TCR and the relevant co-receptor. These genetically modified γδ T cells exerted high levels of antigen-specific cytotoxicity and produced IFN-γ in response to antigen-expressing target cells *in vitro* (van der Veken et al., 2006). *In vivo*, murine γδ T cells that expressed an αβ TCR specific for the model antigen ovalbumin proliferated in an antigen-specific manner, showed prolonged persistence and had the capacity to mount recall responses (van der Veken et al., 2009). Interestingly, the murine TCR-engineered γδ T cells did not show a preferential homing towards intestinal epithelial sites but resided in peripheral blood. Therefore, there is the chance that γδ T cells are an effective alternative to αβ T cells as effector cells to express transgenic TCRs for immunotherapy.

No randomised, controlled clinical trial on HCMV-specific T cell transfer was ever completed and published. Apart from evaluating the efficacy of adoptively transferred HCMV-specific $CD4^+$

or $CD8^+$ T cells, it would be interesting to see whether application of both HCMV-specific $CD4^+$ and $CD8^+$ T cells have a synergistic effect. In a humanised mouse model, HLA-A*02:01-transgenic NOD/SCID/IL-$2rg^{-/-}$ mice were challenged with a chimeric MCMV that was modified to express the NLV-peptide epitope of HCMV (Thomas et al., 2015). The infectious virus load was significantly reduced in spleen and lungs by $CD8^+$ T cells that were engineered to express an NLV-specific TCR, but not by equivalent $CD4^+$ T cells expressing the same TCR. If the same TCR-transgenic $CD4^+$ T cells were co-administered with TCR-transgenic $CD8^+$ T cells – both expressing the same NLV-specific TCR – at a ratio of 1 to 4, the viral load in spleen and lungs dropped to markedly lower levels than TCR-transgenic $CD8^+$ T cells alone. Thus, the TCR-transgenic $CD4^+$ T cells enhanced the antiviral function of TCR-transgenic $CD8^+$ T cells. This strikingly illustrates the benefit through virus-specific $CD4^+$ T cell help, but does not reflect the physiologic situation, in which $CD4^+$ T cells respond to virus-infected target cells that express HLA class II and not HLA class I.

Most of the HCMV-specific, HLA class II-restricted TCRs, whose TCRβ sequences were published, are specific for gB (Abana et al., 2017; Crompton et al., 2008), pp65 (Schwele et al., 2012; Simon et al., 2014) or of unknown specificity (Abana et al., 2017; van Leeuwen et al., 2006). Only few TCRβ sequences of IE-1-specific, HLA class II-restricted TCRs were published (Schwele et al., 2012). Most of those studies analysed the TCRβ repertoire of HCMV-specific T cells from $HCMV^+$ donors *ex vivo* and none of them assessed the function of the described HLA class II-restricted TCRs in recognising HCMV-infected target cells.

HCMV-specific, HLA class II-restricted TCRs should be made available for application in adoptive T cell transfer in order to be able to supply $HCMV^+$ transplant recipients with HCMV-specific, HLA class II-restricted T cells. Nevertheless, the efficacy of HCMV-specific, HLA class II-restricted TCRs that are specific for different antigens in recognition of HCMV infection was neglected to date.

1.3 Aims of this study

HCMV remains a great threat and health burden especially for immunocompromised patients who do not possess HCMV-specific T cells. Adoptive transfer of HCMV-specific, HLA class I-restricted ($CD8^+$) T cells can restore immunity to HCMV and protect patients from HCMV disease. However, many HLA class I molecules succumb to the combined effects of HCMV's immunoevasins resulting in poor recognition of virus infection by HCMV-specific $CD8^+$ T cells. In contrast, T cell clones restricted by HLA-C*07:02 were able to strongly recognise HCMV-infected target cells despite the presence of immunoevasins. Thus, the therapeutic potential of T cells with this HLA restriction needs to be further explored.
HCMV-specific, HLA class II-restricted ($CD4^+$) T cells were identified as important effector cells to control HCMV infection and maintain immunity to HCMV. Since HCMV latently resides in cells of the myeloid lineage that express HLA class II, HCMV-specific $CD4^+$ T cells may be key to detecting viral reactivation from latency early on and to prevent dissemination of the virus and subsequent HCMV disease. However, the efficacy of adoptively transferred HCMV-specific $CD4^+$ T cells alone or in combination with HCMV-specific $CD8^+$ T cells was evaluated only by few studies. Transferring the TCR genes derived from HCMV-specific $CD4^+$ T cells into T cells from the $HCMV^-$ donor (HSCT) or recipient (SOT) can provide HCMV-specific T cells for patients with the highest need. A prerequisite is the identification and functional analysis of HCMV-specific, HLA class II-restricted TCRs and the evaluation of their function in recognition of HCMV infection – both of which have been neglected so far. A schematic illustration of the experiments that were conducted for this study and how they are connected is depicted in fig. 1.6.
The aim of the present study was to address the following questions:

1. Can TCR-transgenic T cells that express an HCMV-specific, HLA-C*07:02-restricted TCR recognise target cells infected with HCMV despite the action of immunoevasins?

2. Can HCMV-specific TCRs that are restricted by HLA class II be functionally expressed on αβ T cells and δ2 T cells? What are the resulting functional differences between the two T cell subsets?

3. Do TCR-transgenic T cells that express an HCMV-specific, HLA class II-restricted TCR recognise HCMV-infected target cells? Does this recognition depend on co-expression of the co-receptor CD4?

4. Do HCMV's immunoevasins affect the presentation of peptides by HLA class II similarly than peptide presentation on HLA class I molecules?

5. Do HLA class II-restricted TCRs that are specific for the HCMV antigens IE-1 or pp65 differ in their recognition of infected cells?

6. Which HCMV-specific, HLA class II-restricted TCRs may be expected to protect from HCMV infection or reactivation and should thus be developed further for clinical application in adoptive T cell therapy?

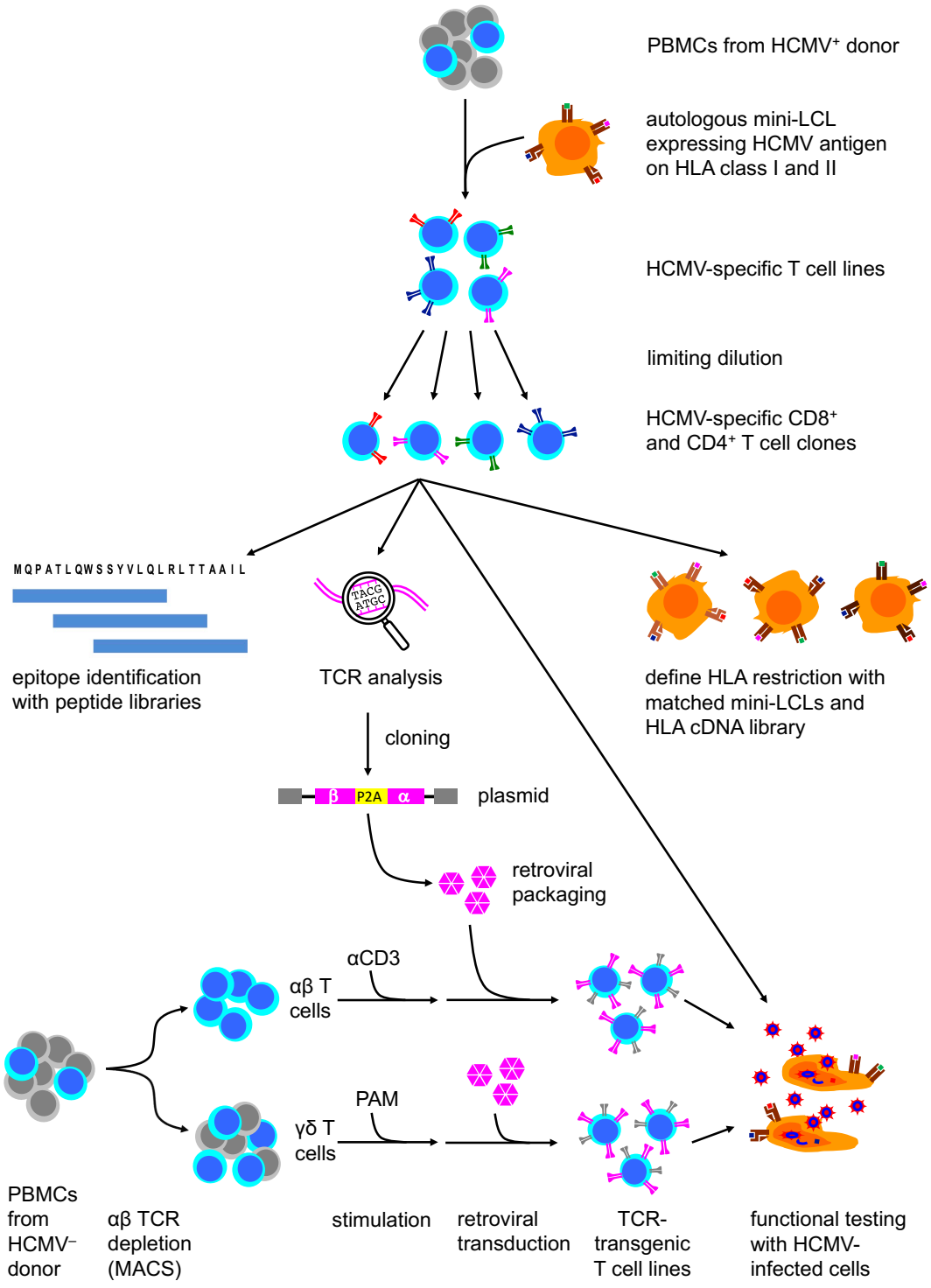

Figure 1.6. Schematic overview of T cell analyses and experiments performed in this study. HCMV-specific T cell lines were derived from PBMCs of healthy HCMV$^+$ donors by stimulation with autologous mini-LCLs that expressed either IE-1 or pp65. HCMV-specific CD4$^+$ and CD8$^+$ T cell clones were obtained by limiting dilution and subjected to (I) epitope identification, (II) TCR sequencing, (III) determination of HLA restriction and (IV) analysis of HCMV-infected target cell recognition. PBMCs from healthy HCMV$^-$ donors as substrates for TCR gene transfer were either used in bulk (not depicted) or after sorting of subpopulations by MACS. The αβ TCR$^+$ fraction ("αβ T cells") and the αβ TCR$^-$ fraction ("γδ T cells") were stimulated with anti-CD3 antibody ("αCD3") or the aminobisphosphonate pamidronate ("PAM"), respectively, prior to transduction with retroviruses coding for HCMV-specific TCRs that were derived from T cell clones. The recognition of HCMV-infected target cells by TCR-transgenic T cell lines was tested identical to that of T cell clones.

2 Materials

2.1 Viruses

The fibroblast adapted HCMV strain **AD169** was kindly provided by Martin Messerle (Hannover, Germany). The **Merlin** strain was acquired from the National Collection of Pathogenic Viruses (Catalogue No. 0302163v), which belongs to Public Health England (Salisbury, United Kingdom). **TB40-BAC4** was obtained from Barbara Adler (LMU Munich, Germany). **CMV-Δall** (Besold et al., 2009, originally named RV-KB6) lacks the genes of the four HCMV-encoded immunoevasive proteins gpUS2, gpUS3, gpUS6 and gpUS11 and was kindly provided by Bodo Plachter (Mainz, Germany). This HCMV mutant was generated through BAC mutagenesis from AD169 as described in Besold et al. (2009). Virus passages of all HCMV virus strains used in this study are shown in table 2.1.

Table 2.1. Stocks of fibroblast supernatant containing laboratory HCMV strains. Supernatants were stored at −80 °C. Producer, date of aliquotation, passage number and virus titer given in $TCID_{50}$ units per ml (see section 3.5.2) are shown.

HCMV strain	**AD169**	**Merlin**	**TB40-BAC4**	**CMV-Δall**
Passage	W22	P4	p5	p5
Date	04.06.2014	04.08.2015	27.07.2015	14.09.2012
Producer	X. Liang	X. Liang	X. Liang	S. Ameres
Titer [$TCID_{50}$/ml]	3.2×10^6	1.8×10^7	8.3×10^6	8.3×10^6

For gene transfer purposes, **MP71** retroviral vectors (Engels et al., 2003) were produced by packaging recombinant, modified MPSV (Myeloproliferative sarcomavirus)-derived vector DNA through co-expression of MuLV (murine leukemia virus)-10A1-env and Mo-MuLV-gag/pol in HEK293T cells (cells and plasmids see sections 2.3.2 and 2.4, respectively).

2.2 Prokaryotic cells

For the propagation of plasmids (see section 2.4), *Escherichia coli* of strain DH5α (Invitrogen, Karlsruhe, Germany) were made chemically transformation-competent. The genotype of the bacteria was F-, Φ80dlacZΔM15, Δ(lacZYA-argF), U169, deoR, recA1, endA1, hsdR17(r_k^-, m_k^-), supE44, thi-I, gyrA96, relA1, λ^-.

2.3 Human cells

2.3.1 Primary cells

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood donations or leukocyte concentrates ("buffy coats", obtained from the Institute for Transfusion Medicine, University of Ulm, Germany) of healthy adult donors, all of which were anonymised for this work by a four to eight digit and letter identifier. The institutional review board (Ethikkommission, Klinikum der Universität München, Marchioninistr. 25, 81377 Munich, Germany) approved the study. All work was conducted according to the principles expressed in the Helsinki Declaration. Low- or

high-resolution HLA genotypes and HCMV serostatus of the PBMC donors are listed in table 2.2.

Table 2.2. HLA type and HCMV serostatus of PBMC donors. Each donor is identified by a unique three to six digit and letter code (1st column). If PBMCs were isolated from a buffy coat instead of whole blood, the additional identifier "BC" was inserted into a four digit and letter identifier (new nomenclature) or "F" was added in front of two digits (old nomenclature). HCMV serostatus (IgG) of the donor is indicated by "+" or "–" (2nd column). For some donors HCMV serostatus or HLA alleles have not been determined ("n.d."). In one case, no HLA-DRB3, DRB4 and DRB5 allele was present in the donor ("a"). Other donors were not analysed for HLA-DRB3/4/5 alleles ("b") although such alleles may be present. Ambiguities of HLA alleles that were not further resolved are depicted by HLA allele codes as published by the National Marrow Donor Program (NMDP, see https://bioinformatics.bethematchclinical.org/hla/alpha.v3.html, accessed on 18.01.2019):
AB = 01/02; AF = 01/09; AFCTE = 02:01/416:01; CKP = 02/04/11;
CWUD = 01/06/09/18/22/52; DPG = 02/17; ENWH = 01/09/19/21/22/24;
KTKP = 01:01/01:01L/01:01N/01:04N/01:22N/01:32/01:37.

Donor	HCMV	HLA							
		A*	B*	C*	DRB	DQA	DQB	DPA	DPB
F46	+	03 11	07 35:01	04:01 07:02	1*13:02 1*15:01 3*03:01 5*01:01	1*01:02	1*06:02 1*06:04	1*01:03	1*04:01 1*04:02
F60	+	02 26	07 38	07:DPG 12:03	1*08:03 1*13:01 3*01:01	n.d.	1*03:01 1*06:03	n.d.	1*02:01 1*04:01
F61	+	02 34	14 44	04:01 08:02	1*01:02 1*07:01 4*01:01	1*01:01 1*02:01	1*02:02 1*05:01	1*01:03 1*02:01	1*04:02 1*14:01
F62	+	03	35:01 52:01	04:AF 1202	1*01:01 1*15:02 5*01:02	1*01:01:01 1*01:03	1*05:01 1*06:01	n.d.	1*01:01 1*04:01
F63[a]	+	03:01:01 24:02:01	18:01:01 40:01:01	03:04:01 07:01:01	1*01:01:01 1*08:03:02	n.d.	1*03:01:01 1*05:01:01	n.d.	1*04:01:01
F64	+	02:01 29:02	15:01 44:03	03:03 16:01	1*07:01 1*13:01 3*01:01 4*01:01	1*01:03 1*02:01	1*02:02 1*06:03	1*01:03 1*02:01	1*02:01 1*11:01
F65	+	24 68	35 44	04:01 15:04	1*14:54 1*15:01 3*02:02 5*01:01	1*01:01 1*01:02	1*05:03 1*06:02	1*01:03	1*02:01 1*03:01
XLBC13	+	11:01 24:02	07:02 35:01	04:01 07:02	n.d.	n.d.	n.d.	n.d.	n.d.

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Table 2.2. HLA type and HCMV serostatus of PBMC donors (continued from previous page)

Donor	HCMV	HLA							
		A*	**B***	**C***	**DRB**	**DQA**	**DQB**	**DPA**	**DPB**
XLBC18	+	03:01	07:02 50:01	06:02 07:02	n.d.	n.d.	n.d.	n.d.	n.d.
0102[b]	–	01:01 02:01	39:06 44:02	07:02 07:04	1*04:04 1*11:01	n.d.	n.d.	n.d.	n.d.
0112[b]	–	01:01 03:01	07:02 08:01	07:01 07:02	1*15:01	n.d.	1*06:02	n.d.	1*04:01
0113	–	01:01:01 26:01:01	35:01:01 57:01:01	04:01:01 06:02:01	1*07:01:01 1*11:04:01 3*02:02 4*01:01	1*0201 1*0505	1*02:02:01 1*03:01:01	n.d.	1*04:01:01 1*04:02:01
0308	–	02:01 03:01	07:02 39:01	07:02 12:03	1*11:01 1*15:01 3*02:02 5*01:01	1*01:02 1*05:05	1*03:01 1*06:02	n.d.	1*04:01 1*04:02
0401	n.d.	02:09 24:02	40:02 52:01	12:02 14:02	1*15:02 5*01:02	n.d.	1*05:03 1*06:AB	n.d.	n.d.
0419	+	03:01 30:03	07:02 58:01	07:02 07:18	1*11:04 1*14:01 3*02:02	1*01:04 1*05:05	1*03:01 1*05:03	n.d.	1*04:02 1*14:01
0506[b]	–	02:01 33:01	14:02 51:01	02:02 08:02	1*04:03 1*07:01	n.d.	1*0202 1*0304	n.d.	1*02:01 1*04:02
0519	+	26:02	35:01 48:01	04:01 08:03	1*11:01 1*15:01 3*02:02 5*01:01	1*01:02 1*05:05	1*03:01 1*06:02	1*01:03 1*02:02	1*02:01 1*04:02
1004[b]	–	11:01 31:01	35:03 40:01	03:04 12:03	1*04:08 1*13:01	n.d.	1*03:04 1*06:03	n.d.	n.d.
1010	+	02:01	07:02 35:03	04:01 07:02	1*03:01 1*14:54 3*01:01 3*02:02	n.d.	n.d.	n.d.	1*01:01 1*02:01
1013	–	02:01 03:01	15:29 51:01	12:03 14:02	1*08:01 1*13:01 3*01:01	1*01:03 1*04:01	1*04:02 1*06:03	n.d.	1*04:01 1*13:01
1014	–	01:01:01 02:01:01	07:02:01 40:01:01	03:04:01 07:02:01	1*13:01:01 1*15:01:01 3*02:02 5*01:01	1*01:02:01 1*01:03	1*06:02:01 1*06:03:01	n.d.	1*04:01:01 1*14:01:01

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Table 2.2. HLA type and HCMV serostatus of PBMC donors (continued from previous page)

Donor	HCMV	HLA							
		A*	B*	C*	DRB	DQA	DQB	DPA	DPB
1021	+	02:01 24:02	07:02 40:01	03:04 07:02	1*07:01 1*13:01 3*02:02 4*01:01	1*01:03 1*02:01	1*02:02 1*06:03	1*01:03	1*04:01 1*20:01
1022	n.d.	02:01:01 31:01:02	27:05:02 40:01:01	02:02:02 03:04:01	1*11:01:01 1*13:02:01 3*02:02 3*03:01	1*01:02 1*05:05	1*03:01:01 1*06:04:01	n.d.	1*02:01:02 1*04:01:01
1107	–	02:05:01 03:01:01	07:02:01 49:01:01	07:01:01 07:02:01	1*11:02:01 1*15:01:01 3*02:02 5*01:01	n.d.	1*03:01:01 1*06:02:01	n.d.	1*03:01:01 1*15:01:01
1201	+	01:01	07:02 08:01	07:01 07:02	1*03:01 1*15:01 3*01:01 5*01:01	n.d.	1*02:01 1*06:02	n.d.	1*03:01 1*04:01
1213	–	02:01:01 03:01:01	14:02:01 35:01:01	04:01:01 08:02:01	1*01:02:01 1*04:01:01 4*01:03	n.d.	1*03:02:01 1*05:01:01	n.d.	1*02:01:02 1*04:01:01
1220	+	02:01	07:02 40:02	02:02 07:02	1*04:01 1*15:01 4*01:03 5*01:01	1*01:02 1*03:03	1*03:01 1*06:02	1*01:03 1*02:01	1*04:01 1*11:01
1223	–	01:01 68:02	27:05 53:01	01:02:01 04:01:01	1*01:01 1*13:02 3*03:01	n.d.	1*05:01:01 1*06:04:01	n.d.	1*04:01 1*04:02
1304	+	01 11	08 15:01	03:03 07:01	1*03:01 1*11:01 3*01:01 3*02:02	1*05:01:01	1*02:01 1*03:01	n.d.	1*02:01 1*04:01
1318	–	02:05 03:01	50:01	n.d.	1*03:01 1*07:01 3*02:02 4*01:03	1*02:01 1*05:01	1*02:01 1*02:02	n.d.	1*01:01 1*02:01
1321[b]	n.d.	11:01 29:02	35:01 45:01	04:01 06:02	1*07:01 1*14:54	n.d.	1*02:02 1*05:03	n.d.	1*02:AFCTE 1*04:02
1613	–	02:01 25:01	18:01 35:01	04:01 12:03	1*11:01 1*15:01 3*02:02 5*01:01	1*01:02 1*05:09	1*03:01 1*06:02	n.d.	1*03:01 1*23:01

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Table 2.2. HLA type and HCMV serostatus of PBMC donors (continued from previous page)

Donor	HCMV	HLA							
		A*	**B***	**C***	**DRB**	**DQA**	**DQB**	**DPA**	**DPB**
1826	+	02:01 29:02	44:02 45:01	06:02	1*04:03 1*15:01 4*01:03 5*01:01	1*01:02 1*03:01	1*03:02 1*06:02	1*01:03	1*02:01 1*03:01
1907	+	01:01 29:02	08:01 44:03	07:01 16:01	1*13:01 1*15:01 3*01:01 5*01:01	n.d.	1*06:02 1*06:03	n.d.	1*02:01 1*04:01
1918	n.d.	01:KTKP 02:05	37:01:01 49:01:01	06:CKP 07:CWUD	1*04:01:01 1*11:02:01 3*02:02 4*01:03	n.d.	1*03:ENWH 1*03:ENWH	n.d.	n.d.

2.3.2 Cell lines

All human cell lines used in this work were available at the Department of Gene Vectors (AGV) of the Helmholtz Zentrum München (Germany). A description of each cell line including HLA typing is given in table 2.3. Mini-lymphoblastoid cell lines (mini-LCLs), T cell clones and T cell lines derived from PBMCs, which were isolated from donors listed in table 2.2, are not included in table 2.3.

T cell clones specific for HCMV antigens pp65 or IE-1 were established by Andreas Moosmann, Martina Wiesner (2005), Andrea Schub (2010) and Stefanie Ameres (2012) from PBMCs of the blood donors shown in table 2.2 (also see section 3.3.5). The nomenclature of these T cell clones indicates the anonymised donor by three to six digits and letters, followed by P or M depending on the target antigen (pp65 or IE-1, respectively), the number of the T cell clone and a three ($CD8^+$ T cell clone) or four letter ($CD4^+$ T cell clone) abbreviation of the recognised peptide in single letter amino acid code. Thus, 1220M #22 EFFT is a $CD4^+$ T cell clone derived from donor 1220 recognising the peptide `EFFT KNSA FPKT TNG` of IE-1 (see section 2.6). If the clone was established from PBMCs of a buffy coat, the sample was coded either by "F" and a number or by XYBC#, with XY being the preparator and # a number.

For the generation of **mini-lymphoblastoid cell lines** (mini-LCL), PBMCs were infected with a mini-Epstein-Barr Virus (mini-EBV), as described in Moosmann et al. (2002). This mini-EBV contains a reduced set of viral genes, but harbours all necessary viral genes and elements for transformation of primary B cells (Kempkes et al., 1995, plasmid 1495.A). In order to generate mini-LCLs that stably express HCMV's pp65 or immediate-early (IE) protein 1, the mini-EBV 1495.A was genetically modified to additionally express the cDNA sequence of either pp65 (Moosmann et al., 2002, plasmid 4014) or IE-1 (Wiesner, 2005, plasmid 3457) under the

simian virus 40 (SV40) promoter derived from the expression plasmid pSG-5 (Stratagene, La Jolla, USA). Mini-LCLs were assigned code names in addition to the donor code: “P” for pp65, “M” for IE-1 and “W” for negative control (no transgene).

Table 2.3. Description and HLA type of other cell lines. Some HLA alleles were not determined (“n.d.”). Ambiguities of HLA alleles that were not resolved by sequencing are presented as HLA allele codes as published by the National Marrow Donor Program (NMDP, see https://bioinformatics.bethematchclinical.org/hla/alpha.v3.html, accessed on 18.01.2019): FE = 01/02/04/05/06/08/09.

Name	Description	HLA						
		A*	B*	C*	DRB	DQA	DQB	DPB
DG-75	human Burkitt’s lymphoma B cell line (derived from metastatic site)	02:01 66:01	41:01 50:01	16:02 17	1*04:04 1*13:01 3*01:01 4*01:03	1*01:03 1*03:03	1*04:02 1*06:03	1*04:01
EBV-B1.11	EBV-transformed B cell line (provided by Josef Mautner, see Nimmerjahn et al., 2003)	02:01 66:01	18:01 41:02	12:03 17:01	1*13:03 1*15:01 3*01:01 5*01:01	1*01:02 1*05:FE	1*03:01 1*06:02	1*03:01 1*04:02
HEK293T	human embryonal kidney cells constitutively expressing the simian virus 40 large T antigen	03:01	07:02	07:02	1*15:01 5*01:01	n.d.	1*06:02	1*04:01
MRC-5	human embryonal fibroblasts (derived from normal lung tissue, see Jacobs et al., 1970)	02:01 29:02	07:02 44:02	05:01 07:02	1*04:08 1*15:01 4*01:03 5*01:01	1*01:02 1*03:03	1*03:01 1*06:02	n.d.
Raji	human Burkitt’s lymphoma B cell line, containing EBV (Pulvertaft, 1964)	03:01	15:10	03:04 04:01	1*03:01 1*10:01 3*02:02	1*01:05 1*05:01	1*02:01 1*05:01	1*01:01

2.3.3 Cell culture media and additives

The media and additives that were used for cultivation of human cells are shown in table 2.4. The cytokines that were used for stimulation, differentiation and cultivation of specific cell types are shown in table 2.5.

Table 2.4. Media and additives that were used for cultivation of human cells.

Name	Application	Manufacturer	Reference No. / Lot No.
Bambanker cryoprotectant (DMSO-based)	Cryopreservation	Nippon Genetics Europe (Düren, Germany)	BB01 / B-020318
DMEM	Cell culture medium for adherent cells (fibroblasts, HEK293T)	Life Technologies (Paisley, United Kingdom)	41966-029 / various
Fetal bovine serum (FBS)	Nutritive substance (used from 04/2018 until 07/2019)	Sigma-Aldrich (St. Louis, USA)	F7524 / BCBT7617
Fetal calf serum (FCS)	Nutritive substance (used from 08/2015 until 04/2018)	Bio&Sell (Feucht / Nuremberg, Germany)	FCS.SAM.0500 / BS225160.5
HEPES (1 M)	pH buffer	Life Technologies (Paisley, United Kingdom)	15630-056 / various
Human serum, off-the-clot, type AB, male	Nutritive substance	PAN Biotech (Aidenbach, Germany)	P40-2701 / P-020317
Non-essential amino acids (100x)	Nutritive substance	Life Technologies (Paisley, United Kingdom)	11140-035 / 1695997
Opti-MEM	Cell culture medium for transfection	Life Technologies (Paisley, United Kingdom)	31985-047 / various
Penicillin (10,000 U/ml) / Streptomycin (10,000 mg/µl)	Antibiotics, inhibition of bacterial growth	Life Technologies (Paisley, United Kingdom)	15140-122 / various
RPMI 1640	Standard cell culture medium	Life Technologies (Paisley, United Kingdom)	21875-034 / various
Sodium selenite (100 mM, sterile filtered)	Supplies an essential element (additive for RPMI 1640 and DMEM)	ICN Biochemicals (Aurora, USA)	not stated
Trypsin-EDTA (0.05%)	Detachment of adherent cells	Life Technologies (Paisley, United Kingdom)	25300-54 / various

Table 2.5. Cytokines used in human cell culture.

Cytokine	Application	Manufacturer
Prostaglandin E_2 (PGE_2)	Maturation of DCs	Tocris Bioscience (Bristol, UK)
Recombinant human GM-CSF(Leukine, sargramostim)	Establishment of DCs	Partner Therapeutics Inc. (Lexington, USA)
Recombinant human IFN-γ	Cultivation of fibroblasts (enhancement of MHC expression)	Miltenyi Biotec (Bergisch Gladbach, Germany)
Recombinant human IL-1β	Maturation of DCs	Miltenyi Biotec (Bergisch Gladbach, Germany)
Recombinant human IL-2 (Proleukin S)	Cultivation of T cells	Novartis (Nürnberg, Germany)
Recombinant human IL-4	Establishment of DCs	R&D Systems (Minneapolis, USA)
Recombinant human IL-6	Maturation of DCs	Miltenyi Biotec (Bergisch Gladbach, Germany)
Recombinant human IL-7	Cultivation of T cells	Miltenyi Biotec (Bergisch Gladbach, Germany)
Recombinant human IL-15	Cultivation of T cells	Miltenyi Biotec (Bergisch Gladbach, Germany)
Recombinant human TNF-α	Maturation of DCs	Miltenyi Biotec (Bergisch Gladbach, Germany)

2.4 Plasmids

All plasmids containing the cDNA of a recombinant HLA allele that were used in this study are shown in table 2.6 and were kindly provided by Josef Mautner (Helmholtz Zentrum München, Germany). The expression of the HLA allele was under the control of the immediate-early enhancer and promoter of HCMV (pCMV).

Tables 2.7 and 2.8 show all plasmids that were used for the generation of MP71 retroviral vectors (see section 3.5.1), which carried different genetic cargo, such as genes coding for CD4, CD8αβ, GFP or TCRα and TCRβ chains.

Table 2.6. Expression plasmids encoding recombinant HLA class II molecules, all of which are controlled by the immediate-early enhancer and promoter of HCMV (pCMV) and were kindly provided by Josef Mautner (Helmholtz Zentrum München, Germany).

Name (including encoded HLA)
pCMV-HLA-DRA1*0101
pCMV-HLA-DRB1*1301
pCMV-HLA-DRB1*1302
pCMV-HLA-DRB3*0101
pCMV-HLA-DRB3*0202
pCMV-HLA-DRB3*0301
pCMV-HLA-DRB5*0101
pCMV-HLA-DQA1*0101
pCMV-HLA-DQA1*0103
pCMV-HLA-DQA1*0501
pCMV-HLA-DQB1*0301
pCMV-HLA-DQB1*0501
pCMV-HLA-DQB1*0601

Table 2.7. Plasmids used for the generation of MP71 retroviral vectors carrying GFP, CD4 or CD8 as genetic cargo. Plasmids that carry a TCRβ–P2A–TCRα construct instead can be found in table 2.8.

Name	Description
pALF-10A1 (env)	Vector for the eukaryotic expression of viral envelope protein of the murine leukemia virus (MuLV) 10A1 (kindly provided by Stitz et al., 2000; Uckert et al., 2000).
pcDNA3.1MLVg/p (gag/pol)	Vector for the eukaryotic expression of group-specific antigens and polymerases of the Mo-MLV retrovirus (Leisegang et al., 2008, originally produced by Christopher Baum (Hannover, Germany)). Kindly provided by Wolfgang Uckert (Berlin, Germany).
MP71Gpre	Retroviral vector with GFP marker gene under MPSV-LTR control. Additionally, a posttranscriptional regulatory element (PRE) of the woodchuck hepatitis virus and a modified mRNA splice site (mSS) have been added (Engels et al., 2003).
MP71-CD4	Retroviral vector based on MP71Gpre, of which the GFP marker gene was replaced by cDNA coding for CD4 (derived from UniProt.org, accession number P01730, accessed 06.03.2017).
MP71-CD8_FYK	Retroviral vector based on MP71Gpre, of which the GFP marker gene was replaced by cDNA coding for CD8α (derived from UniProt.org, acession number P01732, isoform 1, accessed 06.03.2017) and CD8β (derived from UniProt.org, accession number P10966 (accessed 06.03.2017), isoform 1, which is identical to NCBI Gene ID 926 (accessed 06.03.2017), isoform 5, also see Thakral et al. (2008, M-1)), linked by P2A.

Table 2.8. Plasmids encoding for HCMV-specific TCRα and TCRβ chains based on MP71Gpre. The original GFP marker gene of MP71Gpre (see table 2.7) was replaced by cDNA coding for a TCRβ–P2A–TCRα construct (see section 3.2.3). For each construct, the table shows the parental T cell clone, from which the TCRα and TCRβ chains were isolated, and its specificity. The epitope is abbreviated by the first three or four amino acids of the peptide that is recognised by the $CD8^+$ or $CD4^+$ T cell clone, respectively. The origin of each T cell clone is displayed in the last column. Also, if TCR sequences have been identified or modified by someone else, it is indicated in the last column.

Name	parental T cell clone	HCMV antigen	Peptide number	Epitope (abbreviated)	Reference/Origin
MP71pre/AGIL19	F46P #19 and #45	pp65	123	AGIL	T cell clones from Wiesner (2005)
MP71pre/EFFT22	1220M #22	IE-1	54	EFFT	T cell clone from Ameres (2012)
MP71pre/EFFT50	1826M #50	IE-1	54	EFFT	T cell clone from Ameres (2012)
MP71pre/EIMA236	0664M #236	IE-1	62	EIMA	T cell clone from Ameres (2012)
MP71pre/EPDV21	1304P #21	pp65	45	EPDV	T cell clone and TCRβ sequence from A. Schub (2010, plasmid 4054), incorrectly labelled “KVYL”
MP71pre/KYQE16	0662P #16	pp65	128	KYQE	T cell clone from Wiesner (2005)
MP71pre/MSIY40	F46P #40	pp65	28	MSIY	T cell clone from Wiesner (2005)
MP71pre/QPFM17	F46P #17	pp65	66	QPFM	T cell clone from Wiesner (2005) and TCRα & TCRβ sequences from A. Schub (2010, plasmids 4051 and 4052), incorrectly labelled “RPHE”
MP71pre/RRKM18	0665M #18	IE-1	51 & 124	RRKM & KRKM	T cell clone from Ameres (2012)
MP71pre/SVMK6	1021M #6 and #90	IE-1	85	SVMK	T cell clones from Ameres (2012)
MP71pre/SVMK27	1826M #27	IE-1	85	SVMK	T cell clone from Ameres (2012)
MP71pre/YRIQ18	F46P #18	pp65	93 & 94	FTSQ & YRIQ	T cell clone from Wiesner (2005)
MP71pre/YRIQ36	F46P #36	pp65	93 & 94	FTSQ & YRIQ	T cell clone from Wiesner (2005)
MP71pre/CRV1	F46M #165	IE-1	—	CRV	T cell clone and TCRα & TCRβ sequences from X. Liang (unpublished)

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Table 2.8. Retroviral vectors expressing HCMV-specific TCRα and TCRβ chains based on the plasmid MP71Gpre (continued from previous page)

Name	parental T cell clone	HCMV antigen	Peptide number	Epitope (abbreviated)	Reference/Origin
MP71pre/CRV1mu	Same as MP71pre/CRV1, but C region has been replaced by the murine counterpart				Murinisation by F. Lorenz (MDC, Berlin)
MP71pre/CRV2	1021M #75	IE-1	—	CRV	T cell clone and TCRα & TCRβ sequences from X. Liang (unpublished)
MP71pre/CRV2mu	Same as MP71pre/CRV2, but C region has been replaced by the murine counterpart				Murinisation by F. Lorenz (MDC, Berlin)
MP71pre/CRV2α	Same as MP71pre/CRV2, but only coding for TCRα chain				
MP71pre/CRV2β	Same as MP71pre/CRV2, but only coding for TCRβ chain				
MP71pre/CRV3	F46M #36	IE-1	—	CRV	T cell clone from X. Liang (unpublished)
MP71pre/CRV4	1021M #169	IE-1	—	CRV	T cell clone from X. Liang (unpublished)
MP71pre/FRC1	XLBC13 #1, #5, #8 and #13	UL28	—	FRC	T cell clone from X. Liang (unpublished)
MP71pre/FRC6	XLBC13 6#	UL28	—	FRC	T cell clone from X. Liang (unpublished)
MP71pre/FRC32	XLBC18 32#	UL28	—	FRC	T cell clone from X. Liang (unpublished)
MP71pre/FRC93	XLBC18 #93 and #94	UL28	—	FRC	T cell clone from X. Liang (unpublished)
MP71pre/YSE	1304P #19	pp65	—	YSE	T cell clone and TCRα & TCRβ sequences from A. Schub (2010, plasmids 4045 and 4046)

2.5 Oligonucleotides

All oligonucleotides used in this study were synthesised by Metabion (Martinsried, Germany). Their sequences are displayed in tables 2.9 and 2.10.

2.5.1 Primers for the identification of T cell receptor chains

The primers shown in tables 2.9 and 2.10 were used for amplifying parts of the cDNA that coded for **TCRα** or **TCRβ** chains. The primers shown in table 2.9 have been synthesised as listed by Schuster (2008) and Schub (2010), who derived the primer sequences from Steinle et al. (1995) and Zhou et al. (2006). Steinle et al. (1995) stated that they had deduced the sequences of the primers for Vα1 to Vα12 and Vα17 to Vα21 from Davies et al. (1991). However, no primers for Vα19 to Vα21 were listed in that publication and their Vα8 primer (`GGAGAGAATGTGGAGCAG-CATC`, binding nucleotide positions 61 to 82 of TRAV13) was different from the one used by

Schuster (2008) and Schub (2010, TGTGGCTGCAGGTGGACT, binding nucleotide positions 32 to 49 of TRAV13). The origin of the latter primer could not be determined, but this primer was found to encompass an error at nucleotide position 12 of its sequence (5' → 3') showing G instead of C (see section 4.3.2.2), as verified by using the primer TRAV13-2 (tab. 2.10) and comparing the PCR product to IMGT's gene tables of TRAV13-2 (Scaviner and Lefranc, 2000b). Therefore, the primer was corrected ("Vα8corr", G→C in position 12, see table 2.9, marked in red) replacing the erroneous Vα8 primer. The primer names refer to the TCR nomenclature by Arden et al. (1995).

Table 2.9. Primers for identification of T cell receptor chains by PCR as listed by Schuster (2008) and Schub (2010) (see original publications Davies et al., 1991; Steinle et al., 1995; Zhou et al., 2006)**.** Nomenclature of TCR chains is according to Arden et al. (1995). Nucleobases are abbreviated as A = adenosine, C = cytidine, G = guanosine, I = inosine and T = thymidine. Degenerate bases ("wobbles") are displayed as K = G or T, M = A or C, R = A or G, S = C or G, W = A or T and Y = C or T. The original primer Vα8 was replaced by Vα8corr (see description above).

PCR	Name	Sequence (5' —> 3')
TCR Vα	Pan Vα1	AGAGCCCAGTCTGTGASCCAG
	Pan Vα1.1	AGAGCCCAGTCRGTGACCCAG
	Vα2	GTTTGGAGCCAACRGAAGGAG
	Vα3	GGTGAACAGTCAACAGGGAGA
	Vα4	TGATGCTAAGACCACMCAGC
	Vα5	GGCCCTGAACATTCAGGA
	Vα6	GGTCACAGCTTCACTGTGGCTA
	Vα7	ATGTTTCCATGAAGATGGGAG
	Vα8corr	TGTGGCTGCAGCTGGACT
	Vα9	ATCTCAGTGCTTGTGATAATA
	Vα10	ACCCAGCTGCTGGAGCAGAGCCCT
	Vα11	AGAAAGCAAGGACCAAGTGTT
	Vα12	CAGAAGGTAACTCAAGCGCAGACT
	Vα13	GAGCCAATTCCACGCTGCG
	Vα14	CAGTCTCAACCAGAGATGTC
	Vα14.1	CAGTCCCAGCCAGAGATGTC
	Vα15	GATGTGGAGCAGAGTCTTTTC
	Vα16	TCAGCGGAAGATCAGGTCAAC
	Vα17	GCTTATGAGAACACTGCGT
	Vα18	GCAGCTTCCCTTCCAGCAAT
	Vα19	AGAACCTGACTGCCCAGGAA
	Vα20	CATCTCCATGGACTCATATGA
	Vα21	GTGACTATACTAACAGCATGT
	Vα22	TACACAGCCACAGGATACCCTTCC
	Vα23	TGACACAGATTCCTGCAGCTC
	Vα24	GAACTGCACTCTTCAATGC
	Vα25	ATCAGAGTCCTCAATCTATGTTTA
	Vα26	AGAGGGAAAGAATCTCACCATAA
	Vα27	ACCCTCTGTTCCTGAGCATG
	Vα28	CAAAGCCCTCTATCTCTGGTT

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Table 2.9. Primers for identification of T cell receptor chains by PCR (continued from previous page)

PCR	Name	Sequence (5' —> 3')
TCR Vα	Vα29	AGGGGAAGATGCTGTCACCA
	Vα30	GAGGGAGAGAGTAGCAGT
	Vα31NEU	TCGGAGGGAGCATCTGTGACTA
	Vα32	CAAATTCCTCAGTACCAGCA
	3'T-Cα	GGTGAATAGGCAGACAGACTTGTCACTGGA
TCR Cα internal control	P-5'αST	CTGTGCTAGACATGAGGTCT
	P-3'αST	CTTGCCTCTGCCGTGAATGT
TCR Vβ	VP1	GCIITKTIYTGGTAYMGACA
	VP2	CTITKTWTTGGTAYCIKCAG
	CP1	GCACCTCCTTCCCATTCAC

2.6 Peptides

All peptides used in this study were synthesised by JPT (Berlin, Germany) to >70% purity. Peptides were resuspended in 100% dimethy sulfoxide (DMSO) at various concentrations ranging from 1 to 12 mg/ml and stored at −20 °C. For pulsing target cells, peptide concentrations of 1 or 5 µg/ml were used. Peptides used in this study are **YSEHPTFTSQY** ($pp65_{363\text{-}373}$), **CRVLCCYVL** ($IE\text{-}1_{309\text{-}317}$), **FRCPRRFCF** ($UL28_{327\text{-}335}$) or listed in tables 2.11 and 2.12. Peptides are abbreviated by their first three or four amino acids in single letter code indicating whether the peptide is recognised by an HLA class I-restricted TCR or an HLA class II-restricted TCR, respectively. In all T cell effector assays, DMSO was either washed away before co-cultivation of effector and target cells or the DMSO concentration was kept below 0.5% (vol/vol).

For identifying the specificity of pp65- or IE-1-specific T cells, two separate **peptide libraries** were used. Each library contains of a set of peptides that span the entire sequence either of the

Table 2.10. Primers for resolving ambiguity of V region alleles of T cell receptor chains by PCR. The primers listed below were designed using MacVector to produce an amplicon that covers more of the V region than the primers of table 2.9 by binding the cDNA further upstream (i.e. closer to the 5' end). Nomenclature of TCR chains is according to IMGT (Lefranc et al., 1999). The primer names (left column) also show which ambiguities they were designed to resolve. The last column shows which nucleotide positions ("nt pos.") of the respective V region are bound by each primer counting from the first nucleotide of the start codon.

Primer name	Sequence (5' —> 3')	Binding nt pos.
TRAV_9-2*01/*02	TCTCCAGGCTTAGTATCTCTGA	10-32
TRAV13-2	GCAGGCATTCGAGCTTTATT	4-24
TRAV_38-1*01/*04	ACGAGTTAGCTTGCTGTGGG	6-26
TRBV_7-9*01/*02/*03	AGCCTCCTCTGCTGGATG	10-27
TRBV11-2*01_forward	GGGAGCAGAACTCACAGAAG	42-61
TRBV_20-1*01/*02	CTGCTTCTGCTGCTTCTGG	7-25
TRBV25-1*01_forward	TGACTATCAGGCTCCTCTGC	2-21

Table 2.11. The IE-1 peptide library consists of 129 peptides and covers the whole amino acid sequence of HCMV's immediate-early (IE) protein 1, strain AD169 (Peptides no. 1 to 120), and some sequence variants of strains Toledo and TB40. From no. 1 to 120, each peptide is 15 amino acids long and overlaps with the last 11 amino acids of the previous peptide. The peptides no. 121 to 129 contain sequence variants that are found in HCMV strains Toledo and TB40. Each peptide was dissolved in 100% DMSO to 12 mg/ml. Modified from Ameres (2012).

No.	Sequence	No.	Sequence	No.	Sequence
1	MESS AKRK MDPD NPD	44	ELHD VSKG AANK LGG	87	EICM KVFA QYIL GAD
2	AKRK MDPD NPDE GPS	45	VSKG AANK LGGA LQA	88	KVFA QYIL GADP LRV
3	MDPD NPDE GPSS KVP	46	AANK LGGA LQAK ARA	89	QYIL GADP LRVC SPS
4	NPDE GPSS KVPR PET	47	LGGA LQAK ARAK KDE	90	GADP LRVC SPSV DDL
5	GPSS KVPR PETP VTK	48	LQAK ARAK KDEL RRK	91	LRVC SPSV DDLR AIA
6	KVPR PETP VTKA TTF	49	ARAK KDEL RRKM MYM	92	SPSV DDLR AIAE ESD
7	PETP VTKA TTFL QTM	50	KDEL RRKM MYMC YRN	93	DDLR AIAE ESDE EEA
8	VTKA TTFL QTML RKE	51	RRKM MYMC YRNI EFF	94	AIAE ESDE EEAI VAY
9	TTFL QTML RKEV NSQ	52	MYMC YRNI EFFT KNS	95	ESDE EEAI VAYT LAT
10	QTML RKEV NSQL SLG	53	YRNI EFFT KNSA FPK	96	EEAI VAYT LATA GVS
11	RKEV NSQL SLGD PLF	54	EFFT KNSA FPKT TNG	97	VAYT LATA GVSS SDS
12	NSQL SLGD PLFP ELA	55	KNSA FPKT TNGC SQA	98	LATA GVSS SDSL VSP
13	SLGD PLFP ELAE ESL	56	FPKT TNGC SQAM AAL	99	GVSS SDSL VSPP ESP
14	PLFP ELAE ESLK TFE	57	TNGC SQAM AALQ NLP	100	SDSL VSPP ESPV PAT
15	ELAE ESLK TFEQ VTE	58	SQAM AALQ NLPQ CSP	101	VSPP ESPV PATI PLS
16	ESLK TFEQ VTED CNE	59	AALQ NLPQ CSPD EIM	102	ESPV PATI PLSS VIV
17	TFEQ VTED CNEN PEK	60	NLPQ CSPD EIMA YAQ	103	PATI PLSS VIVA ENS
18	VTED CNEN PEKD VLA	61	CSPD EIMA YAQK IFK	104	PLSS VIVA ENSD QEE
19	CNEN PEKD VLAE LVK	62	EIMA YAQK IFKI LDE	105	VIVA ENSD QEES EQS
20	PEKD VLAE LVKQ IKV	63	YAQK IFKI LDEE RDK	106	ENSD QEES EQSD EEE
21	VLAE LVKQ IKVR VDM	64	IFKI LDEE RDKV LTH	107	QEES EQSD EEEE EGA
22	LVKQ IKVR VDMV RHR	65	LDEE RDKV LTHI DHI	108	EQSD EEEE EGAQ EER
23	IKVR VDMV RHRI KEH	66	RDKV LTHI DHIF MDI	109	EEEE EGAQ EERE DTV
24	VDMV RHRI KEHM LKK	67	LTHI DHIF MDIL TTC	110	EGAQ EERE DTVS VKS
25	RHRI KEHM LKKY TQT	68	DHIF MDIL TTCV ETM	111	EERE DTVS VKSE PVS
26	KEHM LKKY TQTE EKF	69	MDIL TTCV ETMC NEY	112	DTVS VKSE PVSE IEE
27	LKKY TQTE EKFT GAF	70	TTCV ETMC NEYK VTS	113	VKSE PVSE IEEV APE
28	TQTE EKFT GAFN MMG	71	ETMC NEYK VTSD ACM	114	PVSE IEEV APEE EED
29	EKFT GAFN MMGG CLQ	72	NEYK VTSD ACMM TMY	115	IEEV APEE EEDG AEE
30	GAFN MMGG CLQN ALD	73	VTSD ACMM TMYG GIS	116	APEE EEDG AEEP TAS
31	MMGG CLQN ALDI LDK	74	ACMM TMYG GISL LSE	117	EEDG AEEP TASG GKS
32	CLQN ALDI LDKV HEP	75	TMYG GISL LSEF CRV	118	AEEP TASG GKST HPM
33	ALDI LDKV HEPF EEM	76	GISL LSEF CRVL CCY	119	TASG GKST HPMV TRS
34	LDKV HEPF EEMK CIG	77	LSEF CRVL CCYV LEE	120	GKST HPMV TRSK ADQ
35	HEPF EEMK CIGL TMQ	78	CRVL CCYV LEET SVM	121	LKAK ARAK KDEL KRK
36	EEMK CIGL TMQS MYE	79	CCYV LEET SVML AKR	122	ARAK KDEL KRKM IYM
37	CIGL TMQS MYEN YIV	80	LEET SVML AKRP LIT	123	KDEL KRKM IYMC YRN
38	TMQS MYEN YIVP EDK	81	SVML AKRP LITK PEV	124	KRKM IYMC YRNV EFF
39	MYEN YIVP EDKR EMW	82	AKRP LITK PEVI SVM	125	LTEF CRVL CCYI LEE
40	YIVP EDKR EMWM ACI	83	LITK PEVI SVMK RRI	126	CRVL CCYI LEET SVL
41	EDKR EMWM ACIK ELH	84	PEVI SVMK RRIE EIC	127	CCYI LEET SVLL AKR
42	EMWM ACIK ELHD VSK	85	SVMK RRIE EICM KVF	128	LEET SVLL AKRP LIT
43	ACIK ELHD VSKG AAN	86	RRIE EICM KVFA QYI	129	ESDE EDAI AAYT LAT

Table 2.12. The pp65 peptide library consists of 138 peptides and covers the whole amino acid sequence of HCMV's phosphoprotein 65 (pp65), strain AD169. Each peptide is 15 amino acids long and overlaps with the last 11 amino acids of the previous peptide (Kern et al., 2002). Each peptide was dissolved in 100% DMSO to 4 mg/ml. Modified from Schub (2010).

No.	Sequence	No.	Sequence	No.	Sequence
1	MESR GRRC PEMI SVL	47	AFVF PTKD VALR HVV	93	FTSQ YRIQ GKLE YRH
2	GRRC PEMI SVLG PIS	48	PTKD VALR HVVC AHE	94	YRIQ GKLE YRHT WDR
3	PEMI SVLG PISG HVL	49	VALR HVVC AHEL VCS	95	GKLE YRHT WDRH DEG
4	SVLG PISG HVLK AVF	50	HVVC AHEL VCSM ENT	96	YRHT WDRH DEGA AQG
5	PISG HVLK AVFS RGD	51	AHEL VCSM ENTR ATK	97	WDRH DEGA AQGD DDV
6	HVLK AVFS RGDT PVL	52	VCSM ENTR ATKM QVI	98	DEGA AQGD DDVW TSG
7	AVFS RGDT PVLP HET	53	ENTR ATKM QVIG DQY	99	AQGD DDVW TSGS DSD
8	RGDT PVLP HETR LLQ	54	ATKM QVIG DQYV KVY	100	DDVW TSGS DSDE ELV
9	PVLP HETR LLQT GIH	55	QVIG DQYV KVYL ESF	101	TSGS DSDE ELVT TER
10	HETR LLQT GIHV RVS	56	DQYV KVYL ESFC EDV	102	DSDE ELVT TERK TPR
11	LLQT GIHV RVSQ PSL	57	KVYL ESFC EDVP SGK	103	ELVT TERK TPRV TGG
12	GIHV RVSQ PSLI LVS	58	ESFC EDVP SGKL FMH	104	TERK TPRV TGGG AMA
13	RVSQ PSLI LVSQ YTP	59	EDVP SGKL FMHV TLG	105	TPRV TGGG AMAG AST
14	PSLI LVSQ YTPD STP	60	SGKL FMHV TLGS DVE	106	TGGG AMAG ASTS AGR
15	LVSQ YTPD STPC HRG	61	FMHV TLGS DVEE DLT	107	AMAG ASTS AGRK RKS
16	YTPD STPC HRGD NQL	62	TLGS DVEE DLTM TRN	108	ASTS AGRK RKSA SSA
17	STPC HRGD NQLQ VQH	63	DVEE DLTM TRNP QPF	109	AGRK RKSA SSAT ACT
18	HRGD NQLQ VQHT YFT	64	DLTM TRNP QPFM RPH	110	RKSA SSAT ACTS GVM
19	NQLQ VQHT YFTG SEV	65	TRNP QPFM RPHE RNG	111	SSAT ACTS GVMT RGR
20	VQHT YFTG SEVE NVS	66	QPFM RPHE RNGF TVL	112	ACTS GVMT RGRL KAE
21	YFTG SEVE NVSV NVH	67	RPHE RNGF TVLC PKN	113	GVMT RGRL KAES TVA
22	SEVE NVSV NVHN PTG	68	RNGF TVLC PKNM IIK	114	RGRL KAES TVAP EED
23	NVSV NVHN PTGR SIC	69	TVLC PKNM IIKP GKI	115	KAES TVAP EEDT DED
24	NVHN PTGR SICP SQE	70	PKNM IIKP GKIS HIM	116	TVAP EEDT DEDS DNE
25	PTGR SICP SQEP MSI	71	IIKP GKIS HIML DVA	117	EEDT DEDS DNEI HNP
26	SICP SQEP MSIY VYA	72	GKIS HIML DVAF TSH	118	DEDS DNEI HNPA VFT
27	SQEP MSIY VYAL PLK	73	HIML DVAF TSHE HFG	119	DNEI HNPA VFTW PPW
28	MSIY VYAL PLKM LNI	74	DVAF TSHE HFGL LCP	120	HNPA VFTW PPWQ AGI
29	VYAL PLKM LNIP SIN	75	TSHE HFGL LCPK SIP	121	VFTW PPWQ AGIL ARN
30	PLKM LNIP SINV HHY	76	HFGL LCPK SIPG LSI	122	PPWQ AGIL ARNL VPM
31	LNIP SINV HHYP SAA	77	LCPK SIPG LSIS GNL	123	AGIL ARNL VPMV ATV
32	SINV HHYP SAAE RKH	78	SIPG LSIS GNLL MNG	124	ARNL VPMV ATVQ GQN
33	HHYP SAAE RKHR HLP	79	LSIS GNLL MNGQ QIF	125	VPMV ATVQ GQNL KYQ
34	SAAE RKHR HLPV ADA	80	GNLL MNGQ QIFL EVQ	126	ATVQ GQNL KYQE FFW
35	RKHR HLPV ADAV IHA	81	MNGQ QIFL EVQA IRE	127	GQNL KYQE FFWD AND
36	HLPV ADAV IHAS GKQ	82	QIFL EVQA IRET VEL	128	KYQE FFWD ANDI YRI
37	ADAV IHAS GKQM WQA	83	EVQA IRET VELR QYD	129	FFWD ANDI YRIF AEL
38	IHAS GKQM WQAR LTV	84	IRET VELR QYDP VAA	130	ANDI YRIF AELE GVW
39	GKQM WQAR LTVS GLA	85	VELR QYDP VAAL FFF	131	YRIF AELE GVWQ PAA
40	WQAR LTVS GLAW TRQ	86	QYDP VAAL FFFD IDL	132	AELE GVWQ PAAQ PKR
41	LTVS GLAW TRQQ NQW	87	VAAL FFFD IDLL LQR	133	GVWQ PAAQ PKRR RHR
42	GLAW TRQQ NQWK EPD	88	FFFD IDLL LQRG PQY	134	PAAQ PKRR RHRY DAL
43	TRQQ NQWK EPDV YYT	89	IDLL LQRG PQYS EHP	135	PKRR RHRY DALP GPC
44	NQWK EPDV YYTS AFV	90	LQRG PQYS EHPT FTS	136	RHRY DALP GPCI AST
45	EPDV YYTS AFVF PTK	91	PQYS EHPT FTSQ YRI	137	DALP GPCI ASTP KKH
46	YYTS AFVF PTKD VAL	92	EHPT FTSQ YRIQ GKL	138	GPCI ASTP KKHR G

antigen pp65 or IE-1 of the HCMV strain AD169. Each of these peptides has a length of 15 amino acids and overlaps in 11 amino acids with the subsequent peptide.

The IE-1 peptide library (see table 2.11) is comprised of 120 peptides covering AD169's IE-1 sequence and nine additional peptides, which contain sequence variants that are found in HCMV strains Toledo and TB40 (peptides no. 121-129, see Ameres, 2012). For handling purposes, the 129 peptides were subpooled in a cross-matrix fashion as described by Kern et al. (1999) and depicted in table 2.13 so that every single peptide was contained within one of the vertical subpools (from 1 to 12) and within one horizontal subpool (from 13 to 23) at 1 mg/ml (Ameres, 2012).

The pp65 peptide library (see table 2.12) is comprised of 138 peptides covering AD169's pp65 sequence (Kern et al., 2002). Similar to the IE-1 peptides, the pp65 peptides were subpooled in a cross-matrix fashion so that every single peptide was contained within one of the vertical subpools (from 1 to 12) and within one horizontal subpool (from 13 to 24) at 0.33 mg/ml (see table 2.13; Kern et al., 2002; Schub, 2010).

Table 2.13. Vertical and horizontal peptide subpools of pp65 or IE-1 peptide libraries. All peptides either of the pp65 library (tab. 2.12) or the IE-1 peptide library (tab. 2.11) were pooled according to the scheme shown in this table. As a result, 12 vertical subpools (bold, numbered 1 to 12) and 12 horizontal subpools (bold, numbered 13 to 24) for pp65 were obtained. Separately, 12 vertical subpools and 11 horizontal subpools were created from the IE-1 peptide library. Each peptide pool contains 12 peptides, except for IE-1 pool no. 23 and pp65 pool no. 24, both of which contain fewer peptides. IE-1 and pp65 peptide numbers (non-bold) refer to the numbers shown in tables 2.11 and 2.12, respectively.

		Vertical subpool											
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Horizontal subpool	**13**	1	2	3	4	5	6	7	8	9	10	11	12
	14	13	14	15	16	17	18	19	20	21	22	23	24
	15	25	26	27	28	29	30	31	32	33	34	35	36
	16	37	38	39	40	41	42	43	44	45	46	47	48
	17	49	50	51	52	53	54	55	56	57	58	59	60
	18	61	62	63	64	65	66	67	68	69	70	71	72
	19	73	74	75	76	77	78	79	80	81	82	83	84
	20	85	86	87	88	89	90	91	92	93	94	95	96
	21	97	98	99	100	101	102	103	104	105	106	107	108
	22	109	110	111	112	113	114	115	116	117	118	119	120
	23	121	122	123	124	125	126	127	128	129	130	131	132
	24	133	134	135	136	137	138						

2.7 Antibodies and beads

Table 2.14. Antibodies used in this study. If not stated otherwise, the antibody is of murine origin.

Antigen (alternative designation)	Conjugated fluorochrome	Manufacturer	Catalogue No.	Clone	Concentration [µg/ml]	Applied dilution
CD3	Alexa Fluor 700	Biolegend (San Diego, USA)	300324	HIT3a	500.0	1:100
CD3	APC	Biolegend (San Diego, USA)	300312	HIT3a	25.0	1:50
CD3	PE	Biolegend (San Diego, USA)	300308	HIT3a	50.0	1:100
CD3	not labelled	Biolegend (San Diego, USA)	317304	OKT3	1,000.0	50 ng/ml
CD4	Alexa Fluor 488	Biolegend (San Diego, USA)	300519	RPA-T4	50.0	1:50
CD4	APC	Biolegend (San Diego, USA)	300514	RPA-T4	12.5	1:50
CD4	PE	Biolegend (San Diego, USA)	300514	RPA-T4	12.5	1:50
CD4	PerCp-Cy5.5	Biolegend (San Diego, USA)	300530	RPA-T4	100.0	1:50
CD8	Alexa Fluor 488	Biolegend (San Diego, USA)	300916	HIT8a	100.0	1:100
CD8	APC-Cy7	Biolegend (San Diego, USA)	301016	RPA-T8	200.0	1:250
CD8	Pacific Blue	Biolegend (San Diego, USA)	301033	RPA-T8	200.0	1:100
CD8	PerCp-Cy5.5	Biolegend (San Diego, USA)	301032	RPA-T8	200.0	1:100
CD11c	PerCp	Biolegend (San Diego, USA)	337233	Bu15	100.0	1:100
CD14	APC-Cy7	Biolegend (San Diego, USA)	301819	M5E2	400.0	1:100
CD14	BV605	Biolegend (San Diego, USA)	301834	M5E2	150.0	1:100
CD14	PE-CF594	BD Biosciences (Heidelberg, Germany)	562334	MΦP-9		1:100
CD19	BV605	Biolegend (San Diego, USA)	302244	HIB19	80.0	1:100

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Table 2.14. Antibodies (continued from previous page).

Antigen (alternative designation)	Conjugated fluorochrome	Manufacturer	Catalogue No.	Clone	Concentration [µg/ml]	Applied dilution
CD25	Pacific Blue	Biolegend (San Diego, USA)	356129	M-A251	50.0	1:50
CD45RA	APC	Biolegend (San Diego, USA)	304112	HI100	25.0	1:50
CD45RA	PE	Biolegend (San Diego, USA)	304108	HI100	1.5	1:50
CD62L	PE-Cy7	Biolegend (San Diego, USA)	304822	DREG-56	200.0	1:50
CD83	FITC	BD Biosciences (Heidelberg, Germany)	556910	HB15e		3:50
CD86	APC	Biolegend (San Diego, USA)	305412	IT2.2	50.0	1:50
CD158b (KIR2DL2/ L3)	FITC	BD Biosciences (Heidelberg, Germany)	559784	CH-L		3:50
CD158b (KIR2DL2/L3)	PE	Biolegend (San Diego, USA)	312606	DX27	50.0	2:50
CD159a (NKG2A)	APC	Beckman Coulter (Krefeld, Germany)	A60797	Z199		4:50
CD197 (CCR7)	Alexa Fluor 488	Biolegend (San Diego, USA)	353206	G043H7	400.0	1:50
CD314 (NKG2D)	PE-Cy7	Biolegend (San Diego, USA)	320812	1D11	200.0	3:100
HLA-DP	not labelled	Leinco Technologies (St. Louis, USA)	H260	B7/21	1,000.0	10 µg/ml
HLA-DQ	not labelled	Bio-Rad (München, Germany)	MCA 379G	SPV-L3	1,000.0	10 µg/ml
HLA-DR	not labelled	Biolegend (San Diego, USA)	307612	L243	1,000.0	40 µg/ml
TCRαβ	APC	Biolegend (San Diego, USA)	306718	IP26	100.0	1:50
TCR Vβ1	FITC	Beckman Coulter (Krefeld, Germany)	IM2406	BL37.2		1:50
TCR Vβ2	FITC	Beckman Coulter (Krefeld, Germany)	IM2407	MPB2D5		3:50
TCR Vβ3	FITC	Beckman Coulter (Krefeld, Germany)	IM2372	CH92		7:50

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Table 2.14. Antibodies (continued from previous page).

Antigen (alternative designation)	Conjugated fluorochrome	Manufacturer	Catalogue No.	Clone	Concentration [µg/ml]	Applied dilution
TCR Vβ5.1	FITC	Beckman Coulter (Krefeld, Germany)	IM1552	IMMU 157		1:10
TCR Vβ5.2	FITC	Beckman Coulter (Krefeld, Germany)	IM1482	36213		1:10
TCR Vβ11	PE	Beckman Coulter (Krefeld, Germany)	IM2290	C21		3:50
TCR Vβ13.1	PE	Beckman Coulter (Krefeld, Germany)	IM2292	IMMU 222		7:50
TCR Vβ13.2	PE	Beckman Coulter (Krefeld, Germany)	IM3603	H132		1:50
TCR Vβ17	PE	Beckman Coulter (Krefeld, Germany)	IM2048	E17.5F 3.15.13		1:10
TCR Vβ21.3	FITC	Beckman Coulter (Krefeld, Germany)	IM1483	IG125		3:50
TCR Vδ2	Pacific Blue	Biolegend (San Diego, USA)	331414	B6	100.0	1:100
TCR Vδ2	PerCp	Biolegend (San Diego, USA)	331410	B6	200.0	1:100

For immunomagnetic separation of cell populations, anti-human CD8 MicroBeads (Order No.: 130-045-201), anti-APC MicroBeads (Order No.: 130-090-855) or anti-FITC MicroBeads (Order No. 130-048-701) by Miltenyi Biotec (Bergisch Gladbach, Germany) have been used.
PE-labelled Calibrite beads (Cat.No. 349502) from BD Biosciences (Heidelberg, Germany) were used to normalise cell counts acquired by flow cytometry.

2.8 Major histocompatibility complex multimers

YSE/HLA-A*01:01 pentamers (unlabelled) and associated R-PE Pro5 fluorotags were purchased from ProImmune (Oxford, United Kingdom).
CRV/HLA-C*07:02 monomers and FRC/HLA-C*07:02 monomers were generated and provided by Fabian Schlott, Michael Neuenhahn and Dirk Busch (Institute for Medical Microbiology, Immunology and Hygiene, TUM, Munich, Germany; see Schlott et al., 2018). Peptide/HLA class I monomers were assembled using MHC OneSTrEPtag-Strep-Tactin (IBA Göttingen, Germany) to generate streptamers (see section 3.4.2 and Knabel et al., 2002; Neudorfer et al., 2007).

Recombinant peptide/HLA class II αβ complexes (hereafter referred to as peptide/HLA class II monomers) were purchased from Immunaware (Copenhagen, Denmark) and applied for staining

Table 2.15. Recombinant peptide/HLA class II monomers (purchased from Immunaware, Copenhagen, Denmark)**.**

Peptide	HLA class II αβ heterodimer	Concentration [nM]	
		of stock	applied
QPFM RPHE RNGF TVL	HLA-DRA1*01:01 / HLA-DRB1*13:02	1,675 nM	failed
YRIQ GKLE YRHT WDR	HLA-DRA1*01:01 / HLA-DRB1*13:02	1,800 nM	60 nM
AGIL ARNL VPMV ATV	HLA-DRA1*01:01 / HLA-DRB3*03:01	2,900 nM	30 nM
MSIY VYAL PLKM LNI	HLA-DRA1*01:01 / HLA-DRB1*15:01	1,600 nM	failed
EFFT KNSA FPKT T	HLA-DRA1*01:01 / HLA-DRB5*01:01	2,870 nM	30 nM

of T cells at concentrations indicated in table 2.15. For QPFM and MSIY monomers sub-optimal folding was reported by Immunaware (70 and 60%, respectively), and indeed, these monomers failed to detect QPFM- or MSIY-specific T cells after tetramerisation, respectively (see table 2.15 and section 4.4.2). Unfortunately, KRKM IYMC YRNV EFF/HLA-DRB3*02:02 monomers failed to fold entirely and were thus not available for staining of RRKM-specific T cells.
Peptide/HLA class II monomers were assembled using PE- (Cat.No. 554061) or APC-labelled streptavidin (Cat.No. 554067) from BD Biosciences (Heidelberg, Germany) to generate tetramers (see section 3.4.2 and Braendstrup et al., 2013, 2014).

2.9 Commercial kits

Table 2.16. Commercial kits.

Name	Manufacturer
Cytofix/Cytoperm	BD Biosciences (Heidelberg, Germany)
Endofree Plasmid Maxi Kit	Qiagen (Hilden, Germany)
HiPure Plasmid Maxiprep Kit	Thermo Fisher Scientific (Waltham, USA)
Human IFN-γELISA Kit (ALP)	Mabtech (Nacka Strand, Sweden)
Human IL-10 ELISA Kit (DuoSet)	R&D Systems (Minneapolis, USA)
NucleoSpin Gel and PCR Clean-up Kit	Macherey-Nagel (Düren, Germany)
QIAshredder	Qiagen (Hilden, Germany)
QuantiTect Reverse Transcription Kit	Qiagen (Hilden, Germany)
RNeasy Mini Kit	Qiagen (Hilden, Germany)

2.10 Chemicals, reagents, enzymes and buffers

Table 2.17. Chemicals, reagents, enzymes and buffers.

Name	Composition, concentration or size	Manufacturer or distributor
Ampicillin		Carl Roth (Karlsruhe, Germany)

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Table 2.17. Chemicals, reagents, enzymes and buffers (continued from previous page).

Name	Composition, concentration or size	Manufacturer or distributor
Aqua ad iniectabilia		Berlin-Chemie (Berlin, Germany)
Cell Trace Violet	5 mM	Thermo Fisher Scientific (Waltham, USA)
Comp Beads, anti-mouse Igκ		BD Biosciences (Heidelberg, Germany)
Dimethyl sulfoxide (DMSO)	≥99.8% p.a.	Carl Roth (Karlsruhe, Germany)
DNA agarose, ultra pure		Life Technologies (Paisley, United Kingdom)
DNA loading dye	6×	Thermo Fisher Scientific (Waltham, USA)
dNTPs	10 mM	Thermo Fisher Scientific (Waltham, USA)
ELISA washing buffer	0.05% Tween 20 in 1× PBS	Freshly produced
Ethanol	≥99.8% p.a.	Carl Roth (Karlsruhe, Germany)
Ethidium bromide	10 mg/ml	Carl Roth (Karlsruhe, Germany)
FACS buffer	2% FCS in 1× PBS	Freshly produced
FACS Clean		BD Biosciences (Heidelberg, Germany)
FACS Flow		BD Biosciences (Heidelberg, Germany)
FACS fixation solution	2% PFA in 1× PBS	Freshly produced or stored at 4 °C
GeneRuler DNA Ladder Mix	1 kb	Fermentas (St. Leon-Rot, Germany)
HCl	≥32%	Merck (Darmstadt, Germany)
Heparin-Natrium	5,000 I E /ml	Rotexmedica (Trittau, Germany)
Ionomycin calcium salt		Tocris Bioscience (Bristol, UK)
Isopropanol	99.9% p.a.	Carl Roth (Karlsruhe, Germany)
$MgSO_4$	25 mM	Thermo Fisher Scientific (Waltham, USA)
NaOH	≥99% p.a.	Carl Roth (Karlsruhe, Germany)
Pamifos (Pamidronate disodium salt), Lot No. K140746E	3 mg/ml	medac (Wedel, Germany)
Pancoll	1.077 g/ml	PAN Biotech (Aidenbach, Germany)
Paraformaldehyde		Carl Roth (Karlsruhe, Germany)
Para-nitrophenyl phosphate (pNPP)		Carl Roth (Karlsruhe, Germany)

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Table 2.17. Chemicals, reagents, enzymes and buffers (continued from previous page).

Name	Composition, concentration or size	Manufacturer or distributor
Para-nitrophenyl phosphate (pNPP)	5× (50% diethanolamine and 5 mg/ml pNPP in sterile water at pH 9.5)	Freshly produced or stored at −20 °C
PBS Dulbecco (without Mg_2^+ & Ca_2^+)	1×, dissolved	Sigma-Aldrich (St. Louis, USA)
PBS Dulbecco (without Mg_2^+ & Ca_2^+)	10×, solid	AppliChem (Darmstadt, Germany)
Protamine sulfate		MP Biomedicals (Illkirch, France)
Protamine sulfate solution	1 mg/ml in sterile H_2O	Freshly produced or stored at 4 °C
RetroNectin	1 mg/ml	Takara Bio Europe (St. Germain en Laye, France)
RetroNectin solution	12.5 µg/ml in sterile H_2O	Freshly produced or stored at −20 °C
Rnase ZAP		Sigma-Aldrich (St. Louis, USA)
TAE (Tris, acetate, EDTA)	10×	Carl Roth (Karlsruhe, Germany)
Taq reaction buffer	10×	New England BioLabs (Ipswich, USA)
Taq DNA polymerase	5,000 U/ml	New England BioLabs (Ipswich, USA)
Tetradecanoylphorbol acetate (TPA)		Sigma-Aldrich (St. Louis, USA)
TransIT-293 transfection reagent		Mirus Bio LLC (Madison, USA)
Tris/HCl	1M	Carl Roth (Karlsruhe, Germany)
Trypan blue		Merck (Darmstadt, Germany)
Tween 20		Carl Roth (Karlsruhe, Germany)

2.11 Consumables

Table 2.18. Consumables.

Name	Brand name, concentration or size	Manufacturer
Cell culture flask	25, 75 or 175 cm^2	Nunc A/S (Roskilde, Denmark)
Cell culture multiwell plate, flat bottom	MaxiSorp Immunoplate, 96-wells	Nunc A/S (Roskilde, Denmark)

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Table 2.18. Consumables (continued from previous page).

Name	Brand name, concentration or size	Manufacturer
Cell culture multiwell plate, flat-bottom	6-, 12-, 48- or 96-wells	Corning Inc. (Corning, USA)
Cell culture multiwell plate, round-bottom	96-wells	Nunc A/S (Roskilde, Denmark)
Cell culture multiwell plate, V-bottom	96-wells	Hartenstein (Würzburg, Germany)
Cell scraper	20 mm width, 30 cm length	TPP Techno Plastic Products (Trasadingen, Switzerland)
Cell strainer	Falcon, 100 µm	Corning Inc. (Corning, USA)
Counting chamber	Neubauer Improved	NanoEnTek (Seoul, Korea)
Cryo tube vial	1.8 ml	Nunc A/S (Roskilde, Denmark)
Electroporation cuvette	GenePulser, 0.4 cm	Bio-Rad (München, Germany)
MACS column	LD	Miltenyi Biotec (Bergisch Gladbach, Germany)
Needle	Multifly, 21G, 240 mm	Sarstedt (Nümbrecht, Germany)
Pasteur pipette (sterile, single use)	3.2 ml	Carl Roth (Karlsruhe, Germany)
Petri dishes	60×15 or 100×20 mm	Corning Inc. (Corning, USA)
Pipette tip	Diamond TowerPack, 2–200 µl or 100–1,000 µl	Gilson Inc. (Middleton, USA)
Pipette tip	epT.I.P.S. Reloads 0.1–10 µl	Eppendorf (Hamburg, Germany)
Pipette tip with filter	MultiGuard Barrier Tips, 10 µl, 20 µl, 200 µl or 1,000 µl	Sorenson BioScience (Salt Lake City, USA)
Plastic pipette	5, 10 or 25 ml	Greiner Bio-One (Kremsmünster, Austria)
Reagent reservoir	Costar, 50 ml	Corning Inc. (Corning, USA)
Reaction tube	0.5, 1.5 or 2.0 ml	Eppendorf (Hamburg, Germany)
Reaction tube for PCR	0.2 µl single tubes or stips	Thermo Fisher Scientific (Waltham, USA)
Syringe	Infuject, 50 ml	Dispomed Witt oHG (Gelnhausen, Germany)
Syringe filter	0.45 µm, SFCA	Thermo Fisher Scientific (Waltham, USA)
Syringe with needle	1 ml, 25G	Terumo (Binan, Philippines)
Tube, conical	Falcon, 15 or 50 ml	Corning Inc. (Corning, USA)
Tube, round-bottom	Falcon, 5 ml (Polystyrene)	Corning Inc. (Corning, USA)

2.12 Laboratory equipment and devices

Table 2.19. Laboratory equipment and devices.

Device / Equipment	Manufacturer / Distributor
Bacteria incubator/shaker innova 4400	New Brunswick Scientific (Enfield, USA)
Centrifuge Avanti J-26 XP	Beckman Coulter (Krefeld, Germany)
Centrifuge 5415 R	Eppendorf (Hamburg, Germany)
Centrifuge Heraeus Pico 21	Thermo Fisher Scientific (Waltham, USA)
Centrifuge Rotanta 46 RSC	Hettich (Bäch, Switzerland)
Centrifuge Rotanta 460 R	Hettich (Bäch, Switzerland)
Centrifuge Rotina 38 R	Hettich (Bäch, Switzerland)
Counting chamber Neubauer Improved	Paul Marienfeld (Lauda-Königshofen, Germany)
Cytofluorometer Canto	BD Biosciences (Heidelberg, Germany)
Cytofluorometer LSR Fortessa	BD Biosciences (Heidelberg, Germany)
Electroporation system GenePulser II	Bio-Rad (München, Germany)
Freezer −20 °C	Liebherr (Biberach an der Riss, Germany)
Freezer −80 °C Igloo Green Line 830L	Telstar (Terrassa, Spain)
Fridge 2–8 °C	Bosch (Gerlingen-Schillerhöhe, Germany)
Gel documentation system Quantum ST4-1100/26LMX	Vilber Lourmat (Eberhardzell, Germany)
Gel electrophoresis chambers	Peqlab Biotechnologie (Erlangen, Germany)
Gel electrophoresis power supply PowerPac 200	Bio-Rad (München, Germany)
Glassware	Schott (Mainz, Germany)
Ice machine AF 206	Scotsman (Milan, Italy)
Incubator	Binder (Tuttlingen, Germany)
Irradiator CIX2, X-ray	xstrahl (Camberley, United Kingdom)
Irradiator Gammacell 40, ^{137}Cs	Atomic Energy of Canada Limited (Ottawa, Canada)
Laminar flow hoods	BDK (Sonnenbühl-Genkingen, Germany)
MACS MultiStand	Miltenyi Biotec (Bergisch Gladbach, Germany)
MACS separator Quadro	Miltenyi Biotec (Bergisch Gladbach, Germany)
Magnetic stirrer Monotherm	Variomag (Daytona Beach, USA)
Microplate reader EL-800	BIO-TEK Instruments (Winooski, USA)
Microplate reader Infinite F200 Pro	Tecan (Männedorf, Switzerland)
Microplate washer hydroSPEED	Tecan (Männedorf, Switzerland)

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Table 2.19. Laboratory equipment and devices (continued from previous page)

Device / Equipment	Manufacturer / Distributor
Microscope (fluorescence) Axiovert 200M	Zeiss (Jena, Germany)
Microscope (inverted) Axiovert 25	Zeiss (Jena, Germany)
Microwave oven R-202	Sharp (Osaka, Japan)
pH-meter pH 526	WTW (Xylem Analytics Germany, Weilheim, Germany)
Pipetboy acu	Integra Biosciences (Fernwald, Germany)
Pipette Transferpette (multichannel, 20–200 µl)	Brand (Wertheim, Germany)
Pipettes (0.1–2.0 µl, 2–20 µl, 20–200 µl, 200–1,000 µl)	Gilson (Mettmenstetten, Switzerland)
Precision scales SPB 55	Scaltec instruments (Göttingen, Germany)
Spectrophotometer Nanodrop ND-1000	Peqlab Biotechnologie (Erlangen, Germany)
Thermocycler Biometra T gradient	Biometra (Göttingen, Germany)
Thermomixer compact	eppendorf (Hamburg, Germany)
Tumble roller mixer TRM-50	IDL (Nidderau, Germany)
Ultra pure water device Aquintus	membraPure (Hennigsdorf, Germany)
UV crosslinker CL-1, 254 nm	Herolab (Wiesloch, Germany)
UV transilluminator UVT 400 M	International Biotechnologies Inc. (New Haven, USA)
Vacuum pump	Fröbel Labortechnik (Lindau, Germany)
Vortex mixer Vortex-Genie 2	Scientific Industries (Bohemia, USA)
Water bath	GFL (Burgwedel, Germany)

2.13 Services

- The **EBV and HCMV IgG serostatus** of blood donors was determined by the Max von Pettenkofer Institute (München, Germany).

- High resolution **HLA typing** of blood donors was performed by the "Zentrum für Humangenetik und Laboratoriumsdiagnostik, Dr. Klein, Dr. Rost und Kollegen" (Martinsried, Germany) using whole blood, isolated PBMCs or established cell lines.

- Sanger **DNA sequencing** of purified PCR products was done by GATC Biotech AG (Konstanz, Germany) and Eurofins Genomics (Ebersberg, Germany).

- **Gene synthesis** and human codon optimisation of TCR constructs as well as cloning of the constructs into pMP71 was done by Thermo Fisher Scientific (Regensburg, Germany).

- **Oligonucleotides** were synthesised by Metabion (Martinsried, Germany).

- **Buffy coats** were obtained from the Institute for Transfusion Medicine of the University of Ulm (Germany)

2.14 Software and databases

For identification of V, CDR3, (D), J and C regions of sequenced TCRα or TCRβ chains the international ImMunoGeneTics information system (IMGT, Lefranc et al., 1999) was used. In particular, the V-QUEST tool (Brochet et al., 2008; Giudicelli et al., 2011, http://www.imgt.org/IMGT_vquest/vquest) was employed and reference sequences were obtained from the TCR repertoire (http://www.imgt.org/IMGTrepertoire/) concerning human TRAV (Scaviner and Lefranc, 2000b) and TRBV (Folch and Lefranc, 2000).

The UniProt database (The UniProt Consortium, 2017, https://www.uniprot.org/) served as source for protein sequences, like that of 65 kDa phosphoprotein (pp65) of HCMV strain AD169 (accession number P06725, accessed 06.03.2017). Additionally, the genetic sequence database

Table 2.20. Software.

Name	Version	Application	Producer
Diva	8.0.1	FACS data analysis & compensation	BD Biosciences (Heidelberg, Germany)
Excel 2016	15.32	Data analysis and table design	Microsoft (Redmond, USA)
FlowJo	10.5.3	FACS data analysis & compensation	TreeStar Inc. (Ashland, USA)
i-control	1.10	ELISA measurement	Tecan (Männedorf, Switzerland)
KC4	2.7	ELISA measurement	BIO-TEK Instruments (Winooski, USA)
MacVector with Assembler	14.5.3	Plasmid visualisation, nucleotide & protein sequence analysis and alignment	MacVector Inc. (Cary, USA)
Mendeley	1.19.2	Reference management	Elsevier Limited (Oxford, United Kingdom)
PowerPoint 2016	15.32	Illustration	Microsoft (Redmond, USA)
Prism	6.0h	Data analysis and display	GraphPad Software (La Jolla, USA)
TeXstudio	2.12.14	Writing	Benito van der Zander, Jan Sundermeyer, Daniel Braun, Tim Hoffmann (https://www.texstudio.org)

GenBank (Benson et al., 2012, https://www.ncbi.nlm.nih.gov/genbank/) of the National Institutes of Health was used to gather nucleic acid sequences, such as the genome of AD169 (accession number FJ527563, accessed 03.06.2019).
All other software, which has been used in this study for experimental design, data analysis and illustration, is shown in table 2.20.

3 Methods

3.1 Microbiological methods

3.1.1 Cultivation and storage of bacteria

Escherichia coli (*E. coli*) strain DH5α (Invitrogen, Karlsruhe, Germany) was used for propagation of plasmids (see section 2.4). They were cultured at 37 °C overnight on LB agar plates or in LB medium under constant agitation at 200 rpm. If the bacteria carried a plasmid, the respective antibiotic was added to the LB agar or medium (100 µg/ml ampicillin, 30 µg/ml chloramphenicol, 30 µg/ml kanamycin) in order to select for bacteria harbouring the plasmid's resistance gene. After overnight incubation, LB plates were stored at 4 °C for up to 7 days.

3.1.2 Transformation of bacteria

For transforming *E. coli* DH5α, a vial of highly concentrated, chemically competent bacteria that had been prepared by L. Schirrmann was thawed on ice. 1 µg of plasmid DNA was added to 50 µl of bacteria, incubating the mixture for 10 to 20 minutes on ice. After a heat shock of 45 seconds at 42 °C, the bacteria suspension was cooled on ice for another 1 to 2 minutes. Next, 1 ml of LB medium without antibiotics was added and the suspension was shaken at 37 °C and 900 rpm. After an hour, the suspension was centrifuged at 3,000 × g for 3 minutes, the majority of the supernatant was discarded and the remaining volume (ca. 50 µl) was used to resuspend the pelleted bacteria and plate them on a pre-warmed LB plate containing the respective antibiotic. Finally, plates were incubated at 37 °C overnight. Well grown, single colonies were used for further propagation.

3.1.3 Propagation of transformed bacteria

One well grown and well separated colony was picked from an overnight LB plate and incubated in 400 ml sterile LB medium including the necessary antibiotic (see section 3.1.1) for selection of transformed bacteria.

3.2 Molecular biology methods

3.2.1 Plasmid DNA purification from *E. coli*

Large scale purification of plasmid DNA for transfection of human cells was performed using the HiPure Plasmid Maxiprep Kit (Thermo Fisher Scientific, Waltham, USA) or Endofree Plasmid Maxi Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. 400 ml of saturated overnight bacteria culture were used for a single preparation providing yields of up to 1 mg DNA. Concentration and purity of the preparation were assessed by absorbency readings at 260 nm and 280 nm using the Nanodrop ND-1000 spectrophotometer (Peqlab Biotechnologie, Erlangen, Germany).

Table 3.1. Primer pools of TCR Vα primers (listed in section 2.5.1, from Davies et al., 1991; Steinle et al., 1995) for amplification of TCRα cDNA.

Pool	Primers included
A	Pan Vα1, Pan Vα1.1, Vα2, Vα3, Vα4
B	Vα5, Vα6, Vα7, Vα8corr
C	Vα9, Vα10, Vα11, Vα12
D	Vα13, Vα14.1, Vα14, Vα15, Vα16
E	Vα17, Vα18, Vα19, Vα20
F	Vα21, Vα22, Vα23, Vα24
G	Vα25, Vα26, Vα27, Vα28
H	Vα29, Vα30, Vα31NEU, Vα32

3.2.2 T cell receptor analysis

Total **RNA** was isolated from T cell clones by using QIAshredder kit and RNeasy mini kit (Qiagen, Hilden, Germany) following the instructions of the manufacturer. T cells were either freshly pelleted or had been frozen at −80 °C and were thawed for RNA extraction. RNA concentration was assessed by absorbency readings at 260 nm with the Nanodrop ND-1000 spectrophotometer (Peqlab Biotechnologie, Erlangen, Germany). cDNA was generated from extracted RNA using the QuantiTect reverse transcription kit (Qiagen, Hilden, Germany). If RNA concentration was higher than or equal to 30 ng/ml, 6 µl were used for reverse transcription. If RNA concentration was lower, 12 µl were used.

For amplification of **TCRα cDNA** by polymerase chain reaction (PCR), 34 forward primers (see Davies et al., 1991; Steinle et al., 1995) were pooled into eight groups as shown in table 3.1. With the forward primer binding in the V region of the TCRα chain and the reverse primer 3'T-Cα specifically binding to the C region of TCRα cDNA, the resulting amplicon was of approximately 400 bp length. Additionally, the primers P-5'αST (forward) and P-3'αST (reverse) generated an amplicon of 547 bp spanning from the C region to the 3' untranslated region (UTR) of TCRα cDNA serving as positive control.
TCRα PCR was carried out on a Biometra T gradient thermocycler (Biometra, Göttingen, Germany). The composition of the PCR mixture is shown in table 3.2. The PCR program consisted of 2 minutes initial denaturation at 95 °C, 38 cycles of (I) 30 seconds at 95 °C, (II) 30 seconds at 56 °C and (III) 60 seconds at 72 °C, followed by a final extension at 72 °C for 10 minutes to allow for the completion of abortive elongation reactions. In the end, the PCR samples were kept at 4 °C until further processing or they were stored at −20 °C.

For amplification of **TCRβ cDNA**, the two degenerate forward primers VP1 and VP2 were used (see Zhou et al., 2006). While VP1 is able to bind to 42 different V regions of TCRβ chains, VP2 is able to bind to 11 additional TCRβ V regions, leaving only Vβ16.1 and Vβ10.1 not to be covered by these two primers. The reverse primer CP1 specifically binds both alleles of the C region of the TCRβ chain (Cβ1 or Cβ2), resulting in an amplicon of approximately 400 bp length.

TCRβ PCR was carried out on a Biometra T gradient thermocycler (Biometra, Göttingen, Germany). The composition of the PCR mixture is shown in table 3.2. The PCR program consisted of 2 minutes initial denaturation at 95 °C, 40 cycles of (I) 30 seconds at 95 °C, (II) 30 seconds at 50 °C and (III) 30 seconds at 72 °C, followed by a final extension at 72 °C for 10 minutes. In the end, the PCR samples were kept at 4 °C until further processing or they were stored at −20 °C.

Table 3.2. Compositions of TCRα and TCRβ PCR mixtures.

PCR	**component**	**concentration**		**required amount for PCR [µl]**	
		stock	**final**	**Vα**	**Vβ**
Vα and Vβ	cDNA	-	-	1.0 - 2.0	1.5
	dNTP	2.0 mM	0.2 mM	4.0	4.0
	Taq Buffer	10×	1×	4.0	4.0
	DMSO	100%	5%	2.0	2.0
	Taq polymerase	5.0 U/µl	2.5 U/40 µl	0.5	0.5
	H_2O	-	-	7.9 - 8.9	19.2
Vα	5' primer subpool A, B, C, ... or H	0.8 µM each	0.2 µM each	10.0	-
	3' primer 3'T-Cα	5.0 µM	0.5 µM	4.0	-
	P-5'αST	2.5 µM	0.175 µM	2.8	-
	P-3'αST	2.5 µM	0.175 µM	2.8	-
Vβ	5' primer VP1 or VP2	10.0 µM	2.0 µM	-	8.0
	3' primer CP1	10.0 µM	0.2 µM	-	0.8
Sum		-	-	40.0	40.0

PCR products were separated according to their size by **agarose gel electrophoresis**. Agarose gels were prepared using 1× TAE buffer, 2% (w/v) ultra pure agarose (Life Technologies, Paisley, UK) and 0.1 µg/ml ethidium bromide (Carl Roth GmbH, Karlsruhe, Germany) for visualisation of the DNA. While all of the sample volume was consumed for gel electrophoresis of from TCRα PCR samples, only 10 µl of the TCRβ PCR samples were used. The samples were mixed with 6× loading dye (Thermo Fisher Scientific, Waltham, USA) and run on a 2% (w/v) agarose gel at 110 V for 45 to 60 minutes using 1× TAE as a running buffer and 1 kb GeneRuler DNA Ladder Mix (Fermentas, St. Leon-Rot, Germany) to determine DNA fragment size. The gel was viewed and documented employing the gel documentation system Quantum ST4-1100/26LMX (Vilber Lourmat, Eberhardzell, Germany). In case of TCRα PCR samples, the gel was additionally viewed on an ultraviolet (UV) transilluminator (UVT 400 M, International Biotechnologies Inc., New Haven, USA) allowing for gel cutting. Cut TCRα bands were extracted from the gel and their DNA was purified using NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. Similarly, DNA products contained in the remaining 30 µl of the TCRβ PCR sample volume were purified using this kit. The DNA concentration of each sample was measured by the Nanodrop ND-1000 spectrophotometer (260 nm) and set to 5 ng/µl for Sanger sequencing by either GATC Biotech AG

(Konstanz, Germany) or Eurofins Genomics GmbH (Ebersberg, Germany).

For **identification of V, J and C regions**, as well as complementarity determining region 3 (CDR3), sequencing results were analysed using IMGT's V-QUEST tool (Brochet et al., 2008; Giudicelli et al., 2011, http://www.imgt.org/IMGT_vquest/vquest). Any ambiguities were resolved by newly amplifying and resequencing the TCR chain using the forward primer specific for the relevant Vα or Vβ region.

3.2.3 Gene synthesis

In order to be able to produce retroviral vectors to transfer HCMV-specific TCR genes into receiving T cells, complete TCRα and TCRβ chains of a T cell clone were assembled *in silico* including the necessary elements for retroviral packaging and strong, equimolar eukaryotic expression. Since the TCRα and TCRβ sequences obtained from the Sanger sequencing service only spanned from the primer binding sites within the V region to the beginning of the C region, but did not fully cover both those regions, the missing parts of the sequences were completed by using IMGT's TCR repertoire (http://www.imgt.org/IMGTrepertoire/) as source for human TRAV (Scaviner and Lefranc, 2000b), TRAC, TRBV (Folch and Lefranc, 2000) and TRBC gene tables (Lefranc et al., 1999). A NotI restriction site (`GCGGCCGC`) and a Kozak sequence (`CACC`) were put in front of the start codon of the complemented TCRβ chain. The stop codon of the TCRβ chain was replaced by a Gly-Ser-Gly linker (`GGCAGCGGC`) and a Picornavirus 2A peptide (P2A) sequence (`GCCACCAACTTCAGCCTGCTGAAACAGGCCGGCGACGTGGAAGAGAACCCTGGGCCC` translating to `ATNFSLLKQAGDVEENPGP`).
The 'self-cleaving' 2A peptide induces a ribosomal 'skip' mechanism between its second to last amino acid (Gly) and its last amino acid (Pro) without affecting the translation of the gene immediately downstream of it, thus allowing for an approximately equimolar separate expression of both the gene upstream and the gene downstream of the 2A peptide within the same ORF (Donnelly et al., 2001). The "cleavage" efficiency is improved by placing the Gly-Ser-Gly linker between NH_2-terminal protein and the 2A peptide (Holst et al., 2006; Szymczak et al., 2004, addendum 01.06.2004). P2A encompassed a BmgBI restriction site in order to be able to exchange TCRα and TCRβ chains from different constructs.
Downstream of P2A, the whole TCRα chain gene including its stop codon was added and an EcoRI restriction site (`GAATTC`) was attached at the end of the sequence. Taken together, every complete construct was of approximately 1,800 bp length and consisted of 5'—NotI–Kozak–**TCRβ**–`GSG`–**P2A**–**TCRα**–Stop–EcoRI—3'. This order was chosen since Leisegang et al. (2008) showed it to be superior to its counterpart (TCRα before TCRβ), probably because TCRβ expression is more limiting.

Two constructs were designed not coding for TCR chains, but co-receptors instead: The first codes for T-cell surface glycoprotein CD4 (see table 2.7). The second codes for T-cell surface glycoproteins CD8α and CD8β linked by P2A (see table 2.7). According to Thakral et al. (2008), CD8β can be spliced into different variants: M-1 is dominant on most thymocytes, peripheral blood $CD8^+$ T cells and naïve $CD8^+$ T cells, M-2 is mainly withheld in internal vesicles, M-3 is barely expressed/found and M-4 is predominant on effector memory $CD8^+$ T cells. The CD8β

splice variant M-1, which is identical to NCBI, Gene ID 926, isoform 5, was chosen. However, a SalI restriction site (`GTCGAC`) at nucleotide position 1373 of the CD8α–P2A–CD8β construct has been included, so that splice variants of CD8β could potentially be edited by PCR and cloning.

Each complete, *in silico* assembled construct was sent to Thermo Fisher Scientific (Regensburg, Germany, formerly "GeneArt") for **commercial gene synthesis and cloning** into plasmid MP71Gpre. Prior to synthesis, however, the construct was subjected to GeneArt's gene optimisation process to allow high and stable expression rates in *Homo sapiens*. During this process the codon usage was adapted to the codon bias of *Homo sapiens* genes, while protecting the intended NotI, EcoRI & BmgBI restriction sites from sequence alteration, but avoiding or removing unintended NotI, EcoRI & BmgBI restriction sites elsewhere. In addition, negative cis-acting sites (such as splice sites, TATA-boxes, etc.), which may negatively influence expression, were avoided where possible and GC content was adjusted to prolong mRNA half life. The synthetic gene was assembled from synthetic oligonucleotides and/or PCR products and cloned into MP71Gpre thereby replacing the GFP marker gene. The sequence congruence within the insertion sites was 100%, as verified by sequencing. The plasmid DNA was purified from transformed bacteria and 5 µg of it were lyophilised and shipped.

3.3 Cell culture methods

3.3.1 Cell culture conditions

All human cells used in this study were cultivated in an incubator at 37 °C, 5% CO_2 and a humidified atmosphere. Handling of the cells was performed in laminar flow hoods, only. In general, for washing and concentration purposes, all cells were pelleted by centrifugation at 300 × g and room temperature for 10 minutes. Before determining the cell count using a Neubauer Improved counting chamber, the cell suspension was diluted 2- to 10-fold with a 0.5% trypan blue solution in order to discriminate live and dead cells. Living cells repel the dye, whereas dead cells take up the dye due to their damaged cell membrane and thus appear in a blue colour when viewed through the microscope.

The standard cell culture medium for Raji and lymphoblastoid cell lines that were created using mini-EBV (mini-LCLs) was RPMI 1650 supplemented with 7% fetal calf serum (FCS), 100 U/ml penicillin, 100 µg/ml streptomycin and 100 nM sodium selenite (henceforth called LCL medium). These cells were cultivated in suspension and split 1:3 up to 1:10 once or twice a week depending on their proliferation rate. DG-75 were cultured as adherent cells and had to be scraped for passaging. Apart from that they were cultured like Raji and mini-LCLs.

Adherent HEK293T cells were cultured in DMEM supplemented with 7% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin and 100 nM sodium selenite, being split approximately 10 to 20-fold once or twice a week, depending on the confluence, by flushing them off the plastic surface with a pipette. MRC-5 human fibroblasts, which also grow adherently, were cultured likewise adding 1% non-essential amino acids and passaged by trypsinisation (0.05% Trypsin/EDTA for 1 to 2 minutes).

3.3.2 Cryopreservation of human cells

For long term preservation of human cells, they were pelleted by centrifugation, the supernatant was discarded and the cells were resuspended in pre-cooled (4 °C) freezing medium composed of RPMI 1640 supplemented with 50% FCS and 10% DMSO. Alternatively, cells were resuspended in the ready-made freezing medium Bambanker (Nippon Genetics Europe, Düren, Germany). The cell suspension was aliquoted in 1.8 ml cryo tubes that were kept on ice for up to 30 minutes. Next, they were stored at −80 °C for several days up to several weeks, after which the tubes were finally moved to the liquid nitrogen tank and stored in the gas phase.
Frozen cells were thawed as fast as possible by pre-warmed (37 °C) RPMI 1640 without any additives transferring the content of the cryo tube into a tube filled with 50 ml RPMI 1640. The cells were pelleted by centrifugation, the supernatant was discarded and the cells were resuspended in the respective medium.

3.3.3 Transfection of human cells with plasmid DNA

For **production of HLA-transgenic cells**, an excess amount of DG-75, Raji or EBV-B1.11 was harvested after splitting the cells 1:2 the previous day. The cells were washed once in RPMI 1640 without any supplements and once in OptiMEM in order to remove any remaining serum. They were resuspended at 16.67×10^6 cells/ml in OptiMEM, of which 0.3 ml were mixed with 10 µg of plasmid DNA in a 1.5 ml reaction tube and transferred into a 0.4 cm Gene Pulser cuvette (Bio-Rad, München, Germany). If two plasmids were transfected simultaneously (e.g. HLA-DQA & HLA-DQB plasmid DNA), 10 µg per plasmid were used. An equal volume of TE buffer from Qiagen's Endofree Plasmid Maxi Kit or 10 µg GFP plasmid DNA (MP71Gpre) served as negative or positive electroporation control, respectively. The cuvette was placed between the contacts of a GenePulser II (Bio-Rad, München, Germany) and electroporated at 230 V using 975 µF. The cells were immediately resuspended in 1 ml pre-warmed FCS and put into one well of a 6-well plate that held 4 ml pre-warmed LCL medium. After overnight incubation at 37 °C, the GFP expression of the positive control was checked and if successful, HLA-transgenic cells were used for determining the HLA restriction of a T cell clone or TCR-transgenic T cell line.

For **production of MP71 retroviral vectors**, adherent HEK293T cells were transfected using TransIT-293 transfection reagent (Mirus Bio LLC, Madison, USA) according to the manufacturer's instructions. In brief, 3×10^5 HEK293T cells in 2.5 ml were plated per well into 6-well plates the day before transfection. On the following day, 250 µl OptiMEM medium, 2.0–4.0 µg of plasmid DNA and 2.5 µl TransIT-293 transfection reagent (pre-warmed to room temperature) per 1.0 µg of plasmid DNA were gently mixed. The amount of total plasmid DNA varied due to different retroviral DNA cargo: (I) 1.0 µg of TCRβ–P2A–TCRα plasmid construct (see section 3.2.3 and tab. 2.8), (II) 1.0 µg of the TCRβ–P2A–TCRα construct and 0.5 µg of co-receptor CD4 or CD8 constructs (DNA ratio 2:1), (III) 1.0 µg of the TCRα and 1.0 µg of the TCRβ constructs (DNA ratio 1:1, only performed with CRV2α and CRV2β), (IV) 1.0 µg of the GFP-encoding plasmid MP71Gpre as transfection and transduction control or (V) no genetic cargo at all ("empty" retroviral vector as mock control). Regardless of the cargo, 1.0 µg of each of the expression plasmids encoding the moloney MLV gag/pol genes (plasmid pcDNA3.1MLVg/p,

kindly provided by Christopher Baum, Hannover, Germany, see Leisegang et al., 2008) and the MLV-10A1 env gene (plasmid pALF-10A1, see Uckert et al., 2000) was used (see section 2.4 and table 2.7). After 30 minutes of incubation at room temperature to allow the formation of TransIT-293/DNA complexes, the solution was added on top of the HEK293T culture medium in a drop-wise manner to different areas of the well while rocking the well plate back-and-forth and side-to-side to ensure an even distribution of TransIT-293/DNA complexes. After 48 hours, the transfection efficiency of the positive control (GFP) was checked usually showing more than 70% GFP-positive cells. Further processing of the supernatant containing the retroviral vectors is described in section 3.5.1. In brief, the supernatant of the HEK293T cells was harvested after 48 hours, filtrated (0.45 µm pore size) and directly used for transduction (Leisegang et al., 2008). A schematic overview of HEK293T transfection in the context of analysing the effector function of TCR-transgenic HLA class I- or HLA class II-restricted T cells is depicted in figure 3.1.

3.3.4 Isolation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) of healthy adult donors were isolated by density gradient centrifugation using Pancoll (PAN Biotech, Aidenbach, Germany) either from heparinised venous blood of voluntary donors or from leukocyte concentrates ("buffy coats") shipped by the Institute for Transfusion Medicine, University of Ulm (Germany). Buffy coats contained enriched leukocytes from standard blood donations (500 ml) suspended in approximately 20 ml. Whole blood samples or buffy coats were pre-diluted 6:1 or 1:1 in 1× PBS, respectively. 30 to 35 ml of diluted blood sample were transferred into 50 ml tubes and carefully underlaid with approximately 12 ml Pancoll (density: 1.077 g/ml). During the following centrifugation for 25 minutes at 1,000 × g, room temperature, low acceleration (level 1 out of 9) and no braking for deceleration, the various components of the blood were separated according to their density. Erythrocytes sediment at the bottom of the tube with Pancoll layering on top, followed by a layer of PBMCs at the interface of Pancoll and plasma. To avoid erythrocyte contamination, PBMCs were carefully aspirated, transferred to new 50 ml tubes and diluted at least 1:5 in 1× PBS for washing of the cells. If necessary, an aliquot of the donor's plasma was collected for determination of the EBV or HCMV IgG serostatus. The cells were pelleted by centrifugation (8–10 min, 300 × g, room temperature, full acceleration and deceleration) and washing was repeated 3 to 4 times in order to remove remaining Pancoll and deplete thrombocytes. Freshly isolated PBMCs were counted using a Neubauer Improved counting chamber (diluted 1:10 in 0.5% trypan blue solution) and either directly frozen or processed for cell culture as described elsewhere in this section.

3.3.5 Establishment and maintenance of T cell clones

The **production of polyclonal T cell lines and subsequent single cell cloning** in order to obtain single T cell clones were performed by Andreas Moosmann, Martina Wiesner (2005), Andrea Schub (2010), Stefanie Ameres (2012) or Xiaoling Liang (unpublished) using PBMCs of HCMV-seropositive ($HCMV^+$) donors (see tab. 2.8). HCMV-specific T cells were expanded *in vitro* by repeated stimulation for several weeks (Moosmann et al., 2002; Wiesner, 2005). Either autologous mini-LCLs endogenously expressing pp65 or IE-1 (see section 2.3.2) or B blasts

that were pulsed with the relevant peptide(s) were irradiated and served as antigen presenting cells (APCs). Also, peptide pulsed, autologous PBMCs were employed. While both mini-LCLs and pulsed APCs can present exogenously loaded antigenic peptides on HLA class I and II molecules, only mini-LCLs can provide endogenously processed peptides of HCMV antigens on HLA molecules. By using autologous APCs, HCMV-specific memory T cells of the donor can be stimulated regardless of their HLA restriction without activating alloreactive T cells.
Resulting polyclonal T cell lines were subjected to limiting dilution giving rise to T cell clones. In some cases, IFN-γ capture and immunomagnetic separation were performed before limiting dilution to further enrich antigen-specific T cells. The T cell clones were maintained and expanded in 96-well plates (round bottom, 200 µl per well) by restimulation with 1,000 U/ml IL-2, 0.1×10^6 irradiated (60 Gy), autologous, antigen-expressing mini-LCL per millilitre and 1.5×10^6 irradiated (60 Gy), allogeneic PBMCs (mixed from at least three donors) per millilitre. The T cell lines were cultivated in RPMI 1640 supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, 100 µg/ml streptomycin and 100 nM sodium selenite (henceforth abbreviated as "10% FCS T cell medium"). One week after restimulation, half of the medium was discarded and replaced by 10% FCS T cell medium supplemented with 1,000 U/ml IL-2. After another week, the T cell lines were restimulated again with the feeder mix described above, frozen or analysed for their epitope specificity using peptide libraries (sections 2.6 and 3.4.4), HLA restriction (section 3.4.3), TCRα and β chain usage (section 3.2.2) and recognition of HCMV-infected target cells (section 3.5.3). As soon as the data from these analyses excluded the possibility of two or more clones being mixed in one T cell line, it was considered a T cell clone.

3.3.6 Generation of monocyte-derived dendritic cells

The recognition of HCMV-infected target cells by $CD4^+$ T cell clones or TCR-transgenic HLA class II-restricted T cell lines was analysed using HLA-matched monocyte-derived dendritic cells (moDCs). moDCs were generated largely according to the published protocol from Jonuleit et al. (2000, 1997). Approximately 5×10^7 freshly isolated PBMCs were incubated in 15 ml RPMI 1640 supplemented with 2% human serum, 100 U/ml penicillin, 100 µg/ml streptomycin and 100 nM sodium selenite per flask (75 cm^2) for at least 2.5 hours in order to let the monocytes adhere to the plastic surface. Next, the supernatant was discarded and the adherent monocytes were rinsed twice using 10 ml pre-warmed RPMI 1640 without any supplements and a plastic pipette (10 ml) to get rid of all remaining non-adherent cells. Immature dendritic cells were generated by cultivating the monocytes for 3 days in 15 ml RPMI 1640 supplemented with 2% human serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 100 nM sodium selenite, 100 ng/ml rhuGM-CSF and 20 ng/ml rhuIL-4. For maturation of moDCs, 10 ng/ml rhuTNF-α, 10 ng/ml rhuIL-1β, 1 µg/ml PGE_2 and 15 ng/ml rhuIL-6 were directly added to the old medium and the cells were incubated for another 24 hours.

3.4 Immunological methods

3.4.1 Immunomagnetic separation of cell populations

In order to separate different populations of immune cells from each other, "magnetic activated cell sorting" (MACS) was applied according to Miltenyi Biotec's instructions. This technique links superparamagnetic, non-toxic and biodegradable nanoparticles ("microbeads") to cells by particle-bound monoclonal antibodies with a defined specificity. Thereby these nanoparticles tag a particular cell population enabling its retention on a column that is placed into a magnetic field. While labelled cells are retained on the column during washing procedures, the unlabelled cells are washed out and can be collected as negative fraction. Once the column has been removed from the magnetic field, the labelled cells can also be removed from the column by flushing and are recovered as the positive fraction.
Freshly isolated or thawed PBMCs (section 3.3.4 or 3.3.2, respectively) were either directly labelled with 2 µl anti-CD8 beads (Miltenyi Biotec, Bergisch Gladbach, Germany) per 10^6 PBMCs or indirectly labelled by using anti-fluorochrome microbeads after the cells had been stained with fluorochrome-tagged antibodies. In the latter case, 10^6 PBMCs were stained with 2 µl APC-labelled anti-TCRαβ antibody (Clone: IP26, Biolegend) or 0.5 µl FITC-labelled anti-TCR Vδ2 antibody (Clone B6, Biolegend, San Diego, USA), after which 2 µl anti-APC or anti-FITC beads (Miltenyi Biotec) were applied per 10^6 PBMCs, respectively. MS MACS columns (Miltenyi Biotec) were used to positively select CD8-positive ($CD8^+$) T cells from PBMCs. LD MACS columns (Miltenyi Biotec) were used to deplete $TCR\alpha\beta^+$ T cells from PBMCs resulting in a negative fraction that contained all PBMC types, except for $TCR\alpha\beta^+$ T cells which were eluted as positive fraction of the separation. In order to check the purity of each fraction, they were stained with antibodies and analysed by flow cytometry (see section 3.4.2). During all steps 2% FCS in 1× PBS was used as equilibration, washing and elution buffer.

3.4.2 Phenotypic analysis of cells by flow cytometry

In order to analyse the **expression of surface proteins** on a cell population of interest, the cells were stained using various monoclonal antibodies conjugated to a fluorochrome (such as FITC or PE, see table 2.14). Usually $0.2–0.3 \times 10^6$ cells were centrifuged at room temperature and 1,000 × g for 5 min in 1.5 ml reaction tubes (Eppendorf, Hamburg, Germany) and the supernatant was discarded. The pelleted cells were resuspended in 50 µl pre-cooled (4 °C) FACS buffer (2% FCS in 1× PBS) containing fluorochrome-labelled monoclonal antibodies at defined dilutions (see table 2.14). The cells were incubated at (4 °C) for 20 minutes, after which they were washed by adding 1 ml of pre-cooled FACS buffer and pelleting the cells as described above. Finally, the cells were resuspended in 0.2 ml pre-cooled FACS buffer for direct analysis in a flow cytometer or fixed with 1% (v/v) paraformaldehyde (PFA) in FACS buffer and stored at 4 °C for up to 7 days.

The **expression of T cell receptors** (TCRs) on T cell clones (section 3.3.5) or TCR-transgenic T cells (section 3.5.1) was assessed by HLA multimers labelled with either PE or APC. This technique employs the natural ligand of the TCR consisting of a peptide that is

bound to an HLA class I α chain/β2m heterodimer or bound to an HLA class II αβ heterodimer. Due to the unstable nature of a TCR-peptide/HLA interaction (Savage et al., 1999) the avidity has to be increased by multimerisation (Altman et al., 1996) to achieve stable binding of the reagent. All peptide/HLA monomers were stored in aliquots at −20 or −80 °C and thawed on ice for multimerisation, while peptide/HLA pentamers were stored at 4 °C.
HLA multimers were never used together with antibodies specific for a particular TCRβ V-region or the αβ TCR (see table 2.14) in one staining in order to avoid competition for binding and sterical inhibition.

CRV- or FRC- specific T cells were stained using an MHC-streptagII-StrepTactin multimer reagent (streptamer) as described in Neudorfer et al. (2007). CRV/HLA-C*07:02 monomers and FRC/HLA-C*07:02 monomers were generated and kindly provided by Michael Neuenhahn and Dirk Busch (Institute for Medical Microbiology, Immunology and Hygiene, TUM, Munich, Germany). Per sample, 0.4 µl of CRV/HLA-C*07:02 monomers (0.93 µg/µl) or 0.7 µl of FRC/HLA-C*07:02 monomers (0.77 µg/µl) were mixed with 0.5 µl PE-labelled StrepTactin (0.5 mg/ml, IBA Göttingen, Germany) in a total volume of 10.0 µl pre-cooled (4 °C) FACS buffer. After 45 minutes of incubation on ice to allow streptamer complexes to form, the solution was diluted 1:3 using pre-cooled FACS buffer and 30 µl of this dilution were used to resuspend and stain 0.2–0.3 × 10^6 pelleted cells for 20 minutes at 4 °C. Next, surface proteins were stained with fluorochrome-labelled monoclonal antibodies as described previously except for two alterations: (I) no washing of the cells was performed after streptamer staining and before surface staining and (II) antibodies were diluted in 20 instead of 50 µl FACS buffer in order to achieve the intended final concentrations as shown in table 2.14 in the total volume of 30+20 µl. The cells were incubated for another 20 minutes at 4 °C to allow the monoclonal antibodies to bind thereby prolonging the streptamer incubation, however, at a reduced concentration. Finally, cells were washed, pelleted and resuspended either for direct or delayed flow cytometrical analysis as described above.
Likewise, **YSE-specific T cells** were incubated with specific multimers first and then counter-stained with a combination of monoclonal antibodies. Differing from the protocol described above, YSE/HLA-A*01:01 pentamers (ProImmune, Oxford, UK) did not have to be multimerised beforehand. Instead, 0.5 µl of them were diluted 1:100 in FACS buffer and used to stain the cells for 10 minutes at room temperature. Afterwards, the cells were washed once and resuspended in FACS buffer containing not only fluorochrome-labelled antibodies, but also 2.0 µl pentamer-binding R-PE Pro5 fluorotag (Proimmune) per 50.0 µl total volume. Other than the alterations described, the procedure was identical.

QPFM-, YRIQ-, AGIL-, MSIY- or EFFT-specific T cells were stained using HLA class II tetramers similar to the protocol described in Braendstrup et al. (2013). QPFM/HLA-DRB1*13:02, YRIQ/HLA-DRB1*13:02, AGIL/HLA-DRB3*03:01, MSIY/HLA-DRB1*15:01 and EFFT/HLA-DRB5*01:01 monomers were purchased from Immunaware (Copenhagen, Denmark, see section 2.8). Peptide/HLA monomers were thawed and coupled to PE-streptavidin (0.5 mg/ml, BD Biosciences, Heidelberg, Germany) or APC-streptavidin (0.2 mg/ml, BD Biosciences) at a molar ratio of 4 to 1. In order to fully saturate streptavidin molecules with peptide/HLA complexes, the required amount of fluorochrome-labelled streptavidin was divided into three equal

portions that were successively added to the solution containing the peptide/HLA monomers every 15 minutes. After each addition, the mix was incubated for 15 minutes at 4 °C. If less than 3 µl of fluorochrome-labelled streptavidin were required, the latter was diluted 1:10 beforehand. Working concentration of peptide/HLA monomers ranged from approximately 1 to 3 µM during tetramerisation (see table 2.15 for concentrations of peptide/HLA monomers). Finally, tetramer concentration was adjusted to 30 or 60 nM and 30 µl of this dilution were applied per staining of usually 0.2–0.3 $\times$ 10^6 cells.

The stained cells were analysed in a Canto or an LSR Fortessa (both by BD Biosciences) flow cytometer and the resulting data was processed using FlowJo software (Tree Star, Ashland, USA). Viable lymphocytes were discriminated according to their characteristic position in the forward/sideward scatter (FSC/SSC) dot plot. The axes of the dot plot diagrams in this study are scaled bi-exponentially, usually comprising -10^3 to 10^5 arbitrary units of fluorescence intensity, except for FSC and SSC diagrams showing intensities from zero to 260,000 on a linear scale. Pseudocolour dot plots indicate increasing density of signals in 2D plots by a colour code ranging from blue (low density) over green, yellow and orange to red (high density).

In order to obtain absolute cell counts by flow cytometric measurement, 20,000 PE-labelled Calibrite beads (BD Biosciences) were added to the cells after they had been stained as described in the paragraphs above. Prior to the staining procedure, cells were resuspended in cell culture plates first and then a particular volume of resuspended cells that was standardised within one experiment to maintain comparability was taken for staining of cells (e.g. 500 µl from a well of a 24-well plate containing 2.0 ml in total and, if applicable, 1.0 ml from a well of a 12-well plate containing 4.0 ml in total, thereby taking 25% of the cells per well regardless of the total volume). The absolute cell count was calculated after flow cytometric measurement according to the following formula:

$$\text{absolute cell count} = \frac{\text{cell count}}{\text{bead count}} \cdot 20{,}000 \tag{1}$$

3.4.3 Determination of human leukocyte antigen class II restriction

The nomenclature of HLA alleles describes (I) the gene name, (II) the allele group, (III) the specific HLA protein, (IV) silent DNA substitutions within the coding region and (V) differences in non-coding regions (see http://hla.alleles.org/nomenclature/naming.html, accessed on 21.01.2019). As an example, the allele HLA-DRB1*13:02:01 codes for an HLA-DRβ chain of the allele group “13”, precisely the specific HLA-DRB1 protein “13:02”, with the precise coding nucleic acid sequence “01”. Consequently, the alleles HLA-DRB1*13:02:01, DRB1*13:02:02, DRB1*13:02:03, etc. have different coding nucleic acids sequences, but code for the same amino acid sequence. In contrast, HLA-DRB1*13:01 and DRB1*13:02 produce slightly different proteins with alterations in the amino acid sequence (in the given example at amino acid position 86: DRB1*13:01 = Val, DRB1*13:02 = Gly).

T cell clones or TCR-transgenic T cell lines (abbreviated as “effector cells” below) were co-

cultured with HLA-genotyped APCs in three different approaches to ascertain the HLA class II restriction of the TCR:

1. Presentation of endogenously processed antigens on HLA-DR, HLA-DQ or HLA-DP by autologous mini-LCLs was blocked by respective anti-human HLA antibodies (see table 2.14) to see which of the three HLA class II isoforms provided the epitope.
2. Effector cells were tested on a panel of HLA-matched as well as HLA-mismatched allogeneic mini-LCLs to narrow the variety of HLA candidates that could be presenting the epitope.
3. HLA-mismatched, transformed B cells were transfected to transiently express a certain HLA class II molecule (see section 3.3.3 and table 2.6) and pulsed with the peptide epitope (only possible after determining the TCR's epitope, as described in section 3.4.4).

Different effector-to-target cell (e:t) ratios were used, depending on the kind of effector cell, with T cell clones requiring a lower cell number for a sufficient response than TCR-transgenic T cell lines, and depending on the expression level of the HLA transgene on a transformed B cell line. Using T cell clones, the effector-to-target cell ratio of approach 1 and 2 usually was 10,000:20,000, whereas the ratio was 20,000:40,000 for HLA-transgenic DG-75 or 20,000:80,000 for HLA-transgenic Raji and EBV-B1.11. If TCR-transgenic T cell lines were used as effector cells, their amount was usually doubled while target cell numbers were kept because in general only 20 to 40% of the TCR-transgenic T cell lines are positive for the transduced TCR. If effector-to-target cell ratio was different than stated here, it is noted in the figure caption.
Each co-culture was incubated at 37 °C for 16–21 hours in 200 µl 10% FCS T cell medium per well in 96-well plates (V-bottom) in duplicates or triplicates. Secreted IFN-γ levels were analysed by enzyme-linked immunosorbent assay (ELISA, see section 3.4.5) to quantify the activation of T cells by recognition of their target peptide/HLA complex.

3.4.4 Epitope mapping using peptide libraries

In order to determine the epitope specificity of T cell clones or TCR-transgenic T cell lines, autologous or allogeneic, HLA-matched mini-LCLs that do not express any HCMV antigen ("control mini-LCLs") were pulsed with synthetic peptides of 15 amino acids length derived from the HCMV antigen pp65 (UL83). After pulsing control mini-LCLs with 24 different pp65 peptide pools (see section 2.6 and tables 2.12 & 2.13, final concentration: 1 µg/ml per 15-mer) for 1 hour at 37 °C, cells were washed three times in 1× PBS and co-cultured with T cell clones at a usual e:t ratio of 10,000:20,000 (i.e. 10,000 effector cells and 20,000 target cells in each individual 96-well reaction in a volume of 200 µl) in duplicates for 16–20 hours. If TCR-transgenic T cell lines were chosen as effector cells, the e:t ratio varied from 20,000:20,000 to 40,000:40,000, depending on availability of effector cells. Co-culture supernatants were analysed for IFN-γ secretion by ELISA (see section 3.4.5).
Epitopes of IE-1-specific $CD4^+$ T cell clones were determined by Stefanie Ameres (2012) using the IE-1 peptide library (table 2.11) organised in peptide pools as shown in table 2.13.

3.4.5 Enzyme-linked immunosorbent assay (ELISA) for quantification of cytokine release

IFN-γ production by effector cells (TCR-transgenic T cells or T cell clones) was analysed to quantify the antigen-specific recognition of target cells. Effector and target cells were co-

cultivated at different ratios for 16–21 hours in 200 µl 10% FCS T cell medium per well in 96-well plates (V-bottom) in duplicates or triplicates at 37 °C, after which the plates were either frozen at −20 °C or directly used in a "sandwich" ELISA according to the manufacturer's instructions (Mabtech, Nacka Strand, Sweden) with a few modifications. 10% FCS T cell medium was always used for co-cultivation of effector and target cells, because 5% HS T cell medium caused high and irregular background levels of IFN-γ. As a positive control for IFN-γ secretion, effector T cells were sometimes stimulated with 50 ng/ml tetradecanoylphorbol acetate (TPA) and 0.5 µM Ionomycin (Iono). Every step of the protocol was performed at room temperature unless noted otherwise.

Highly adsorbent, 96-well ELISA plates (MaxiSorp Immunoplate, Nunc A/S) were coated at 4 °C with 50 µl/well of the IFN-γ-specific monoclonal capture antibody 1-D1K diluted to 2 µg/ml in 1× PBS overnight. The following day, plates were washed twice with 200 µl ELISA washing buffer (0.05% Tween 20 in 1× PBS) per well to remove excess antibody, and were blocked for one hour at 37 °C using 100 µl 10% FCS T cell medium per well to cover non-specific binding sites. Plates were washed twice with 200 µl ELISA washing buffer per well before applying 50 µl of the co-culture supernatant to be analysed per well and incubating the plates for two hours to allow the plate-bound antibody to bind soluble IFN-γ. In parallel, a blank reaction and a cytokine standard series (Mabtech) spanning from 20 to 20,000 pg/ml were also set up in each of the plates to allow for quantification. Next, the plates were washed four times with 300 µl ELISA washing buffer per well and incubated with 50 µl/well of the biotinylated monoclonal detection antibody 7-B6-1 diluted to 1 µg/ml in 1× PBS. After one hour incubation, during which the antibody 7-B6-1 bound to IFN-γ, the plates were washed four times with 300 µl ELISA washing buffer per well and incubated for another hour with 50 µl/well of Streptavidin-ALP (diluted 1:1000 in 1× PBS) that attached to 7-B6-1 via biotin. Once again, the plates were washed four times with 300 µl ELISA washing buffer per well and 100 µl/well of alkaline phosphatase (ALP) substrate solution were added. The ALP substrate solution was freshly prepared by diluting 5× pNPP stock solution (50% diethanolamine and 5 mg/ml pNPP in sterile water at pH 9.5, stored at −20 °C) to 1× solution with sterile water, and adding $MgSO_4$ solution (stock concentration 200 mM; final concentration 0.8 mM), as a co-factor of ALP. ALP catalyses the hydrolysis of colorless pNPP to yellow para-nitrophenol, which has a maximum of absorbance at 405 nm that is measured by a spetrophotometer. The standard curve for calculating the concentration of each sample as well as the calculation itself were performed with Prism (GraphPad Software, La Jolla, USA) employing a sigmoidal four parameter logistic curve fit with the average blank value serving as lower asymptote of the curve fit.

The ELISA for quantification of **IL-10** secretion (R&D Systems, Minneapolis, USA) in the co-culture supernatant was performed analogously to that of IFN-γ, except that the biotinylated goat anti-human IL-10 detection antibody was diluted to 150 ng/ml in 1× PBS, and the cytokine standard spanned a range from 31.3 to 2,000.0 pg/ml.

3.5 Virus-based methods

3.5.1 Gene transfer via retroviral vectors

On day zero of the protocol, PBMCs were isolated from whole blood (section 3.3.4) of HCMV-seronegative ($HCMV^-$) donors and resuspended at 5×10^5 cells per millilitre in RPMI 1640 supplemented with 5% human serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 100 nM sodium selenite, 50 U/ml interleukin (IL)-2, 5 ng/ml IL-7 and 5 ng/ml IL-15. Bulk PBMCs were stimulated by either 50 ng/ml anti-CD3 antibody (Clone: OKT3, Biolegend, San Diego, USA) or 10 µM pamidronate (medac, Wedel, Germany) to trigger proliferation of total T cells or of δ2 T cells, respectively. Depending on the required number of stimulated T cells, 8, 4 or 2 ml of the resuspended cells were seeded into 6-, 12- or 24-well plates, respectively, and incubated for 72 hours at 37 °C.
In order to transduce selected T cell subpopulations, PBMCs were separated by MACS using an αβ TCR-specific antibody (section 3.4.1). The αβ T cells of the αβ TCR-enriched (positive) MACS fraction and $V\delta2^+$ T cells of the αβ TCR-depleted (negative) MACS fraction were stimulated with anti-CD3 antibody and pamidronate, respectively, as described above.

On day one, adherent HEK293T cells were transfected with plasmids encoding for the moloney MLV gag/pol genes, the MLV-10A1 env gene and MP71 retroviral vector plasmids coding either for a TCRβ–P2A–TCRα construct, CD4, CD8 or the GFP marker gene (see section 3.3.3). As a negative ("mock") control the plasmid carrying the TCR, co-receptor or GFP was omitted. 48 hours after transfection (i.e. on day three of the workflow), the supernatant of the HEK293T cells was harvested, filtrated (0.45 µm pore size) and directly used for transduction (Leisegang et al., 2008). If identical retrovirus supernatant was harvested from more than one well, the volume was pooled and mixed before filtration.
To prepare T cell transduction by spinoculation (centrifugal inoculation), on day two, non-tissue culture treated 24-well plates (tab. 2.18) were coated with 400 µl per well of retronectin (Takara Bio Europe, St. Germain en Laye, France) diluted in sterile H_2O to 12.5 µg/ml. After overnight incubation at 4 °C, the retronectin solution was discarded, the wells were blocked for 30 minutes at 37 °C using 500 µl per well of 2% FCS in 1× PBS and washed using 1.5 ml per well of 2.5% (v/v) HEPES in 1× PBS. Next, the washing solution was discarded, 1 ml of each filtrated retrovirus supernatant was added per well into the retronectin-coated 24-well plate and each well was supplemented with 4 µg/ml protamine sulfate, 1% (v/v) HEPES, 100 U/ml IL-2, 5 ng/ml IL-7 and 5 ng/ml IL-15 (all concentrations calculated for a final volume of 2 ml per well). In order to mix, the plate was carefully rocked back-and-forth and from side-to-side. Anti-CD3- or pamidronate-stimulated cell cultures were harvested, pooled, pelleted by centrifugation, counted and resuspended in RPMI 1640 without any supplements at a defined cell concentration in order to match the multiplicity of infection (MOI) for both stimulations. Finally, 1 ml of resuspended cell suspension per well was added drop-wise on top of the virus supernatant. After centrifuging the plate for 90 minutes at 1,000 × g in a pre-warmed (32 °C) centrifuge with low acceleration (level 1 out of 9) and switched-off brakes, the cells were incubated overnight at 37 °C.
On day four, each of the different transduction reactions was harvested, pooled if applicable, and the cells were pelleted and resuspended in the same amount of medium than before using RPMI

1640 supplemented with 5% human serum (HS), 100 U/ml penicillin, 100 µg/ml streptomycin, 100 nM sodium selenite and 100 U/ml IL-2 (henceforth abbreviated as "5% HS T cell medium"). The cells were incubated in 12- or 24-well plates at 37 °C for up to 8 days. Every three to four days, half of the cell culture medium was replaced by fresh medium or the cells were expanded 1:2 (i.e. from one to two wells) if rapid acidification was noticed (orange to yellow discolouration of the medium).
In order to assess the expression of the transgene, cells were usually stained with multimers and fluorochrome-labelled monoclonal antibodies and analysed by flow cytometry on day nine or ten. Testing of the transduced cells in any kind of cellular assay was generally done on day ten (7 days after transduction). If no assay was performed, the cells were either frozen as described in section 3.3.2 or restimulated by using an autologous or HLA-matched mini-LCL expressing the relevant HCMV antigen. For this purpose, the pp65- or IE-1-expressing mini-LCL was harvested and irradiated with 60 Gray, and 0.5×10^6 of them were combined with 1.5×10^6 transduced T cells per well of a 24-well plate. Cells were co-cultured in 2 ml 5% HS T cell medium per well at 37 °C for up to 8 days replacing cell culture medium or expanding the cells as described above. If the number of cells was higher, the cell counts were 2-fold up-scaled for 12-well plates, keeping the cell concentrations constant (4 ml/well).

A schematic overview of the whole process is depicted in figure 3.1.

3.5.2 Stocks of human cytomegalovirus

Stocks of HCMV strains AD169, TB40-BAC4, Merlin or CMV-Δall (see section 2.1) were generated by virus passage on human MRC-5 fibroblasts. Semi-confluent fibroblasts were infected with HCMV at a MOI of 0.1. HCMV replicates lytically in fibroblasts causing the release of virus progeny, NIEPs and dense bodies into the cell culture supernatant. 14-18 days later, when most cells displayed a cytopathic effect, cell culture supernatant was harvested. After clearing aspirated cells and cellular debris from the supernatant by two rounds of centrifugation at 300 × g and room temperature for 10 minutes, virus stocks were aliquoted and frozen at −80 °C.
The concentration of infectious virus particles in HCMV stock preparations ("virus titer") was determined by calculation of the tissue culture infection dose 50 ($TCID_{50}$), describing the amount of virus required to infect and pathologically change 50% of the wells in limiting dilution. Virus titers typically ranged from 10^6 to 10^7 $TCID_{50}$/ml. Multiplicity of infection (MOI) was defined as $TCID_{50}$ units per cell.
Well grown MRC-5 fibroblasts were diluted 1:3, 1:6 and 1:9 and seeded in 96-well plates (flat bottom) in 100 µl/well DMEM supplemented with 7% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, 100 nM sodium selenite and 1% non-essential amino acids. Virus stocks were serially diluted from 10^{-2}–10^{-9} using the same medium and 100 µl/well of diluted virus supernatant were applied to each dilution of MRC-5 fibroblast cells (12 wells per virus dilution for each MRC-5 dilution). Cytopathic effect of MRC-5 fibroblasts was evaluated no earlier than two weeks after infection by microscopic examination. $TCID_{50}$ per millilitre was calculated according to the Spearman-Kärber method (1908; 1931) using the following formula:

$$\log_{10}(\mathrm{TCID}_{50}) = x_0 - \frac{d}{2} + \frac{d}{n} \cdot \sum x_i + \log_{10} \frac{1}{v} \tag{2}$$

with

x_0	=	positive value of the exponent of the highest dilution, at which all test objects react positively
d	=	dose distance (factor separating dilutions given as log_{10}; in this case for 10×10^{-2}, 10×10^{-3}, 10×10^{-4}, etc. it is $log_{10}10 = 1$)
n	=	amount of test objects per dilution (in this case 12)
$\sum x_i$	=	sum of all test objects reacting positively starting from and including x_0
v	=	volume of virus supernatant applied per test object given in the same unit as TCID_{50} (in this case millilitre)

3.5.3 Recognition of HCMV-infected target cells by T cell clones or transduced T cell lines

In order to test the capability of T cell clones or TCR-transgenic T cell lines to recognise HCMV-infected target cells, either MRC-5 fibroblasts or mature monocyte-derived dendritic cells (moDCs) were used as target cells. Prior to infection, MRC-5 fibroblasts were treated with 300 U/ml IFN-γ for 72 hours and moDCs were differentiated from monocytes and matured (section 3.3.6). Target cells were infected with various strains of HCMV (table 2.1) at an MOI of 5. After 48 hours, infected target cells were washed three times in 1× PBS in order to remove residual free virus, cytokines, etc. Typically, 20,000 effector cells were co-incubated with 10,000 target cells for 16–21 hours in 200 µl 10% FCS T cell medium per well in 96-well plates (V-bottom) in duplicates or triplicates at 37 °C. Co-culture supernatants were analysed for IFN-γ secretion by effector cells in an ELISA (section 3.4.5). If T cell clones were tested, e:t ratio usually was 10,000:10,000, instead. As positive or negative control, non-infected target cells were peptide-pulsed with 1 or 5 µg/ml at 37 °C for one hour or were likewise incubated without applying any peptides, respectively. If two or more peptides were restricted by the same HLA, such as CRV and FRC both of which are HLA-C*07:02-restricted, control target cells were split and loaded with each peptide separately. If a different e:t ratio was chosen or any other parameter was altered, it is indicated in the caption of the respective figure.
Figure 3.1 provides a schematic overview of target cell preparations in the context of analysing the effector function of TCR-transgenic HLA class I- or HLA class II-restricted T cells.

Since DCs actively take up extracellular material, they may present viral peptides by ingesting and processing cellular debris, virus-like particles or defective virions without being infected themselves. As a result, these DCs could trigger IFN-γ release by HCMV-specific effector T cells just like truly HCMV-infected DCs. In order to discriminate between the recognition of viral antigens newly synthesised in the infected host cell and antigens that were introduced into the host cell by uptake of virions and their materials (e.g. structural proteins), control cells were infected with UV-inactivated HCMV supernatant. The UV irradiation damages viral DNA rendering it defective in translation and replication. UV inactivation of virus supernatant was performed at room temperature using the CL-1 Crosslinker (Herolab, Wiesloch, Germany) applying 1 J/cm^2. Virus supernatant was irradiated for 231 seconds in 6 well plates (max. 1.5 ml

per well) without the lid covering the plate.

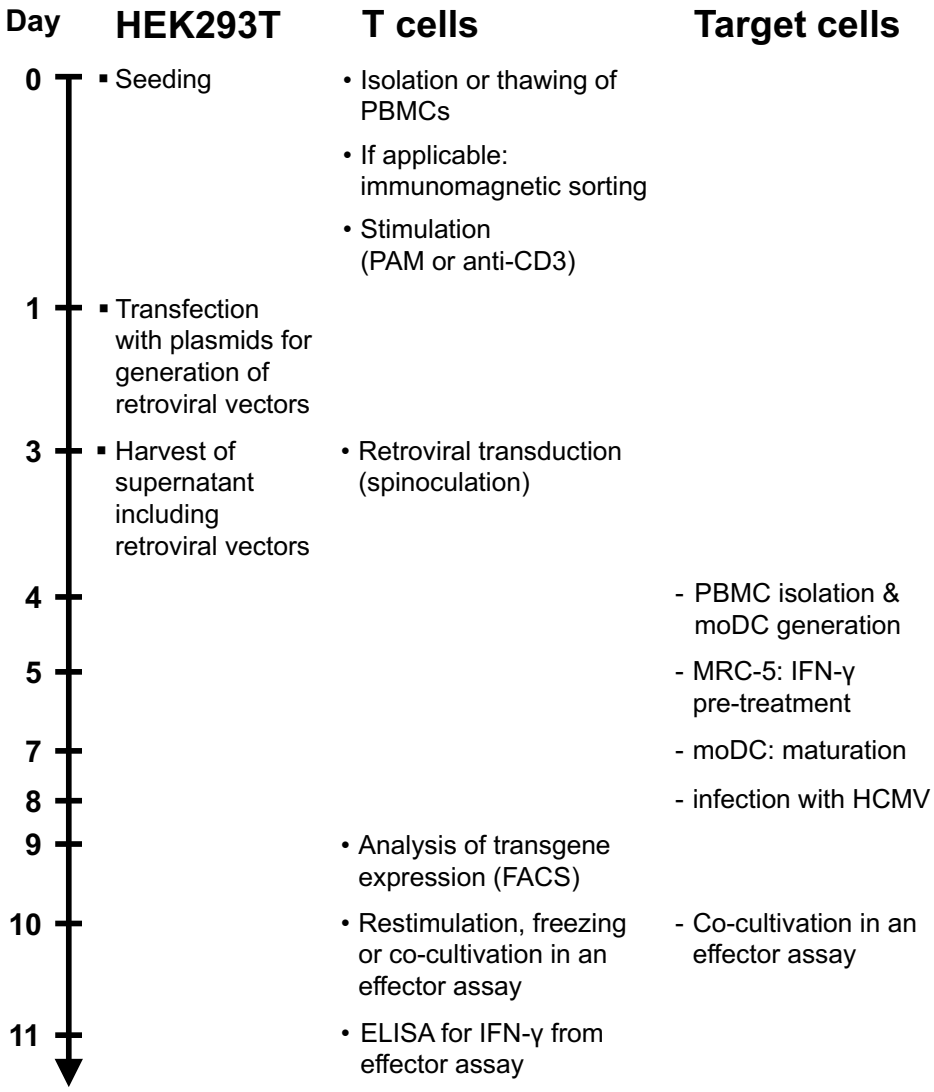


Figure 3.1. Schematic timeline of retroviral transduction of T cell lines and subsequent analysis of their function on HCMV-infected target cells. The timeline shows (I) production of retroviral vectors in HEK293T cells (left), (II) generation and use of TCR-transgenic effector cells (centre), and (III) generation and use of HCMV-infected target cells (MRC-5 fibroblasts or moDCs, right). More detailed information can be found within sections 3.3.3, 3.3.6, 3.5.1 and 3.5.3.

4 Results

HCMV-specific $CD8^+$ CTLs kill HCMV-infected target cells and play a crucial role in limiting replication of the virus (Einsele et al., 2002; Reddehase, 2002). If HCMV-specific $CD8^+$ immunity is restored by adoptive T cell transfer in immunocompromised patients after HSCT, they are protected from HCMV disease (Cobbold et al., 2005; Einsele et al., 2002; Riddell et al., 1992; Schmitt et al., 2011; Walter et al., 1995). Recently, IE-$1_{309\text{-}317}$ (CRV)-specific T cell clones restricted by HLA-C*07:02 were reported to strongly recognise HCMV-infected target cells despite the presence of immunoevasins, while other IE-1-specific T cell clones restricted by HLA-A or -B failed to recognise infection (Ameres et al., 2014, 2013). Thus, the TCRs from such T cells are clearly of interest for therapy development. Consequently, here the TCRs of HCMV-specific, HLA-C*07:02-restricted T cell clones were sequenced, transferred into primary T cells via retroviral vectors and their efficiency in mediating recognition of HCMV-infected target cells was assessed.

HCMV-specific $CD4^+$ T cells also play an important role in immunity against HCMV. They maintain the HCMV-specific $CD8^+$ T cell response (Riddell and Greenberg, 1995; Walter et al., 1995), provide help to $CD8^+$ T cells (Gabanti et al., 2014) thereby contributing to memory formation, and directly control HCMV infection by cytotoxic activity and secretion of pro-inflammatory molecules (Crompton et al., 2008; Jackson et al., 2017; Pachnio et al., 2016). Therefore, it is generally believed that they should be included in adoptive T cell transfer (Einsele et al., 2002; Sellar and Peggs, 2012). There is little published information on HCMV-specific, HLA class II-restricted TCRs, and none of them has been tested on HCMV-infected target cells. In the present study, a set of pp65-specific or IE-1-specific TCRs restricted by HLA class II was sequenced, transferred into primary T cells via retroviral vectors, and the TCRs' efficacy in mediating recognition of HCMV-infected target cells was assessed.

4.1 Analysis of HCMV-specific T cell clones

The $CD4^+$ and $CD8^+$ T cell repertoires against HCMV have been intensively studied (Ameres et al., 2015; Elkington et al., 2003; Khan et al., 2007; Nastke et al., 2005; Slezak et al., 2007; Sylwester et al., 2005, to name but a few). Nevertheless, HCMV-specific T cell clones were often characterised incompletely, and occasionally they seem to have been assigned an incorrect HLA restriction or epitope specificity, as will become clear in the following description. Some IE-1-specific $CD4^+$ T cells have been analysed in detail by Ameres et al. (2015) including epitopes and HLA-restrictions, but their TCR sequences were not determined.
In earlier work of this group, pp65-specific $CD4^+$ T cell clones were characterised (Schub, 2010; Wiesner, 2005), but with some lack of detail of their epitope specificity and HLA restriction and with no information about their TCR sequences. Such analyses were now performed for this thesis.

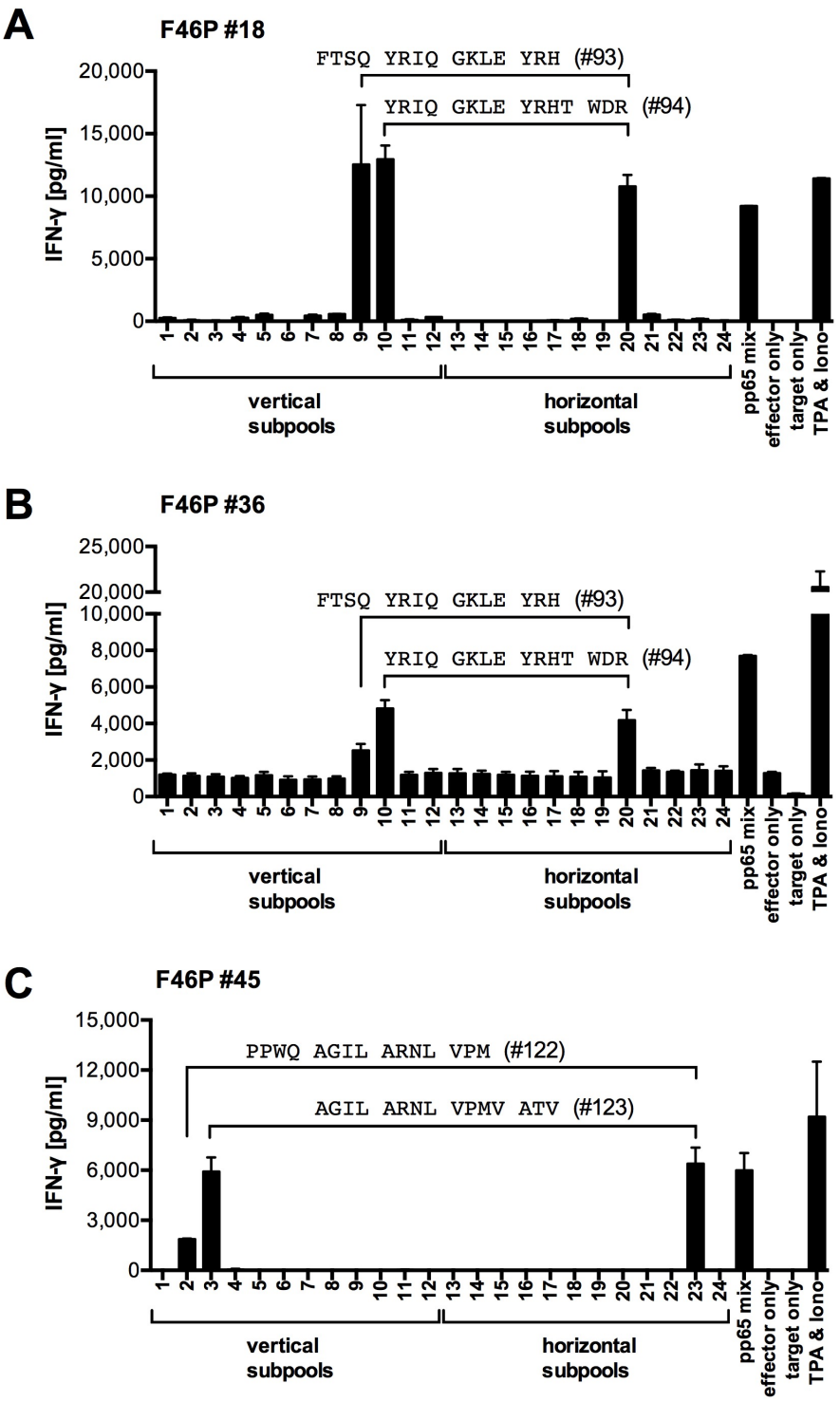


Figure 4.1. Epitope mapping of pp65-specific CD4$^+$ T cell clones F46P #18 (A), #36 (B) & #45 (C). After pulsing autologous mini-LCLs with 24 different pools of pp65 peptides (see tab. 2.12 & 2.13) they were co-incubated overnight with three different pp65-specific T cell clones derived from donor F46 at an effector-to-target (e:t) ratio of 10,000:20,000 (i.e. 10,000 T cells and 20,000 B cells in each individual 96-well reaction in a volume of 200 µl). The graphs show the concentration of secreted IFN-γ in the supernatant as evaluated by ELISA. The mean and standard deviation of duplicate samples are shown. As positive controls, mini-LCLs were pulsed with a peptide mixture containing all pp65 peptides of the pp65 library ("pp65 mix") and T cell clones were stimulated by TPA & ionomycin in the absence of target cells. Effector and target cells were incubated separately from each other to serve as negative controls ("effector only" and "target only").

4.1.1 Epitope determination of T cell clones

All HCMV-specific T cell clones analysed and used in this study were generated by Andreas Moosmann and Martina Wiesner (2005), Andrea Schub (2010), Stefanie Ameres (2012) or Xiaoling Liang (unpublished). As described in section 3.3.5, they isolated PBMCs from HCMV$^+$ donors and stimulated them with autologous mini-LCLs endogenously expressing either pp65 or IE-1. Resulting T cell lines were subjected to limiting dilution resulting in numerous T cell clones. The epitope specificity of T cell clones was determined by co-incubation with autologous or HLA-matched mini-LCLs that do not express any HCMV antigen, but had been pulsed with synthetic peptides before (see section 3.4.4). These peptides were derived from HCMV strain AD169's pp65 (UL83, tab. 2.12) or IE-1 (UL123, tab. 2.11) protein and were pooled in a cross-matrix fashion as described by Kern et al. (1999) and as depicted in table 2.13. Every single peptide was contained within one of the vertical subpools (from 1 to 12) and within one horizontal subpool (from 13 to 23 for pp65 or from 13 to 24 for IE-1). After overnight incubation, IFN-γ secretion by T cell clones was analysed by ELISA to quantify their antigen-specific recognition of target cells (see section 3.4.5).
The epitopes of all CD8$^+$ T cell clones were identified by Martina Wiesner (2005), Andrea Schub (2010) or Xiaoling Liang (unpublished).

Figure 4.1 shows examples of this epitope determination for three pp65-specific CD4$^+$ T cell clones. The CD4$^+$ T cell clones F46P #18 and #36 reacted to pools 9, 10 and 20 (fig. 4.1 A and B, respectively). The peptide pool no. 20 shares peptides no. 93 (`FTSQ YRIQ GKLE YRH`) and 94 (`YRIQ GKLE YRHT WDR`) with pools 9 and 10, respectively, so both clones recognised the 11-mer `YRIQ GKLE YRH` and were hence tagged with the abbreviation YRIQ. While F46P #18 secreted a similar amount of IFN-γ in response to each of these three peptide pools, F46P #36 showed an approximately 2-fold reduced IFN-γ secretion in response to pool no. 9 in comparison to pool no. 10 indicating a preference for peptide no 94 that was later confirmed using TCR-transgenic T cells (data not shown). F46P #45 reacted to pools 2, 3 and 23 (fig. 4.1 C), which share peptides no. 122 (`PPWQ AGIL ARNL VPM`) and 123 (`AGIL ARNL VPMV ATV`). While the 11-mer `AGIL ARNL VPM` contained in both peptides seems to be sufficient for recognition of pulsed mini-LCLs by the T cell clone, peptide no. 123 provoked an approximately 3-fold increased IFN-γ secretion. As a result, F46P #45 was tagged with the epitope specificity abbreviated as AGIL.

The epitopes of all IE-1-specific CD4$^+$ T cell clones shown in this work (see tab. 4.2) have been identified by Stefanie Ameres (2012).
Martina Wiesner (2005) determined the epitopes of the pp65-specific T cell clones F46P #17, #19, #40 and F62P #16 as (`QPFM`) `RPHE RNGF TVL` (`CPKN`), (`AGIL`) `ARNL VPMV ATV` (`QGQN`), `MSIY VYAL PLKM LNI` and (`GQNL`) `KYQE FFWD AND` (`IYRI`), respectively. Except F46P # 40, these T cell clones each recognised two peptides overlapping in 11 amino acids (shown between the brackets). The epitope specificity of F46P #17 had to be corrected later to (`TRNP`) `QPFM RPHE RNG` (`FTVL`), as demonstrated later in this section (fig. 4.7 A). Also, the epitope specificity of F46P #19 had to be corrected because this T cell clone was shown to express the identical TCR chains as F46P #45 (tab. 4.2) and F46P #45 recognised (`PPWQ`) `AGIL ARNL VPM` (`VATV`), but not peptide

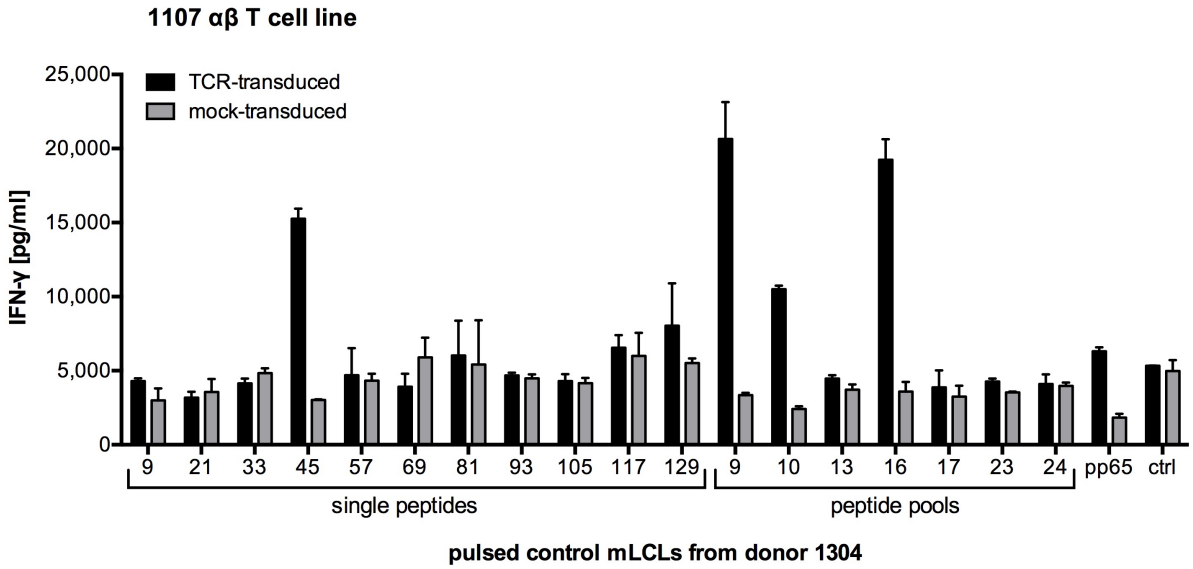


Figure 4.2. Epitope mapping of the TCR from pp65-specific CD4$^+$ T cell clone 1304P #21 using TCR-transgenic αβ T cell lines. After pulsing control (ctrl) mini-LCLs from donor 1304 that do not express any HCMV antigen with one of 11 single peptides (tab. 2.12) or one of 7 peptide pools of pp65 peptides (tab. 2.13) they were co-incubated overnight with αβ T cells from donor 1107 that had either been TCR-transduced (black bars) or mock-transduced (grey bars) 7 days before this assay. Effector cells have also been tested on mini-LCLs from donor 1304 endogenously expressing pp65 ("pp65") or not expressing any HCMV antigen ("ctrl"). The graphs show the concentration of secreted IFN-γ in the supernatant as evaluated by ELISA. e:t ratio was 30,000:20,000. The mean and standard deviation of duplicate samples are shown.

no. 124 (fig. 4.1 C) as suggested by Wiesner (2005) for F46P #19. Since both T cell clones express the same TCR, F46P #19 certainly recognises the same epitope as F45P #45 and was consequently re-labelled as AGIL-specific. F46P #40 could not be established after thawing the last remaining aliquot of it, hence the epitope specificity of this T cell clone could not be checked. The specificity of F62P #16 for peptide no. 128 could be verified in this study (fig. 4.8 A).

Andrea Schub (2010) generated and analysed the CD4$^+$ T cell clone 1304P #21, but tested its reactivity against a selection of pp65 peptides only, using APCs pulsed with peptide pools 1-9, 11, 12, 14, 15 and 17-22. She found this T cell clone to strongly react to pool no. 9 and very weakly to pool no. 17, which share peptide no. 57 (`KVYL ESFC EDVP SGK`), and consequently reported peptide no. 57 as the epitope.
Due to the scarcity of frozen clone aliquots, further analyses of 1304P #21 within the present study, except for HLA blocking (fig. 4.3 F) and HLA mismatch (fig. 4.6 B), were performed using TCR-transgenic T cell lines. For this purpose, the gene sequences of the TCRα and TCRβ chains that were expressed by this T cell clone were analysed (section 4.1.3) and assembled *in silico* to construct a retroviral vector plasmid, which coded for both TCR chains within a single gene (section 4.1.4). Recombinant retroviruses were produced by HEK293T cells and carried the designated TCR construct as cargo DNA (section 3.5.1). PBMCs were isolated from healthy, HCMV$^-$ adults and the T cells were stimulated to become susceptible for transduction with a retrovirus encoding the TCRα and TCRβ chains that had been isolated from T cell clone 1304P

#21. As a control for unspecific reactivity, such as allogeneic recognition of peptide-loaded APCs by the transduced T cell line, another T cell line was tested simultaneously that had been transduced with "empty" MP71 retrovirus (no genetic cargo DNA = "mock-transduced"). The same procedure was performed to analyse other HCMV-specific TCRs if the T cell clones from which the TCRs had been isolated were scarce or not available any more. T cells that had been transduced with a retrovirus encoding the TCR isolated from 1304P #21 only recognised peptide no. 45 (`EPDV YYTS AFVF PTK`) out of eleven peptides contained in pool no. 9 well above background recognition by mock-transduced T cells (fig. 4.2). Also peptide pools no. 9, 10 and 16 were recognised by TCR-transgenic T cells above background, while pools no. 13, 17, 23 and 24 did not elicit a specific response. Pool no. 16 shares peptides no. 45 (`EPDV YYTS AFVF PTK`) and 46 (`YYTS AFVF PTKD VAL`) with pools no. 9 and 10, respectively. Since pool no. 9 was recognised about twice as strong as pool no. 10 and peptide no. 46 failed to activate TCR-transgenic T cells in a separate experiment (data not shown), the T cell clone and its TCR were tagged with the abbreviation of epitope 45 (i.e. EPDV).

4.1.2 HLA restriction of T cell clones

T cells can become active when their TCR recognises their cognate antigen, which is a complex of specific peptide bound to a particular HLA. Binding of both elements by the TCR is required. Therefore, in adoptive T cell therapy, the patient must have the appropriate HLA for the T cells to work. This makes it important to precisely identify the required HLA – in other words, the HLA restriction of the TCR.

The **HLA class I restriction** of $CD8^+$ T cell clone MDP #19 YSE was identified as HLA-A*01:01 by Andrea Schub (2010). IE-$1_{309\text{-}317}$ (CRV)-specific and $UL28_{327\text{-}335}$ (FRC)-specific T cell clones were found to be restricted by HLA-C*07:02 (Ameres et al., 2013; Kim et al., 2011b, respectively).

There are three different isoforms of HLA class II, namely HLA-DR, HLA-DQ and HLA-DP. Each isoform consists of two different polypeptide chains, α and β, that are encoded by separate genes in the HLA class II region and are very polymorphic except for HLA-DRA (= HLA-DRα). In contrast to HLA class I molecules, both chains of an HLA class II heterodimer participate in the presentation of antigenic peptides and are thus relevant for determining HLA restriction. The HLA class II restriction of $CD4^+$ T cell clones was identified in a three step consecutive process.
First, the restriction was narrowed down to one of the three HLA class II isoforms. **Anti-HLA-DR, -HLA-DQ or -HLA-DP antibodies** were used to block interactions of the peptide/HLA complexes on autologous antigen-expressing mini-LCLs with TCRs of $CD4^+$ T cell clones. Blocking of an irrelevant HLA class II isoform did not affect the recognition of presented epitopes by a T cell clone, whereas blocking the particular HLA class II isoform that can present the epitope of a T cell clone strongly decreased or obviated the IFN-γ secretion. In this manner, F46P #17, #18, #40 and #45 were identified as HLA-DR-restricted, while F62P #16 and 1304P #21 were restricted by HLA-DQ (fig. 4.3). F46P #36 could not be tested because the clone died before

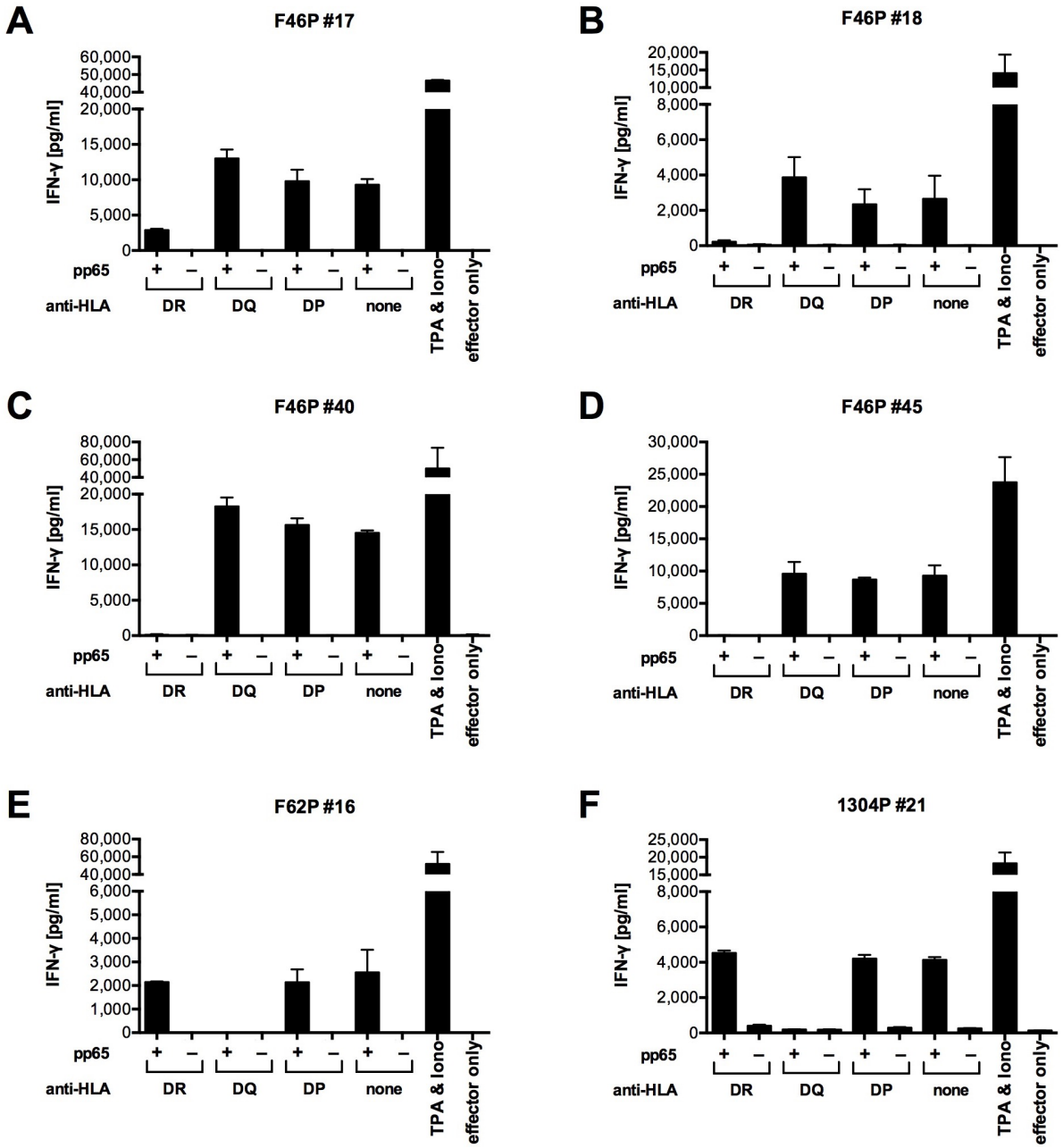


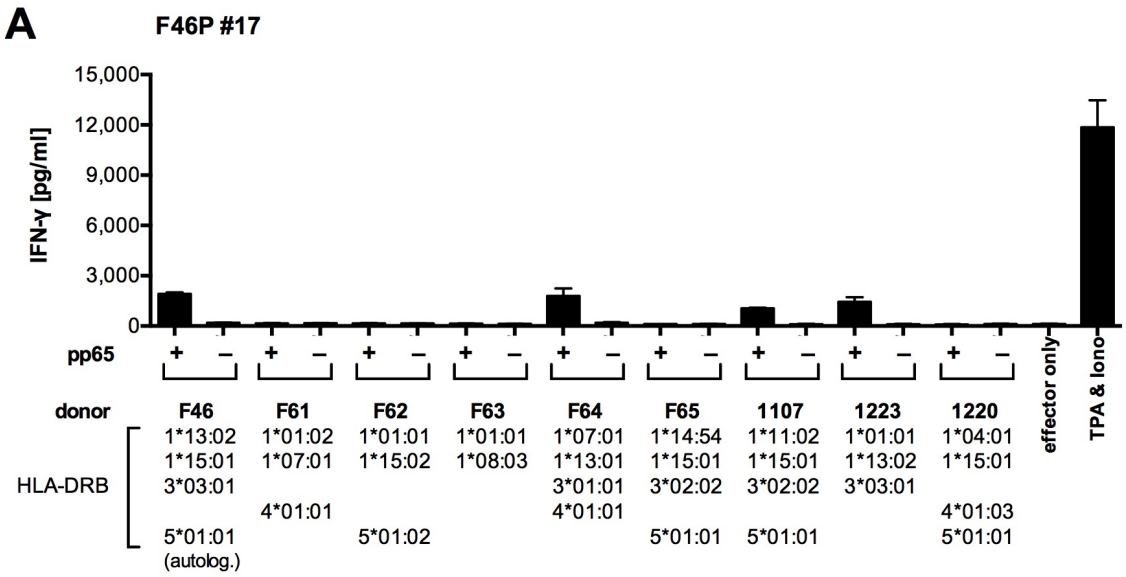
Figure 4.3. Blocking of HLA-DR, -DQ or -DP on pp65-expressing mini-LCLs to narrow HLA restriction of CD4$^+$ T cell clones. After blocking autologous pp65-expressing mini-LCLs with anti-human HLA antibodies for 30 minutes at 37 °C, they were co-incubated overnight with T cell clones at an e:t ratio of 10,000:20,000. Stimulation by TPA and ionomycin served as positive control, while effector cells in the absence of mini-LCLs were evaluated as negative control ("effector only"). The graphs show the concentration of secreted IFN-γ in the supernatant as evaluated by ELISA. The mean and standard deviation of duplicate samples are shown. Each experiment has been repeated once with equivalent results.

any assay could be performed.

Next, T cell clones were challenged by a **panel of HLA-matched as well as HLA-mismatched allogeneic mini-LCLs** in order to identify HLA molecule candidates that could be presenting the epitope. Apart from pp65-expressing mini-LCLs, control mini-LCLs from the same donors were also included in the panel as negative controls and to identify potential alloreactivity of T cell clones. No reactivity against control mini-LCLs, whether matched or mismatched, was observed for the T cell clones described in this section.
The HLA-DR-restricted T cell clone F46P #17 reacted to pp65-expressing mini-LCLs from donors F46 (autologous), F64, 1107 and 1223 (fig. 4.4 A). 1223 matches F46 in DRB1*13:02 and F64 harbours HLA-DRB1*13:01, which is very similar in amino acid sequence to DRB1*13:02 (>99% homology, Marsh et al., 2000). 1107 matches F46 in HLA-DRB1*15:01 and DRB5*01:01, but two other donors (F65 and 1220) with the same HLA alleles did not elicit a response. While recognition of mini-LCLs from donor 1107 could not be explained, HLA-DRB1*13:01 and DRB1*13:02 were identified as the most likely restricting HLA alleles for F46P #17.
Similar to F46P #17, T cell clone F46P #18 responded to pp65-expressing mini-LCLs from donors F46 (autologous), F64 and 1223 (fig. 4.4 B). Thus, HLA-DRB1*13:01 and DRB1*13:02 are most likely the restricting HLA alleles for F46P #18, too.
In contrast, F46P #45 secreted IFN-γ after encountering pp65-expressing mini-LCLs from donors F46 (autologous), F65, 1107 and 1223 (fig. 4.4 C). The donor 1223 matches F46 in HLA-DRB3*03:01. F46, F65 and 1107 share HLA-DRB1*15:01 and DRB5*01:01, but 1220 argues against this HLA restriction because 1220 also harbours these two alleles, but has not been recognised by F46P #45. F65 and 1107 both have HLA-DRB3*02:02, which is similar to HLA-DRB3*03:01 (>95% homology, Marsh et al., 2000). Thus, T cell clone F46P #45 is likely restricted by HLA-DRB3*03:01 and DRB3*02:02.

Due to a lack of frozen aliquots of T cell clone F46P #40, further analyses of its HLA restriction were performed using TCR-transgenic T cell lines. For this purpose, αβ T cells had been transduced with the retrovirus MP71 carrying the TCRα and TCRβ genes isolated from F46P #40 (MSIY40, tab. 4.2). These TCR-transgenic T cells from donor 0112 recognised pp65-expressing mini-LCLs from two donors that were positive for HLA-DRB1*15:01 and DRB5*01:01 (F46 & F65, fig. 4.5). The pp65-expressing mini-LCL from donor F64 was also recognised by TCR-transgenic T cells despite the fact that it does not share any HLA-DRB allele with F46. In another experiment using the same effector cells, a pp65-expressing mini-LCL from donor 1626 that is positive for HLA-DRB1*15:01 and DRB5*01:01 was also recognised (data not shown). Again, pp65-expressing mini-LCLs from donor F64 were recognised despite the HLA mismatch, whereas HLA-mismatched mini-LCLs from donor 1223 were not recognised by MSIY40-transgenic T cells. While recognition of mini-LCLs from donor F64 could not be explained, HLA-DRB1*15:01 or DRB5*01:01 were identified as the most likely restricting HLA alleles for F46P #40.

The HLA-DQ-restricted T cell clone F62P #16 recognised four out of four pp65-expressing mini-LCLs, all from different donors that were positive for HLA-DQB1*05:01 (fig. 4.6 A). pp65-expressing mini-LCLs from five donors who lacked this HLA allele were not recognised, and


A
F46P #17
IFN-γ [pg/ml]
15,000
12,000
9,000
6,000
3,000
0
pp65
+ – + – + – + – + – + – + – + – + –
donor
HLA-DRB
F46
1*13:02
1*15:01
3*03:01
5*01:01
(autolog.)
F61
1*01:02
1*07:01
4*01:01
F62
1*01:01
1*15:02
5*01:02
F63
1*01:01
1*08:03
F64
1*07:01
1*13:01
3*01:01
4*01:01
F65
1*14:54
1*15:01
3*02:02
5*01:01
1107
1*11:02
1*15:01
3*02:02
5*01:01
1223
1*01:01
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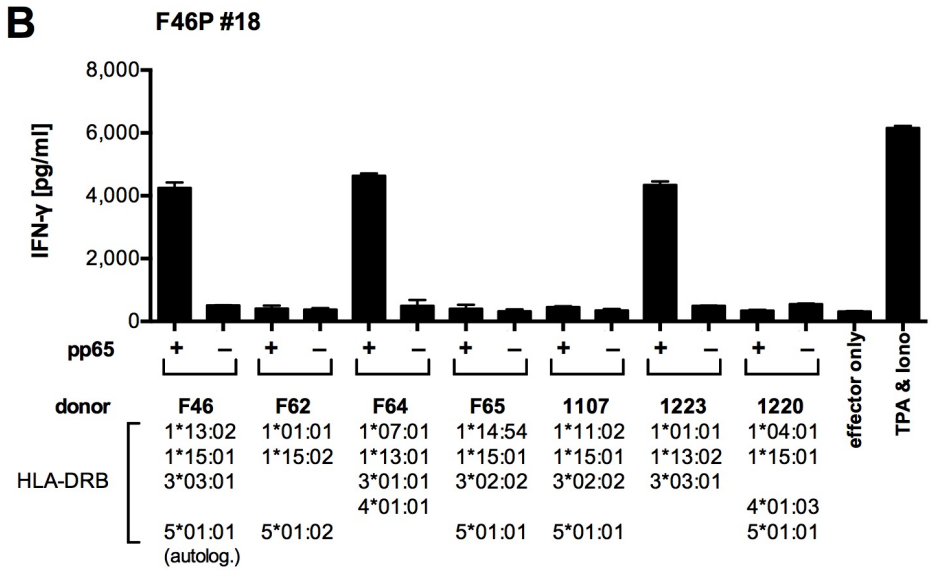
1220
1*04:01
1*15:01
4*01:03
5*01:01
effector only
TPA & Iono


B
F46P #18
IFN-γ [pg/ml]
8,000
6,000
4,000
2,000
0
pp65
+ – + – + – + – + – + – + –
donor
HLA-DRB
F46
1*13:02
1*15:01
3*03:01
5*01:01
(autolog.)
F62
1*01:01
1*15:02
5*01:02
F64
1*07:01
1*13:01
3*01:01
4*01:01
F65
1*14:54
1*15:01
3*02:02
5*01:01
1107
1*11:02
1*15:01
3*02:02
5*01:01
1223
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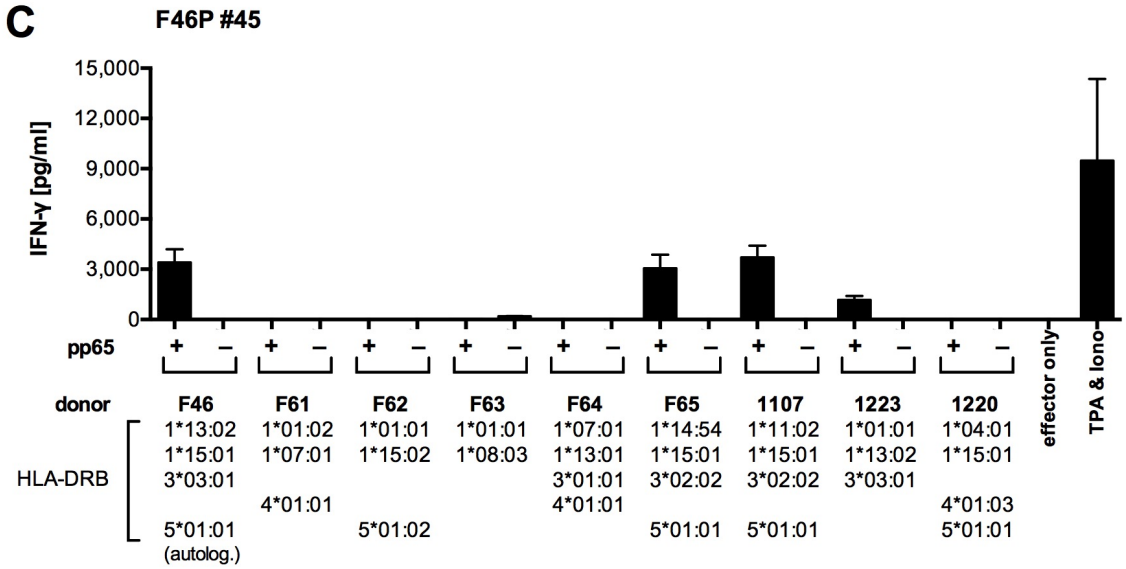
5*01:01
effector only
TPA & Iono


C
F46P #45
IFN-γ [pg/ml]
15,000
12,000
9,000
6,000
3,000
0
pp65
+ – + – + – + – + – + – + – + – + –
donor
HLA-DRB
F46
1*13:02
1*15:01
3*03:01
5*01:01
(autolog.)
F61
1*01:02
1*07:01
4*01:01
F62
1*01:01
1*15:02
5*01:02
F63
1*01:01
1*08:03
F64
1*07:01
1*13:01
3*01:01
4*01:01
F65
1*14:54
1*15:01
3*02:02
5*01:01
1107
1*11:02
1*15:01
3*02:02
5*01:01
1223
1*01:01
1*13:02
3*03:01
1220
1*04:01
1*15:01
4*01:03
5*01:01
effector only
TPA & Iono

no control mini-LCL was recognised. Therefore, the experiment strongly suggested that the T cell clone was restricted through HLA-DQB1*05:01, and its specificity for pp65 and absence of alloreactivity was confirmed. Because HLA-DQA was not known for all target cells, the restricting DQA chain was not identified with the same high level of confidence, but the results were consistent with restriction through DQA1*01:01. Since most HLA haplotypes with DQB1*05:01 have DQA1*01:01 (Klitz et al., 2003), the T cell clone and TCR are likely to recognise pp65 in a majority of donors who express this DQB1 chain.
T cell clone 1304P #21 recognised the autologous and two HLA-matched pp65-expressing mini-LCLs, all of which shared HLA-DQB1*03:01 (fig. 4.6 B). In this experiment, the transformed B cell lines Raji and EBV-B1.11 were pulsed with the wrong peptide (pp65 peptide no. 57 = KVYL, tab. 2.12) because the error in epitope determination of 1304P #21 was identified and corrected later (pp65 peptide no. 45 = EPDV, see section 4.1.1). As a result, neither Raji nor EBV-B1.11 could be recognised by the T cell clone after peptide loading despite the fact that EBV-B1.11 cells have HLA-DQB1*03:01. HLA-DQA1*05:01 is most likely the corresponding DQA1 chain that presents the peptide to T cell clone 1304P #21 along with DQB1*03:01, since no other HLA-DQA allele was found to be expressed by donor 1304. As two thirds of HLA haplotypes with DQB1*03:01 have DQA1*05:01 (Klitz et al., 2003), this TCR is likely to recognise pp65 in the majority of donors who express this DQB1 chain. However, EPDV peptides may also be presented by DQB1*03:01 in association with other DQA1 molecules, since donors 0519 and 1220 do not possess DQA1*05:01 but DQA1*01:02 & DQA1*05:05 and DQA1*01:02 & DQA1*03:03, respectively (fig. 4.6 B).

Certain HLA alleles, such as HLA-DRB1*13:02 and HLA-DRB3*03:01, are often in close linkage disequilibrium as elements of the same HLA haplotype, making these HLA restrictions difficult to distinguish in a match/mismatch setting. Thus, HLA candidates were individually studied by **transfection of HLA-mismatched, transformed B cells with plasmids coding for recombinant HLA molecules**. B cell lines Raji, DG-75 and EBV-B1.11 were used. After externally loading (also called "pulsing") the transiently HLA-transfected B cells with the chemically synthesised peptide epitope, T cell clones or TCR-transgenic T cell lines were co-incubated and IFN-γ secretion was analysed in order to ascertain the HLA restriction. Since HLA-DRA is not polymorphic, it was assumed that endogenous DRA would be sufficient, and recombinant DRA was not co-transfected together with plasmids coding for HLA-DRB chains. Figure 4.7 C shows an experiment that confirmed this assumption, since there was similarly good recognition of targets when transfected with DRB regardless of DRA co-transfection.

Figure 4.4 . ***(displayed on preceding page)***
Recognition of HLA-matched and -mismatched mini-LCLs by the CD4$^+$ T cell clones F46P #17 (A), #18 (B) and #45 (C). pp65-expressing mini-LCLs ("+") and respective control mini-LCLs ("–") from several donors were co-incubated overnight with T cell clones at an e:t ratio of 10,000:20,000. "autolog." marks the donor from which the T cell clone originated. Stimulation by TPA and ionomycin served as positive control, effector cells in the absence of mini-LCLs as negative control ("effector only"). The HLA-DRB type of each donor is given below each graph (blanks indicate absence of HLA alleles). The graphs show the concentration of secreted IFN-γ in the supernatant as evaluated by ELISA. The mean and standard deviation of duplicate samples are shown.

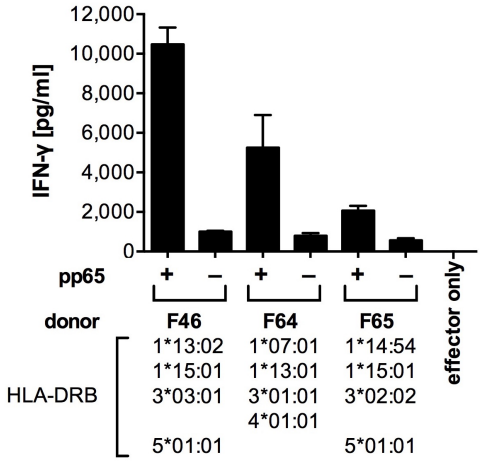


Figure 4.5. Recognition of HLA-matched and -mismatched mini-LCLs by a TCR-transgenic αβ T cell line that expressed the TCR from pp65-specific CD4$^+$ T cell clone F46P #40. pp65-expressing mini-LCLs ("+") and respective control mini-LCLs ("–") from three donors were co-incubated overnight with αβ T cells from donor 0112 that had been transduced with a retrovirus coding for MSIY40 seven days before this assay. e:t ratio was 40,000:40,000. The HLA-DRB type of each donor is given below each graph (blanks indicate absence of HLA alleles). The graphs show the concentration of secreted IFN-γ in the supernatant as evaluated by ELISA. The mean and standard deviation of duplicate samples are shown.

The T cell clone F46P #17 was reported to be specific for overlapping peptides no. 66 (`QPFM RPHE RNGF TVL`) and 67 (`RPHE RNGF TVLC PKN`) of the pp65 library (tab. 2.12), which overlap in the sequence `RPHE RNGF TVL` (Wiesner, 2005). However, multiple re-evaluations of the specificity of F46P #17 using autologous control mini-LCLs pulsed with peptides no. 65 (`TRNP QPFM RPHE RNG`), 66 or 67 contradicted this and suggested a somewhat different specificity: Peptide no. 65 caused a profound IFN-γ secretion by the T cell clone F46P #17 (2,200 pg/ml), peptide no. 66 provoked a strong secretion (19,000 pg/ml) and peptide no. 67 resulted in negligible secretion (10 pg/ml, fig. 4.7 A). Thus, the epitope of this T cell clone centres on the sequence `QPFM RPHE RNG` with a clear preference for peptide no. 66 over 65 and consequently F46P #17 was tagged with the epitope's abbreviation QPFM.

The T cell clone F46P #17 QPFM responded to HLA-mismatched Raji cells transfected with HLA-DRB1*13:01, but not to Raji cells transfected with HLA-DRB1*13:02 (fig. 4.7 A). The experiment was repeated four times using different aliquots of the T cell clone and different target cells with equivalent results. The amino acid sequence of mature HLA proteins DRB1*13:01 and DRB1*13:02 is identical except for a single amino acid which is located within the β1 domain: DRB1*13:01 has valine at amino acid position 86, whereas DRB1*13:02 has glycine (Bondinas et al., 2007; Marsh et al., 2000). Amino acid no. 86 of HLA-DRB contributes to peptide binding and is located close to the end of the α-helix that lines the binding cleft of the αβ HLA-DR heterodimer. Due to the high homology of both HLAs and because DRB1*13:02 was the molecule expressed by the original T cell donor, it seemed unlikely that this lack of responsiveness of the T cell clone to HLA-DRB1*13:02-transfected and peptide-loaded target cells meant that the epitope could only be presented by its close allogeneic variant HLA-DRB1*13:01. Instead,

it seemed more plausible that expression of transfected HLA-DRB1*13:02 or its peptide binding were corrupted. Sequencing data from Josef Mautner revealed that the plasmid that was used for transfection (tab. 2.6) coded for an Asp-to-Tyr mutation at amino acid position 70 of HLA-DRB1*13:02. Since the amino acid at position 70 of mature HLA-DRB1 protein contributes to peptide and TCR binding simultaneously (Bondinas et al., 2007; Marsh et al., 2000), any amino acid replacement is likely to affect both. Replacement of an acidic residue by a bulky aromatic residue will likely have a strong effect on the binding of peptides and TCRs that usually interact with HLA-DRB1*13:02. Further analyses were conducted without transfection of erroneous HLA-DRB1*13:02 and HLA-DRB1*13:01 was used as a surrogate due to high sequence similarity. Nevertheless, F46P #17 is likely restricted by both HLA-DRB1*13:01 and DRB1*13:02 because two target cell lines with endogenous expression of DRB1*13:02, but not DRB1*13:01 were recognised, and DRB1*13:01-transfected target cells were recognised as well (fig. 4.4 A and 4.7 A, respectively).

At the time of performing the HLA transfections, F46P #18 and #36 T cell clones were not available any more. So TCR-transgenic αβ T cell lines from donor 0112 were used as effector cells for determination of HLA restriction, instead. DG-75 cells were transfected with HLA-DRB1*15:01 or DRB5*01:01 and Raji cells were transfected with DRB1*13:01 or DRB3*03:01, covering all HLA-DRB alleles of donor F46 except for DRB*13:02. Both YRIQ18- and YRIQ36-transgenic T cell lines only responded to HLA-DRB1*13:01-transfected, peptide-pulsed cells (fig. 4.7 B). For reasons explained in the above paragraph, DRB1*13:02 could not be tested, but because of the close similarity between autologous DRB1*13:02 and the related molecule DRB1*13:01, it is likely that either HLA presents the peptide to the T cell clone.

The putative HLA restriction of F46P #40 MSIY was HLA-DRB1*15:01 (Wiesner, 2005), but TCR-transgenic T cells that expressed the TCR MSIY40, which was isolated from F46P #40, were not activated by target cells transfected with HLA-DRB1*15:01 and loaded with pp65 peptide no. 28 (MSIY, tab. 2.12) in three independent experiments (data not shown). Neither the variant HLA-DRB1*15:02 of this allotype, nor the molecule DRB5*01:01, whose gene is in strong linkage to DRB1*15:01 and therefore sometimes difficult to distinguish from it as a restriction element, activated TCR-transgenic T cells. Since the HLA-DRB1*15:01 tetramer also failed to bind to T cell lines transgenically expressing the TCR MSIY40 (section 4.4.2, data not shown), several lines of evidence indicate that HLA-DRB1*15:01 is probably not the restriction element for this T cell clone. As strong binding of MSIY peptide to HLA-DRB1*15:01 was reported in an artificial *in vitro* assay (Muixí et al., 2008), it is also possible that MSIY is not the epitope which is recognised by T cell clone F46P #40. Thus, the HLA restriction of TCR MSIY40 remains elusive and its epitope specificity should be reviewed.

For determining the HLA restriction of the T cell clone F46P #45 AGIL, EBV-B1.11 cells were transfected with HLA-DRB3*01:01, DRB3*02:02 or DRB3*03:01. As a separate condition, HLA-DRA1*01:01 and HLA-DRB3*03:01 were transfected simultaneously to assess the effect of co-transfected HLA-DRA on antigen recognition by F46P #45. The T cell clone produced IFN-γ in response to peptide-pulsed EBV-B1.11 cells if they were transfected with HLA-DRB3*02:02

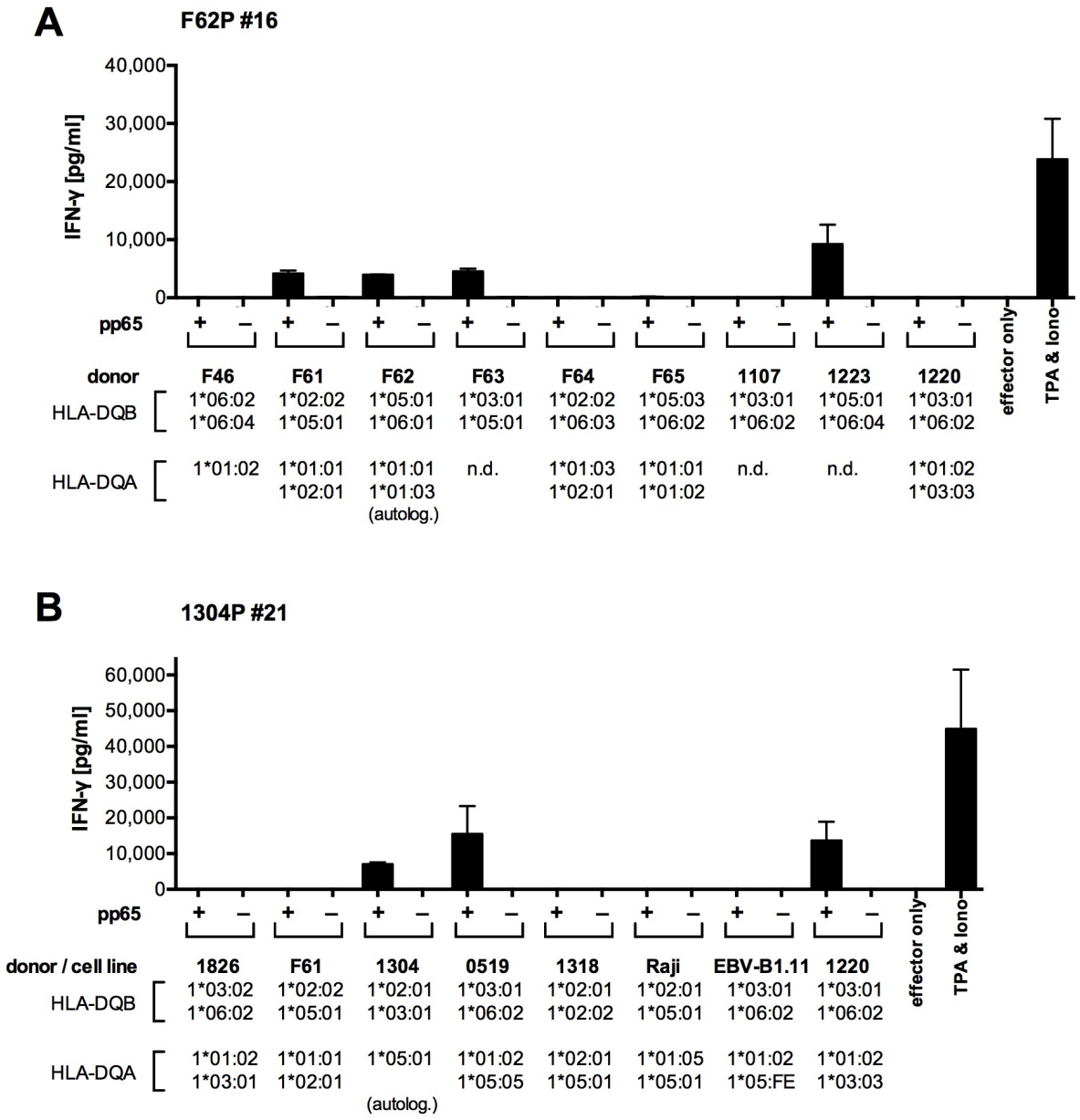
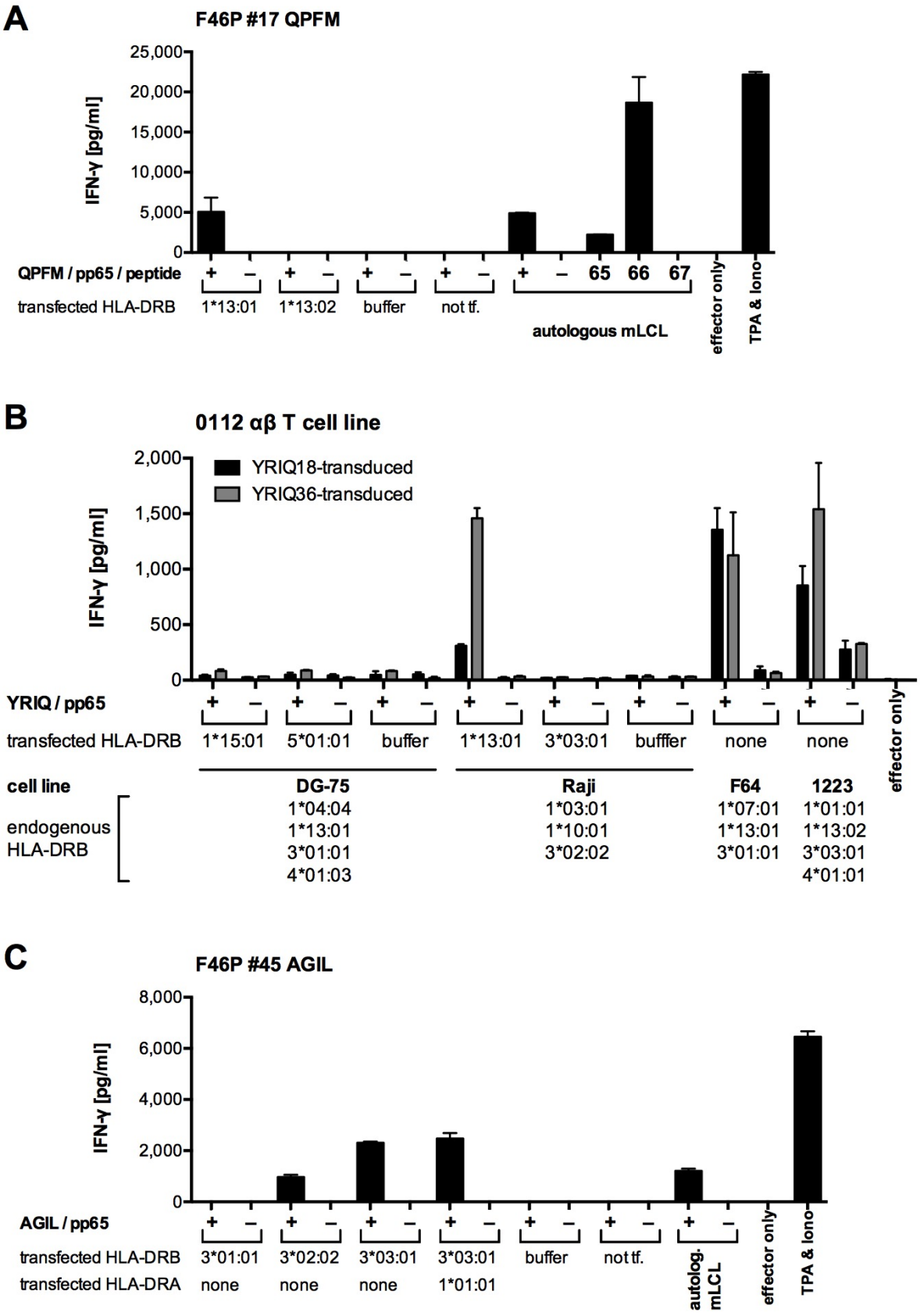


Figure 4.6. Recognition of HLA-matched and -mismatched mini-LCLs by the CD4$^+$ T cell clones F62P #16 (A) and 1304P #21 (B). pp65-expressing mini-LCLs ("+") and respective control mini-LCLs ("–") from several donors were co-incubated overnight with T cell clones at an e:t ratio of 10,000:20,000. Raji and EBV-B1.11 cells had been loaded with peptide no. 57 ("+", tab. 2.12) or were left untreated to serve as negative control ("–") prior to co-incubation with effector cells. "autolog." marks the donor from which the T cell clone originated. Stimulation by TPA and ionomycin served as positive control, effector cells in the absence of mini-LCLs as negative control ("effector only"). The HLA-DQB and -DQA type of each donor is given below each graph (blanks indicate absence of HLA alleles; "n.d." marks HLA alleles that have not been determined by HLA typing). The graphs show the concentration of secreted IFN-γ in the supernatant as evaluated by ELISA. The mean and standard deviation of duplicate samples are shown.

or DRB3*03:01, but not DRB3*01:01 (fig. 4.7 C). While the amount of secreted IFN-γ was not notably changed by HLA-DRA co-transfection (2,500 pg/ml), presentation of the peptide by HLA-DRB3*03:01 (2,300 pg/ml) resulted in more than twice the amount of cytokine as presentation by DRB3*02:02 (1,000 pg/ml). This experiment was independently reproduced four times, providing equivalent results. Thus, F46P #45 AGIL is restricted by HLA-DRB3*03:01 (autologous allele), but is also able to bind its epitope when presented by DRB3*02:02. The amino acid sequence of both HLA-DRB chains differs in ten amino acids (Marsh et al., 2000), four of which participate in peptide binding (amino acid positions 30, 37, 57 & 86, Bondinas et al., 2007). Although amino acid exchanges at positions 30, 57 and 86 are non-conservative (His–Tyr, Asp–Val and Gly–Val, respectively), AGIL peptide was presented by HLA-DRB3*02:02 as well as DRB3*03:01 and was recognised well despite differences in HLA-DRB sequence.

In contrast to HLA-DR, the analysis of **HLA-DQ restrictions** requires identification of the restricting DQA and DQB chains, since both are polymorphic. The T cell clone F62P #16 KYQE and EPDV21-transgenic δ2 T cell lines were tested in such experiments. Donor F62 has HLA-DQA1*01:01 and DQB1*05:01, as well as DQA1*01:03 and DQB1*06:01; these two pairings represent frequent HLA haplotypes (Klitz et al., 2003). EBV-B1.11 cells that had been transfected with DQA1*01:01 & HLA-DQB1*06:01 or DQA1*01:03 & DQB1*06:01 and that had been loaded with KYQE peptide were not recognised by the T cell clone F62P #16 (data not shown). If EBV-B1.11 cells had been transfected with DQB1*05:01, however, F62P #16 clearly recognised peptide-loaded target cells (fig. 4.8 A). Transfecting EBV-B1.11 cells with HLA-DQB1*05:01 alone and loading them with antigenic peptide resulted in robust IFN-γ production by the T cell clone (4,000 pg/ml), but co-transfections of the same DQB molecule together with either DQA1*01:01 (16,000 pg/ml) or DQA1*01:03 (14,000 pg/ml) resulted in a more than 3.5-fold increase of IFN-γ levels. The endogenous HLA haplotypes of EBV-B1.11 cells were identified as DQA1*01:02 & DQB1*06:02 and DQA1*05:FE & DQB1*03:01 (tab. 2.3). As noted earlier, two thirds of HLA haplotypes with DQB1*03:01 have DQA1*05:01 and the other third has DQA1*03:02 apart from a few other less frequent combinations (Klitz et al., 2003). Thus, EBV-B1.11 cells very likely expressed DQA1*05:01 apart from DQA1*01:02, neither of which are present in donor F62. Apparently, endogenous expression of DQA1*01:02 and DQA1*05:01 allowed presentation of the epitope to the TCR, but an even stronger response was achieved by co-transfection of one of the other two DQA1*01 variants DQA1*01:01 or DQA1*01:03 (fig. 4.8 A). Since DQA1*05:01 shares just 87.9% of its amino acid sequence with DQA1*01:01, whereas DQA1*01:02 and DQA1*01:03 share 99.1 and 97.9% sequence homology with DQA1*01:01 (Marsh et al., 2000), DQA1*01:02 rather than DQA1*05:01 probably served as a substitute for presentation of KYQE peptide in EBV-B1.11 cells transfected with DQB1*05:01 alone. Endogenous DQA1*01:02, however, may have been expressed at lower levels than the transfected molecules or may bind the KYQE peptide or the TCR with lower affinity than DQA1*01:02 and DQA1*01:03. Taken together, target cells were recognised by T cell clone F62P #16 if KYQE peptide was presented through DQB1:05:01 together with any of the three DQA1 chains DQA1*01:01, DQA1*01:02 or DQA1*01:03. As most HLA haplotypes with DQB1*05:01 have DQA1*01:01 (Klitz et al., 2003), the T cell clone and the TCR derived from it are likely to recognise pp65 in a majority of donors who express this DQB1 chain.


A
F46P #17 QPFM
IFN-γ [pg/ml]
25,000
20,000
15,000
10,000
5,000
0
QPFM / pp65 / peptide
+ – + – + – + – + – 65 66 67
transfected HLA-DRB
1*13:01
1*13:02
buffer
not tf.
autologous mLCL
effector only
TPA & Iono
B
0112 αβ T cell line
YRIQ18-transduced
YRIQ36-transduced
IFN-γ [pg/ml]
2,000
1,500
1,000
500
0
YRIQ / pp65
transfected HLA-DRB
1*15:01
5*01:01
buffer
1*13:01
3*03:01
bufffer
none
none
effector only
cell line
DG-75
Raji
F64
1223
endogenous HLA-DRB
1*04:04
1*13:01
3*01:01
4*01:03
1*03:01
1*10:01
3*02:02
1*07:01
1*13:01
3*01:01
1*01:01
1*13:02
3*03:01
4*01:01
C
F46P #45 AGIL
IFN-γ [pg/ml]
8,000
6,000
4,000
2,000
0
AGIL / pp65
transfected HLA-DRB
3*01:01
3*02:02
3*03:01
3*03:01
buffer
not tf.
transfected HLA-DRA
none
none
none
1*01:01
autolog. mLCL
effector only
TPA & Iono

Since aliquots of the T cell clone 1304P #21 EPDV were scarce, its HLA restriction was analysed by employing TCR-transgenic T cell lines, as done for its epitope determination described above (section 4.1.1). For this experiment, αβ TCR-depleted PBMCs from donor 0506 were stimulated with pamidronate to expand the population of δ2 T cells (see section 4.2 for more information on this procedure) and render them susceptible to transduction with a retrovirus encoding the TCRα and TCRβ chains from T cell clone 1304P #21. The δ2 T cell line was transduced with the TCR EPDV21 seven days before co-incubation with HLA-transfected DG-75 cells that were used as target cells because they do not express HLA-DQB1*03:01, which is the putative HLA restriction of this TCR (fig. 4.6 B). DG-75 cells were transfected with HLA-DQB1*03:01 alone or in combination with DQA1*05:01 and loaded with antigenic peptide. The TCR-transgenic δ2 T cells, but not GFP-transgenic δ2 T cells that were prepared in parallel as controls, secreted a large amount of IFN-γ in response to DG-75 cells co-transfected with HLA-DQA1*05:01 and DQB1*03:01 (fig. 4.8 B). Target cells that had been transfected with DQB1*03:01 alone were not recognised. Thus, the endogenous HLA chains DQA1*01:03 and DQA1*03:03 of DG-75 cells did not contribute to epitope presentation under these conditions. As described in the paragraph above, DQA1*01:01, DQA1*01:02 and DQA1*01:03 are very similar in amino acid sequence (≥98% homology), whereas DQA1*05:01 differs in 28 of 232 amino acids from DQA1*01:01 (Marsh et al., 2000), which may easily explain this preference. As two thirds of HLA haplotypes with DQB1*03:01 have DQA1*05:01 (Klitz et al., 2003), the T cell clone and the TCR derived from it are likely to recognise pp65 in a majority of donors who express this DQB1 chain.

Previously, the epitope EPDV was described as HLA-DR7-restricted by Li Pira et al. (2004), but that study did not consider the possibility that $CD4^+$ T cells may be restricted through HLA-DQ or DP rather than DR. Thus, DR7 may represent an alternative HLA restriction of EPDV. Alternatively, the HLA restriction may have been erroneously determined in the earlier study (Li Pira et al., 2004). Since DR7 is linked to DQB1*03 alleles in about a quarter of haplotypes

Figure 4.7 . ***(displayed on preceding page)***
Verifying the HLA-DR restriction of TCR-transgenic T cell lines or T cell clones. DG-75, EBV-B1.11 or Raji cells were either transfected with HLA-encoding plasmids (given below each graph, also see tab. 2.6), transfected without plasmid DNA ("buffer") or not transfected ("not tf."). Afterwards, they were pulsed with antigenic peptides ("+") or left untreated to serve as control ("–"). In each experiment, a target cell was used that was negative for the HLAs to be tested. Autologous ("autolog.") mini-LCLs or HLA-matched allogeneic mini-LCLs either expressing pp65 endogenously ("+") or respective control mini-LCLs ("–") were included as controls. Stimulation by TPA and ionomycin served as positive control for T cell clones. Concentration of secreted IFN-γ in the supernatant as evaluated by ELISA after overnight co-incubation at an e:t ratio of 20,000:80,000 is shown. The mean and standard deviation of duplicate (A & B) or triplicate (C) samples are shown. Each experiment was independently performed at least twice. **A**: T cell clone F46P #17 QPFM was tested on Raji cells. Epitope specificity was re-checked by pulsing autologous control mini-LCLs with peptides no. 65, 66 or 67 of the pp65 library (tab. 2.12). **B**: αβ T cells from donor 0112 had been transduced with TCR YRIQ18 (black bars) or YRIQ36 (grey bars) 14 days before this assay, in which they were tested on Raji and DG-75 cells. **C**: T cell clone F46P #45 AGIL was tested on EBV-B1.11 cells including a comparison of transfecting HLA-DRB3*03:01 alone or together with HLA-DRA1*01:01.

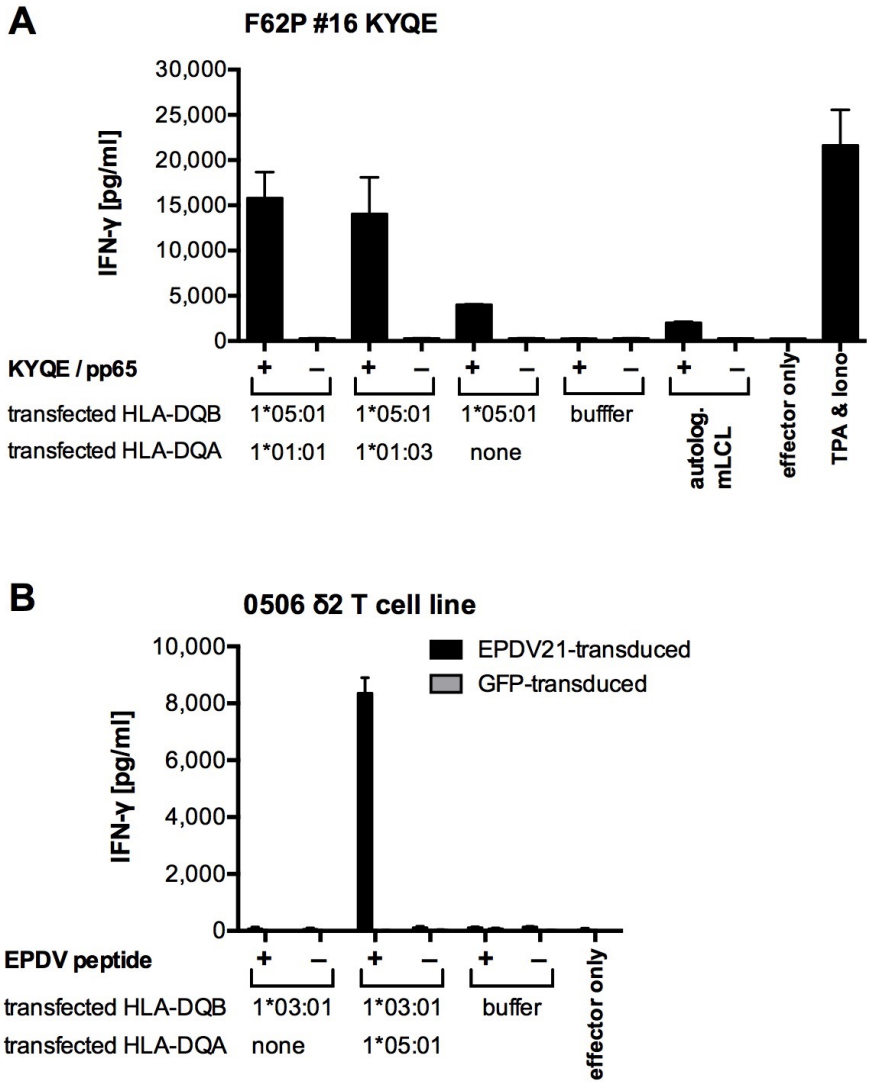


Figure 4.8. Verifying the HLA-DQ restriction of TCR-transgenic T cell lines or T cell clones. DG-75 or EBV-B1.11 cells were either transfected with HLA-encoding plasmids (given below each graph, also see tab. 2.6) or transfected without plasmid DNA ("buffer"). Afterwards, they were pulsed with antigenic peptides ("+") or left untreated to serve as control ("–"). In each experiment, a target cell was used that was negative for the HLAs to be tested. Autologous ("autolog.") mini-LCLs or HLA-matched allogeneic mini-LCLs either expressing pp65 endogenously ("+") or respective control mini-LCLs ("–") were included as controls. Concentration of secreted IFN-γ in the supernatant as evaluated by ELISA after overnight co-incubation is shown as the mean and standard deviation of duplicate samples. Each experiment was independently performed at least twice. **A**: T cell clone F62P #16 KYQE was tested on HLA-transfected and peptide-loaded EBV-B1.11 cells at an e:t ratio of 20,000:40,000. IFN-γ secretion by the T cell clone in response to target cells transfected either with HLA-DQB1*05:01 alone or in combination with DQA1*01:01 or 1*01:03 was compared. Stimulation by TPA and ionomycin served as positive control. **B**: δ2 T cells from donor 0506 had been transduced with TCR EPDV21 (black bars) or GFP (grey bars) 7 days before this assay, in which they were tested on HLA-transfected and peptide-loaded DG-75 cells at an e:t ratio of 20,000:80,000.

(Knipper et al., 2000), such an error does not seem unlikely. As the T cell clone 1304P #21 was undoubtedly restricted by HLA-DQ (fig. 4.3 F) and this restriction was confirmed by expression of recombinant HLA-DQ molecules, surely the TCR of 1304P #21 and maybe the EPDV epitope in general are restricted by HLA-DQA1*05:01 and DQB1*03:01.

Taken together, the HLA class II restriction of six out of seven pp65-specific $CD4^+$ T cell clones could be determined, either by testing the original T cell clone, or, when these were no longer available, using TCR-transduced T cells. Table 4.1 gives an overview about the HLA restrictions that were identified and the corresponding method that was used, as described earlier in this section. Moreover, table 4.1 shows the population frequency of each HLA in the Caucasian population (coverage). This percentage can be used to predict the probability that a particular TCR may be applied for adoptive T cell therapy to a Caucasian patient.

HLA restrictions of all IE-1-specific $CD4^+$ T cell clones, whose TCRs are described in this

Table 4.1. Summary of HLA restrictions of pp65-specific $CD4^+$ T cell clones that were identified in this study. Pairs of HLA α and β chains that were identified as the restricting elements for each T cell clone are shown. For some of the analyses T cell clones were no longer available, so experiments were performed with TCR-transgenic T cell lines (marked by "a"). The epitope specificity and HLA restriction of F46P #40 (marked with "b") could not be confirmed. Restricting HLAs were either identified by testing T cells on a panel of HLA-matched and -mismatched mini-LCLs ("match/mismatch") or on transformed B cells that were transiently transfected with HLA-encoding plasmids and loaded with pp65 peptides ("HLA transfection"). Allele frequencies (AFs) of Caucasians were gathered from Gragert et al. (2013). Percentages of phenotype coverage were calculated by assuming distribution according to the Hardy-Weinberg equilibrium: Population coverage (i.e. probability that a specific allele is present in both or one of the two sets of chromosomes of a person) = $(1-(1-\mathrm{AF})\cdot(1-\mathrm{AF}))$. Population coverage of HLA-DQ restrictions was calculated involving the AF of DQB only (regardless of the frequency of the respective DQA allele; marked by "c").

T cell clone	HLA restriction		Method of identification	population coverage [%]
	α chain	β chain		
F46P #17 QPFM	DRA1*01:01	DRB1*13:01	HLA transfection	12.0
		DRB1*13:02	match/mismatch	9.1
F46P #18 YRIQ[a]	DRA1*01:01	DRB1*13:01	HLA transfection	12.0
		DRB1*13:02	match/mismatch	9.1
F46P #36 YRIQ[a]	DRA1*01:01	DRB1*13:01	HLA transfection	12.0
F46P #40 MSIY[ab]	DRA1*01:01	DRB1*15:01	match/mismatch	24.2
		DRB5*01:01	match/mismatch	24.3
F46P #45 AGIL	DRA1*01:01	DRB3*02:02	HLA transfection	36.2
		DRB3*03:01	HLA transfection	9.2
F62P #16 KYQE	DQA1*01:01 DQA1*01:02 DQA1*01:03	DQB1*05:01	HLA transfection	21.9[c]
1304P #21 EPDV[a]	DQA1*05:01	DQB1*03:01	HLA transfection	35.4[c]

work, have been analysed by Stefanie Ameres et al. (2015), by means of testing their reactivity against HLA-matched and -mismatched target cells and, in some cases, target cells transfected with specific HLAs. The HLA restrictions postulated by Ameres et al. (2015) were confirmed in experiments that assessed the function of TCR-transgenic T cell lines on HLA-matched and HCMV-infected target cells (section 4.4.5) with one exception: The TCRs that were isolated from T cell clones 1021M #6 & #90 SVMK and 1826M #27 SVMK mediated recognition of HCMV-infected target cells if these expressed HLA-DPB1*03:01 (donor 1613, fig. 4.28), but not if target cells expressed HLA-DPB1*02:01 and DPB1*04:01 (donor 1022, data not shown). Since both TCRs were only tested once on target cells from two different donors, further investigation is required to clarify whether the postulated HLA restriction (DPB1*02:01 & DPB1*03:01) needs to be corrected to DPB1*03:01 and possibly DPB1*14:01 & DPB1*20:01, which are very similar to DPB1*03:01 in protein sequence (99.6% homology each, Marsh et al., 2000).

4.1.3 Determination of HCMV-specific TCRα and TCRβ sequences

Identifying the gene sequences ot both TCRα and β chains is a prerequisite for TCR gene transfer. Hence, total mRNA was isolated from previously established T cell clones and reversely transcribed into cDNA (see section 3.2.2). A fragment of TCRα-encoding cDNA was selectively amplified by PCR using 34 primers organised in 8 primer pools covering all human TCRα V regions and one primer specific for the Cα region. Likewise, a fragment of TCRβ-encoding cDNA was selectively amplified by PCR using two degenerated primers covering most TCRβ V regions and a Cβ region-specific primer that is fully complementary to each of the two possible variants of the Cβ region. This amplified fragment ("amplicon") was expected to be sufficient to deduce the entire TCRα or TCRβ sequence, since it covered the complete CDR3 and J region and a sufficient part of the V region to identify the V segment that is used by the respective TCR chain (section 3.2.2). The amplicons were sent for commercial sequencing and analysed by utilising IMGT's V-QUEST tool (section 2.14). These amplicons were sufficient to determine the sequence of 36 TCR chains, but in 8 cases there were ambiguities of V region determination listing several V region alleles that identically matched the sequence of the amplicon. Such ambiguities were resolved through a second PCR with newly designed V region primers (tab. 2.10). These new primers enabled the amplification of a larger part of the V region than the previously used primers (tab. 2.9). This technique was also used to clarify amino acid mismatches between IMGT's reference alleles and initial sequencing results.

Table 4.2 shows the antigenic epitope, the composition of TCRα and TCRβ chains and the HLA restriction of all T cell clones whose TCR chains were used to construct vectors for TCR gene transfer and recombinant expression in T cells. All epitope specificities had already been reported and most HLA restrictions were known, too (tab. 4.2, last column and notes in the caption). HLA restrictions of the T cell clones F46P #17 QPFM & #19/#45 AGIL, F62P #16 KYQE and of the TCRs YRIQ18, YRIQ36 and EPDV21 were determined in this study, as shown in the previous sections.

Table 4.2. Paternal T cell clones, epitope specificities, TCR sequences and HLA restrictions of all TCRs that were cloned into retroviral vector plasmids (see tab. 2.8)**.** TCRs marked with "a" or "b" were sequenced by X. Liang (unpublished) or A. Schub (2010), respectively. Peptide sequences are given as single letter amino acid code (AA). HLA restrictions identified or verified in this work are marked with "c". References are sorted chronologically for each epitope (reported HLA restrictions in parentheses).

Donor	clone #	TCR name	CMV Ag	AA pos.	Peptide epitope	TCRα AV	TCRα AJ	TCRβ BV	TCRβ BD	TCRβ BJ	TCRβ BC	HLA restriction	References
F46M	165	**CRV1**[a]	IE-1	309-317	CRVLCCYVL	38-1*01	50*01	25-1*01	1*01	2-1*01	1	C*07:02	Kern et al. (B7, 1999); Khan et al. (B*07:02, 2007); Slezak et al. (C*07, 2007); Ameres et al. (C*07:02, 2013)
1013M	75	**CRV2**[a]				12-1*01	8*01	7-8*01		2-1*01	1		
F46M	36	**CRV3**				26-2*01	49*01	6-5*01	1*01	1-1*01	1		
1013M	169	**CRV4**				12-1*01	50*01	25-1*01	2*01	2-1*01	2		
XLBC13	1, 5, 8, 13	**FRC1**	UL28	327-335	FRCPRRFCF	5*01	16*01	20-1*01	1*01	1-1*01	1		Kim et al. (C*07:02, 2011b)
XLBC13	6	**FRC6**				14/DV4*02	49*01	28*01	1*01	1-6*02	1		
XLBC18	32	**FRC32**				12-2*01	48*01	28*01	1*01	2-1*01	2		
XLBC18	86	**FRC86**				13-2*01	40*01	6-2*01 /6-3*01	2*01	2-7*01	2		
XLBC18	93, 94	**FRC93**				25*01	40*01	28*01	2*02	2-1*01	2		
F46P	17	**QPFM17**[b]	pp65	261-275	QPFMRPHERNGFTVL	36/DV7*01	32*02	5-1*01	1*01	1-1*01	1	DRB1*13:01[c] /1*13:02	Li Pira et al. (DR13, 2004)
F46P	18	**YRIQ18**	pp65	369-387	(FTSQ)YRIQG-KLEYRH(TWDR)	6*02	36*01	7-9*01	1*01	2-5*01	2	DRB1*13:01[c] /1*13:02	Khattab et al. (DR3, 1997); Gallot et al. (DR1302, 2001); Li Pira et al. (DR3, 2004)
F46P	36	**YRIQ36**				6*02	29*01	7-9*01	2*02	2-2*01	2		
F46P	40	**MSIY40**	pp65	109-123	MSIYVYALPLKMLNI	26-1*01	9*01	28*01	2*02	2-3*01	2	DRB1*15:01	Wiesner et al. (DRB1*15, 2005); Nastke et al. (DRB1*07, 2005)
F46P	19, 45	**AGIL19**	pp65	489-503	AGILARNLVPMVATV	21*02	36*01	20-1*01	2*01	2-5*01	2	DRB3*02:02[c] /3*03:01[c]	Khattab et al. (DR3, 1997); Gallot et al. (DR1401, 2001); Li Pira et al. (DR3 or DR11, 2004); Slezak et al. (DRB3*02, 2007); Pachnio et al. (DRB3*02:02, 2016)
F62P	16	**KYQE16**	pp65	509-523	KYQEFFWDWNDIYRI	23/DV6*01	54*01	30*01	1*01	2-7*01	2	DQB1*05:01[c]	Khattab et al. (DR3, 1997); Li Pira et al. (DR1 or DR3, 2004); Slezak et al. (DRB3*01:01 or DQB1*05:02, 2007)
1304P	21	**EPDV21**[b]	pp65	177-191	EPDVYYTSAFVFPTK	20*02	35*01	5-6*01	2*02	2-7*01	2	DQB1*03:01[c]	Li Pira et al. (DR7, 2004)
F64M	236	**EIMA236**	IE-1	241-259	(CSPD)EIMAYA-QKIFK(ILDE)	17*01	29*01	11-2*01	2*02	2-7*01	2	DRB1*13:01	Ameres et al. (DRB1*13:01, 2015)
F65M	18	**RRKM18**	IE-1	201-215	RRKMMYMCYRNIEFF	9-2*01	43*01	7-3*01	1*01	2-3*01	2	DRB3*02:02	Ameres et al. (DRB3*02:02 and possibly 3*01:01, 2015)
1220M	22	**EFFT22**	IE-1	213-225	EFFTKNSAFPKTT	25*01	6*01	9*01	1*01	1-5*01	1	DRB5*01:01	Braendstrup et al. (DRB5*01:01, 2013); Ameres et al. (DRB5*01:01, 2015)
1826M	50	**EFFT50**				25*01	49*01	7-2*02/*03	2*01	2-7*01	2		
1021M	6, 90	**SVMK6**	IE-1	337-351	SVMKRRIEEICMKVF	9-2*02	40*01	19*01	1*01	1-5*01	1	DPB1*03:01	Ameres et al. (DPB1*02:01, 1*03:01, 1*20:01 and possibly 1*14:01, 2015)
1826M	27	**SVMK27**				38-1*01	40*01	6-5*01	2*02	2-4*01	2		

Analysis of the composition of these TCRs led to an interesting observation about gene segment usage of human TCRs. One T cell clone (1304P #21, specific for EPDV, tab. 4.2) used a Jα segment called TRAJ35*01. First identified by Koop et al. (1994), TRAJ35*01 was considered "non-functional", and annotated accordingly in IMGT, due to a non-canonical J-motif (i.e. C-G-X-G instead of F-G-X-G, Scaviner and Lefranc, 2000a). On 23.01.2019, I informed Marie-Paule Lefranc from IMGT of the identification of a TCRα chain using TRAJ35*01 and recombinant proof of its function. As a result, the allele's status was switched from "ORF" (lacking proof of functional expression) to "functional" (see IMGT's human TRAJ gene table online, IMGT note no. 6, accessed on 20.02.2019). This finding has two interesting implications: First, it demonstrates that the canonical phenylalanine within the J motif, which traditionally defines the C-terminal end of the CDR3, is not absolutely required to form a functional TCRα chain by gene recombination, but can be replaced by a cysteine. Second, other TCRs using the TRAJ35*01 motif will no longer be automatically dismissed as non-functional or eliminated from TCR analyses. This will make future analyses of human TCR repertoires more complete.

4.1.4 Generation of bicistronic vector plasmids coding for TCRα and TCRβ chains

For simultaneous transduction of TCRα and TCRβ genes and their expression in the receiving T cell, it is advantageous when both TCR genes are encoded by the same retroviral vector. The 'self-cleaving' 2A peptide P2A that is derived from the porcine teschovirus-1 is suited very well for facilitating equimolar expression of both genes (Donnelly et al., 2001, also see section 3.2.3). P2A showed the best cleavage efficiency among several variants of 2A peptides (Kim et al., 2011a), is likely not immunogenic (Arber et al., 2013) and is short (19 amino acids), wasting no precious DNA cargo capacity. Thus, it is reliable, effective and likely safe to apply in a clinical context. In contrast, an internal ribosome entry site (IRES) is larger and its downsteam gene is translated less efficient than the one upstream (Mizuguchi et al., 2000).
A Gly–Ser–Gly linker separating P2A and the gene downstream of it was shown to further improve cleavage efficiency (Holst et al., 2006; Szymczak et al., 2004, addendum 01.06.2004) and was therefore incorporated in the TCR constructs described in this thesis. *In silico*, TCRα and TCRβ genes were fused as 5'—TCRβ–`GSG`-P2A–TCRα—3', since Leisegang et al. (2008) showed this arrangement to be superior to its reverse order. All TCR sequences except for CRV1mu and CRV2mu (tab. 4.2) were fully human and not murinised or altered in any other way than codon optimisation, which was done by Thermo Fisher Scientific (Regensburg, Germany) prior to gene synthesis. The TCR constructs were commercially synthesised and cloned into MP71 (section 2.13). All plasmids that were generated for this study and that code for an HCMV-specific TCR are shown in table 2.8.

4.2 Establishment of δ2 T cells as recipients for TCR transfer

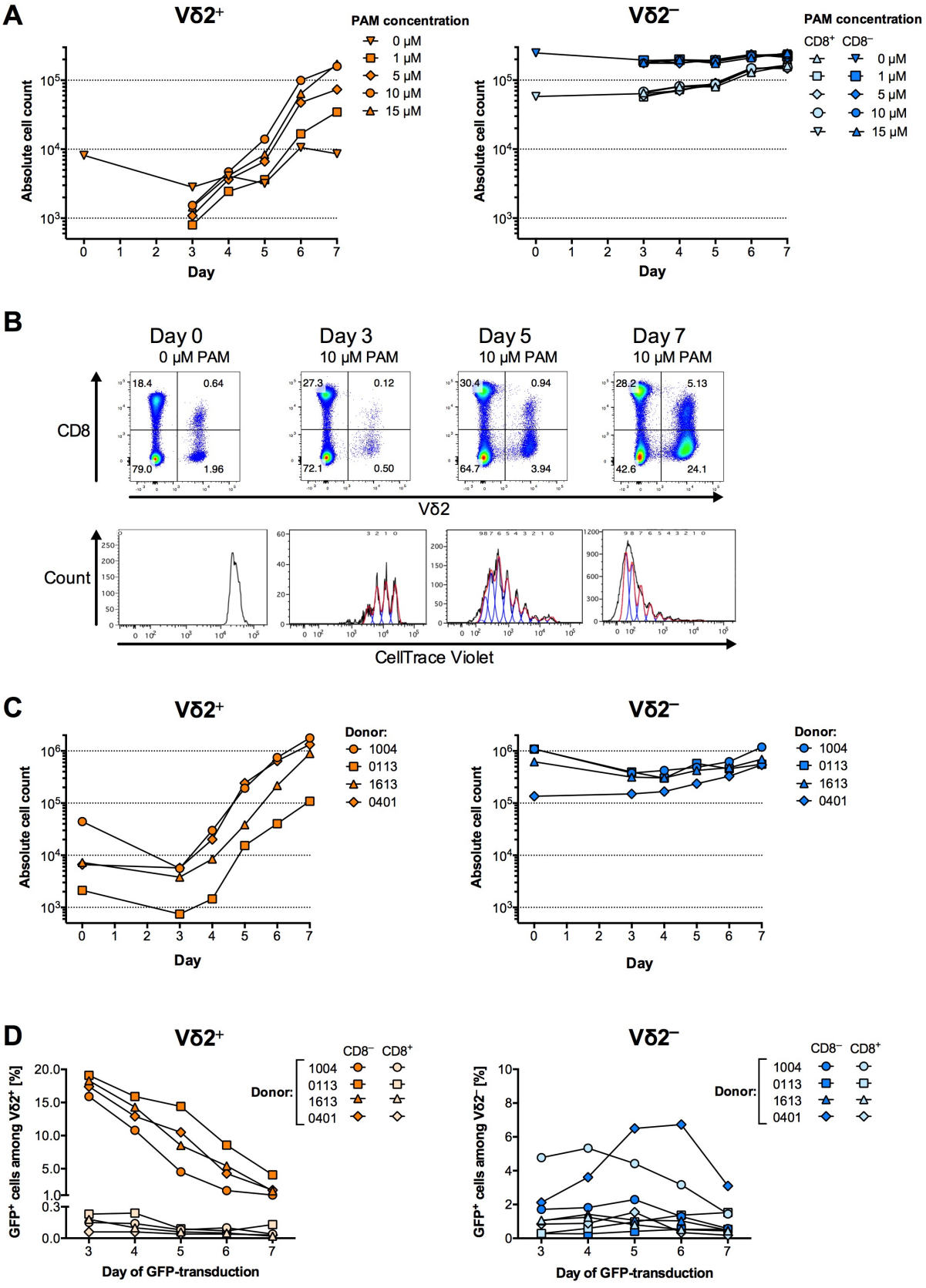
Gammadelta (γδ) T cells are attractive targets for the transfer of an αβ TCR because they do not express mis-paired TCR dimers (Koning et al., 1987; Saito et al., 1988). After adoptive transfer of HCMV-specific T cells into a patient, TCR-transgenic δ2 T cells may be stimulated by aminobisphosphonates to maintain and expand this population in the absence of stimulation via the transgenic TCR. Moreover, γδ T cells are a convenient tool to analyse TCR function in

the absence of co-stimulation by CD4 or CD8, both of which are not expressed by most γδ T cells (Groh et al., 1989; Janeway, 1992; van der Veken et al., 2006). However, γδ T cells are not as abundant in peripheral blood as αβ T cells and may not behave comparably if stimulated and retrovirally transduced. Focussing on the largest fraction of γδ T cells in peripheral blood, the specific enrichment, stimulation and transduction of Vγ9Vδ2 T cells (abbreviated as δ2 T cells) was studied in order to establish a quick and easy protocol for obtaining αβ TCR-engineered δ2 T cells.

4.2.1 Optimising pamidronate concentration for the stimulation of δ2 T cells

In order to be susceptible to retroviral transduction, target cells need to be proliferating. Since treatment of PBMCs with aminobisphosphonates was shown to drive activation and proliferation of δ2 T cells *in vitro* (Gober et al., 2003; Khan et al., 2014; Morita et al., 2007), the aminobisphosphonate pamidronate was chosen as stimulating agent. 50 U/ml Interleukin (IL)-2 were simultaneously administered to provide δ2 T cells with co-stimulation in addition to Vγ9Vδ2 TCR stimulation through pamidronate (Khan et al., 2014; van der Veken et al., 2006, applied 300 or 100 U/ml, respectively). Since survival and proliferation of γδ T cells were shown to benefit from the presence of IL-7 (van der Veken et al., 2009, 5 ng/ml) and IL-15 (Khan et al., 2014; van der Veken et al., 2006, 10 or 20 ng/ml, respectively), both cytokines were supplemented during δ2 T cell stimulation and retroviral transduction at 5 ng/ml each. All PBMCs were incubated in 5% HS T cell medium containing human instead of fetal calf serum throughout experimental proceedings irrespective of PBMC sorting or chosen stimulation. However, all effector assays during which T cells were co-incubated with target cells were set up in 10% FCS T cell medium because HS contained human IFN-γ at levels that negatively affected analyses of T cell function by ELISA.

The concentration of pamidronate (PAM) in the cell culture was titrated by stimulating bulk PBMCs with different amounts of PAM. After an initial drop of cell numbers at days 3 and 4, $V\delta2^+$ T cells proliferated best at 10 or 15 µM PAM (fig. 4.9 A & B, one out of two donors shown) leading to an increase in $V\delta2^+$ T cell counts of at least 20-fold from day 0 to day 7. The initial decline in $V\delta2^+$ T cell numbers could be caused by impaired detection via FACS as a result of down-regulation of TCR expression after stimulation. $V\delta2^-$ $CD8^-$ T cells, which mainly consist of $CD4^+$ αβ T cells, did not proliferate. In contrast, $V\delta2^-$ $CD8^+$ T cells, which mostly are composed of $CD8^+$ αβ T cells, more than doubled in numbers from day 0 to day 7. This could be caused by proliferation of $CD8^+$ CTLs in response to presentation of an unknown antigen by monocytes or even $V\delta2^+$ T cells that may function as APCs (Khan et al., 2014).
A higher PAM concentration of 45 µM did not improve cell proliferation, but resulted in a pronounced loss of $V\delta2^+$ T cells (data not shown). Since PAM interferes with the mevalonate pathway, a high concentration of PAM may be toxic for cells by preventing the synthesis of downstream products of the pathway, such as isoprenoids, steroids and cholesterol. This may affect δ2 T cells directly or cells, like monocytes, that are required to stimulate δ2 T cells by PAM. Bearing this in mind, 10 µM PAM were chosen for future stimulations because δ2 T cell proliferation did not seem to benefit from higher concentrations (15 µM), but was less pronounced


A
Vδ2+
Vδ2−
PAM concentration
0 µM
1 µM
5 µM
10 µM
15 µM
CD8+ CD8−
Absolute cell count
Day
B
Day 0
0 µM PAM
Day 3
10 µM PAM
Day 5
10 µM PAM
Day 7
10 µM PAM
CD8
Vδ2
Count
CellTrace Violet
C
Donor:
1004
0113
1613
0401
D
GFP+ cells among Vδ2+ [%]
GFP+ cells among Vδ2− [%]
Day of GFP-transduction

if concentration was lower (1 or 5 µM).
The effect of 10 µM PAM on proliferation of Vδ2$^-$ and Vδ2$^+$ T cells from four different donors was evaluated. While the four donors showed a 40- to 200-fold increase of Vδ2$^+$ T cell numbers from day 0 to day 7 (fig. 4.9 C), the numbers of Vδ2$^-$ T cells in general stayed constant or were reduced except for one donor (0401, 4-fold increase). As observed before, the numbers of Vδ2$^+$ T cells dropped from day 0 to day 3, before recovering on day 4 or 5. Taken together, these experiments confirmed that PAM stimulation specifically and strongly expanded the Vδ2$^+$ T cell-fraction, and established an optimal dose for their short-term *in vitro* expansion from PBMCs.

4.2.2 Optimal time point for retroviral transduction of δ2 T cells after stimulation by pamidronate

After 3, 4, 5, 6 or 7 days of PAM stimulation, PBMCs were transduced with retroviruses coding for GFP to determine the optimal time point for retroviral transduction. Another four days after transduction, GFP-expression of Vδ2$^+$ and Vδ2$^-$ T cells was analysed by FACS. For all four donors, the proportion of GFP-expressing Vδ2$^+$ T cells was highest if PBMCs were transduced after 3 days of PAM stimulation (fig. 4.9 D). Prolonged PAM stimulation for 4, 5, 6 or 7 days resulted in gradually decreased proportions of GFP$^+$ Vδ2$^+$ T cells. Since transduction efficiency was best after 3 days of PAM stimulation, this scheme was applied in all future retroviral transductions of δ2 T cells. Most GFP$^+$ Vδ2$^+$ T cells were CD8$^-$ because the large majority of Vδ2$^+$ T cells does not express CD8.
Although pamidronate specifically activates Vδ2$^+$ T cells, some Vδ2$^-$ T cells were transduced with GFP as well ranging from less than 0.6% (donor 0113) to more than 6% (donor 0401) GFP$^+$ Vδ2$^-$ T cells. This simultaneous, non-intended transduction of Vδ2$^-$ T cells, which presumably are mainly composed of αβ T cells, did not generally favour the CD4$^+$ or CD8$^+$ subpopulation over the other: While mainly CD8$^-$ Vδ2$^-$ T cells, but few CD8$^+$ Vδ2$^-$ T cells, expressed GFP

Figure 4.9 . ***(displayed on preceding page)***
Characterisation of optimal conditions for stimulation and subsequent transduction of Vδ2 T cells. Bulk PBMCs from healthy donors were stimulated with pamidronate (PAM) and analysed by FACS. Graphs A, C & D show cell numbers for Vδ2$^+$ T cells (left graph) and Vδ2$^-$ T cells (right graph). Absolute cell numbers were determined by FACS using a pre-defined number of PE-labelled beads that were acquired simultaneously as a standard. Results for CD8$^+$, CD8$^-$ or all T cells of the respective T cell subset without discrimination by CD8 expression are shown. All T cells were pre-gated as CD14$^-$ CD19$^-$ CD3$^+$. **(A)** Cell numbers were assessed before (day 0) and after stimulating 10^6 PBMCs from donor 1107 with 0, 1, 5, 10 or 15 µM PAM for 3, 4, 5, 6 or 7 days and **(B)** dilution of the intracellular dye CellTrace Violet due to proliferation was followed (top row: pseudocolour dot plots demonstrating δ2 T cell expansion; bottom row CellTrace Violet histograms showing model sum (red line) and model components (blue line) calculated by FlowJo). Dead cells were excluded from analysis by using a live/dead marker (TOPRO). 10^6 PBMCs from four additional donors were stimulated with 10 µM PAM for 3, 4, 5, 6 or 7 days. On those days, cell numbers were assessed **(C)**. PBMCs that had been stimulated with PAM for 3 to 7 days were transduced with a GFP-encoding retrovirus, and the proportion of GFP$^+$ T cells in the Vδ2$^+$ or Vδ2$^-$ subset was determined by FACS (**D**, the x-axis indicates the day of transduction after PAM stimulation, the measurement was made four days after transduction in each case).

in the PBMC culture from one donor (0401), this ratio was inverted in the PBMC culture from another donor (1004).

4.2.3 Phenotypic analysis of δ2 T cells after stimulation by pamidronate

TCR-transgenic T cells need to possess a certain degree of proliferative capacity in order to exert desired effector functions when encountering targets. Thus, T cells produced for application in adoptive T cell therapy of HCMV should originate from an effective T cell subpopulation, which is capable of driving the immune response and is not terminally differentiated. αβ T cells have been grouped into naïve (N, i.e. not antigen-experienced), central memory (CM), effector memory (EM) and effector memory re-expressing CD45RA (EMRA) subpopulations according to their differentiation status (N → CM → EM → EMRA, from early to late). The efficacy of a T cell product will likely benefit from a high T_{CM} and T_{EM} content (Busch et al., 2016) because transfer of less-differentiated T cell subsets was highly correlated with clinical responses (Klebanoff et al., 2012).
One pair of surface markers to monitor T cell subpopulation distribution consists of CD45RA, which is expressed by T_N and T_{EMRA}, and CD62L, which is expressed by T_N and T_{CM}. PBMCs from three healthy donors were simultaneously stained by monoclonal antibodies specific for CD62L and CD45RA to assess the differentiation status of Vδ2 T cells before and after stimulating them with 10 µM pamidronate. Upon encounter of APCs, the fraction of naïve $V\delta2^+$ T cells diminished in all three donors over the course of 7 days after PAM stimulation (fig. 4.10 B). The fraction of central memory $V\delta2^+$ T cells enlarged from day 0 to 3 and from day 3 to 5. At the end of the evaluated time period (from day 5 to day 7), T_{CM} $V\delta2^+$ T cells decreased slightly, probably resulting from continued antigen presentation driving differentiation further. Effector memory $V\delta2^+$ T cells made up the largest (donor 1004 & 1613) or second-to-largest (donor 0113) fraction on day 0, declining in numbers over the next several days, but recovering at day 7. This may also be caused by continued antigen presentation that promotes differentiation from T_N to T_{CM} to T_{EM} $V\delta2^+$ T cells. Terminally differentiated T_{EMRA} $V\delta2^+$ T cells were found in fairly large numbers on day 0 in two out of three donors (21.2% & 24.9%), but their numbers strongly decreased in PBMC cultures from all three donors following PAM stimulation (<3% on days 5 and 7).
In summary, PBMCs stimulated with 10 µM pamidronate offered a favourable T cell composition of mainly T_{CM} and T_{EM} Vδ2 T cells that likely retain proliferative capacity and functional efficacy. If the definitions of T_N, T_{CM}, T_{EM} and T_{EMRA} subpopulations originally identified on αβ T cells can also be applied to γδ T cells (as suggested by Dieli et al., 2003), the demonstrated composition of PAM-stimulated $V\delta2^+$ T cells strongly supports engineering them with transgenic TCRs and using them in adoptive T cell therapy.

4.3 Characterisation of HCMV-specific TCRs restricted by HLA class I

$CD8^+$ CTLs are an essential part of HCMV-specific immunity that limits replication of HCMV (Einsele et al., 2002; Reddehase, 2002) by killing infected host cells. Recently, IE-1-specific $CD8^+$ T cell clones that are restricted by HLA-C*07:02 were shown to strongly recognise AD169-infected target cells, which other T cell clones restricted by HLA-A or -B could not (Ameres et al., 2014,

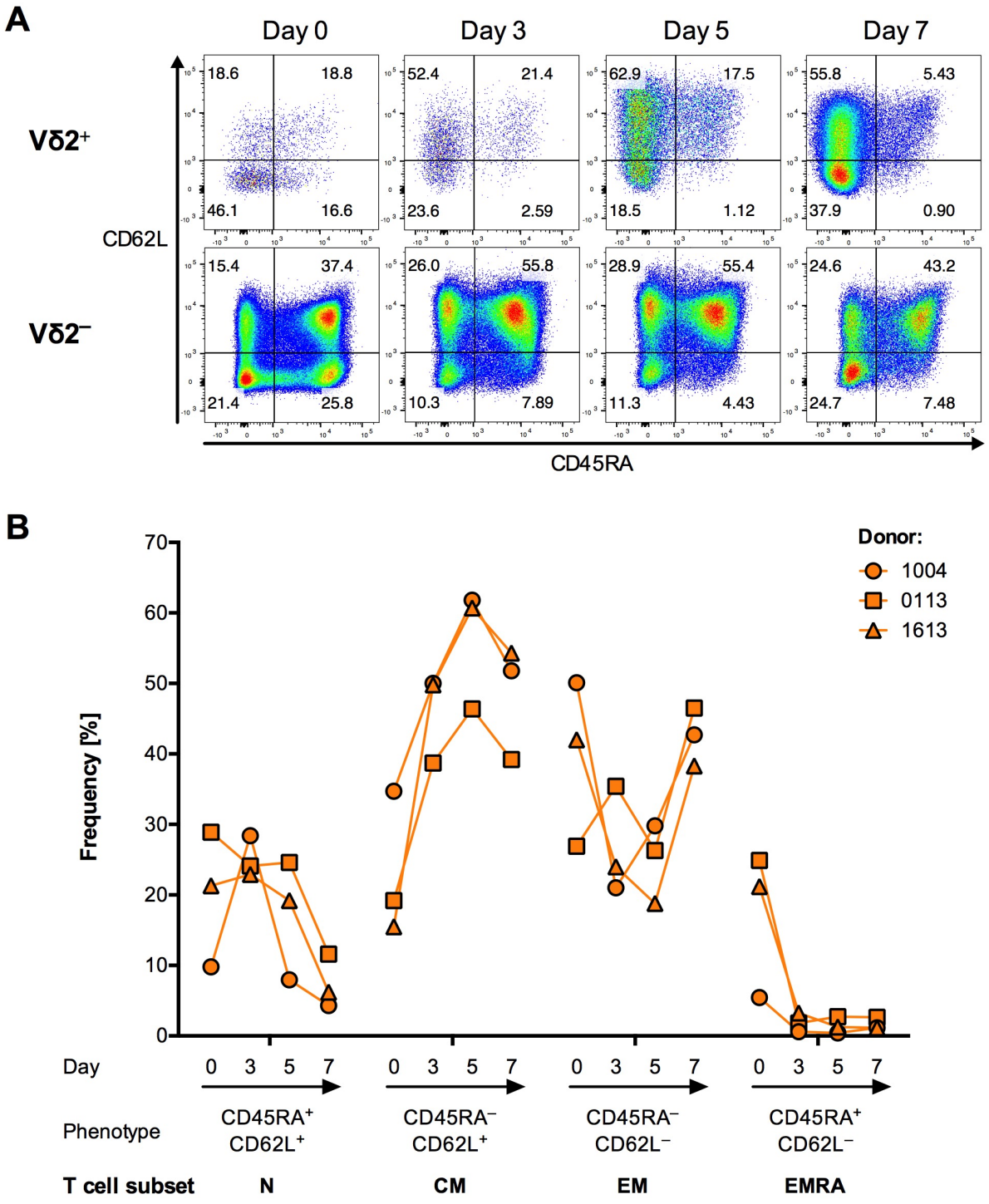


Figure 4.10. Differentiation phenotype of T cells after stimulation with pamidronate. 10^6 bulk PBMCs from healthy donors were stimulated with 10 µM pamidronate (PAM) and analysed on day 0, 3, 5 and 7 by FACS. All T cells were pre-gated as $CD14^-$ $CD19^-$ $CD3^+$. **A:** Pseudocolour dot plots show the expression of CD62L and CD45RA on $V\delta2^+$ (top row) and $V\delta2^-$ (bottom row) T cells (representative data from one donor (1613) out of three). **B:** Distribution of differentiation phenotypes of $V\delta2^+$ T cells from donors 1004 (circles), 0113 (squares) & 1613 (triangles). $CD45RA^+$ $CD62L^+$, $CD45RA^-$ $CD62L^+$, $CD45RA^-$ $CD62L^-$ or $CD45RA^+$ $CD62L^-$ $V\delta2^+$ T cells were identified as naïve (N), central memory (CM), effector memory (EM) or effector memory T cells re-expressing CD45RA (EMRA), respectively.

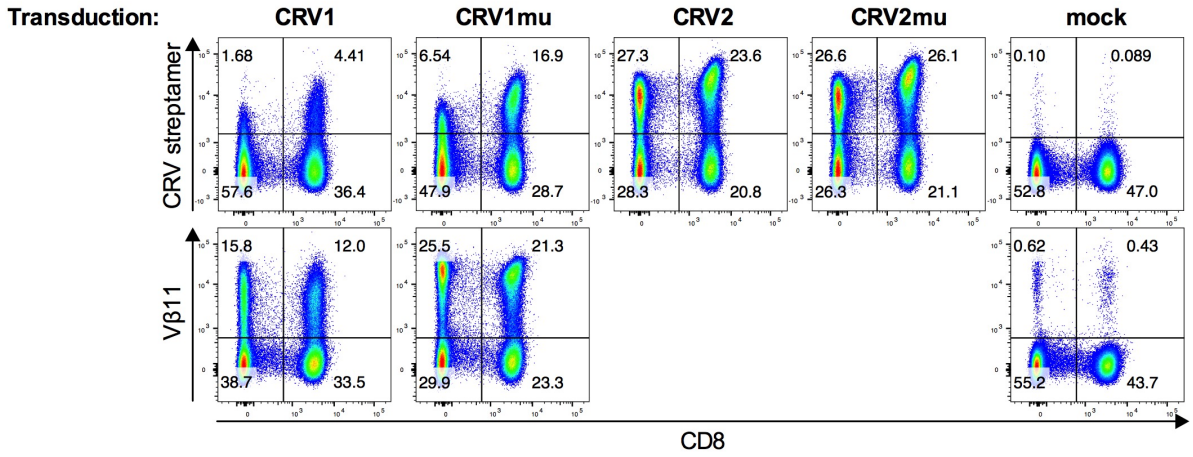


Figure 4.11. Expression of CRV1, CRV1mu, CRV2 or CRV2mu by T cells four days after transduction. Bulk PBMCs from donor 1107 were stimulated with anti-CD3 antibody before transduction. Stainings with CRV streptamer (top row) and Vβ11 (bottom row) against CD8 are shown as pseudocolour dot plots. Vβ6-specific antibodies for staining CRV2 and CRV2mu were not available. Mock-transduced (using retroviruses devoid of genetic cargo) T cells served as negative control. T cells were pre-gated as $CD14^-$ $V\delta2^-$ $CD158b^-$ $CD3^+$ cells.

2013).

4.3.1 Expression of HLA class I-restricted, HCMV-specific TCRs on T cells

Before evaluating the efficacy of TCR-transgenic T cell lines in recognising HCMV-infected target cells, the expression of HCMV-specific TCR genes on the cell surface was assessed by FACS analysis four to seven days after transduction. Different levels of TCR expression were detected depending on the individual TCR (e.g. one CRV-specific TCR in comparison with another CRV-specific TCR), but also depending on (I) co-expression of CD8 on TCR-transgenic T cells (e.g. $CD4^+$ vs. $CD8^+$ T cells), (II) the T cell subpopulation that received the TCR genes (αβ vs. δ2 T cells), and (III) the applied staining method (HLA multimer vs. Vβ-specific antibody). Exemplary data of these observations are provided for the three TCRs CRV1, CRV2 and YSE below.

Focussing on bulk PBMCs transduced with one of the two TCRs CRV1 or CRV2 after stimulation by anti-CD3 antibody, CRV1 was functionally expressed by 6.1% of $CD14^-$ $V\delta2^-$ $CD158b^-$ $CD3^+$ (presumably αβ) T cells (as assessed by CRV streptamers, fig. 4.11, 1st column). Interestingly, the large majority of all CRV $streptamer^+$ T cells also expressed CD8 after transduction with a CRV1-encoding retrovirus. The proportion of CRV $streptamer^+$ $CD8^-$ T cells was low, as was the intensity of CRV streptamer staining of this subpopulation. In contrast, the TCRβ chain of the CRV1 TCR was strongly expressed by a considerable fraction of $CD8^-$ T cells, as revealed by counter-staining with a Vβ11-specific antibody. Taken together, this implies that the co-expression of CD8 in TCR-transgenic T cells enhances the binding of CRV1 TCR to peptide/HLA complexes. Consequently, this property of CRV1 may affect TCR functionality in $CD8^-$ T cells, such as recognising HCMV-infected cells (see section 4.3.2). The discrepancy between streptamer and Vβ11 staining may be caused by mis-pairing of TCR chains in αβ T cells, since both TCR chains of CRV1 are completely human and can therefore form mixed TCR

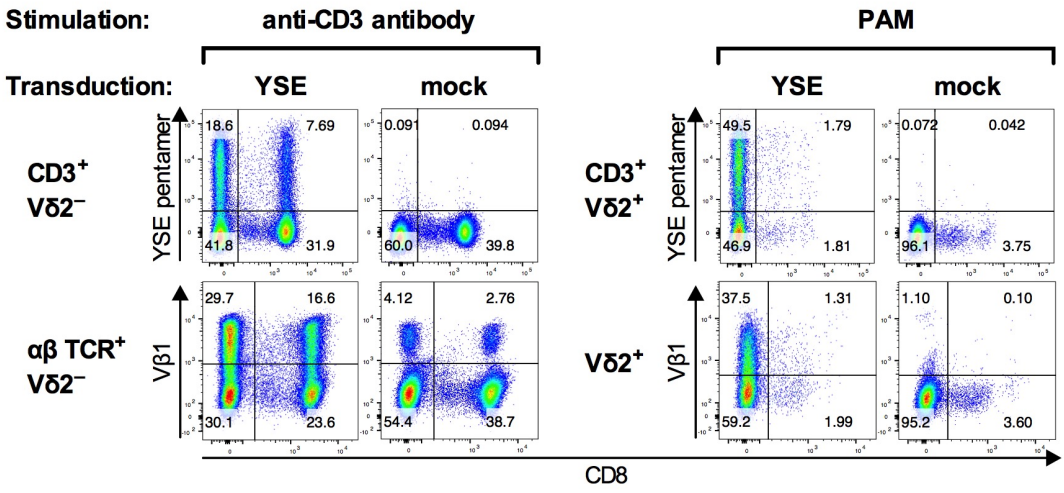


Figure 4.12. Expression of YSE TCR by T cells four days after transduction. Bulk PBMCs from donor 1223 were stimulated with anti-CD3 antibody or PAM before transduction. Stainings with YSE pentamer (top row) and Vβ1 (bottom row) against CD8 are shown as pseudocolour dot plots. Mock-transduced (using retroviruses devoid of genetic cargo) T cells served as negative control. Cells were pre-gated as $CD14^-$.

dimers together with the endogenous TCR chains of αβ T cells as reported by van Loenen et al. (2010). When the constant region of both TCR chains of CRV1 was exchanged with the respective murine ("mu") constant regions (a process called "murinisation"), this TCR (i.e. CRV1mu) still relied on CD8 co-receptor for successful streptamer binding, but the difference between CRV $streptamer^+$ and $Vβ11^+$ proportions became less pronounced (fig. 4.11, 2^{nd} column), possibly indicating less TCR mis-pairing. Despite the murinisation that should facilitate preferential TCR binding, the large majority of CRV $streptamer^+$ T cells expressed CD8.
In contrast to CRV1, CRV2 could be stained by CRV streptamers on 50.9% of T cells with comparable proportions of $CD8^+$ and $CD8^-$ cells carrying the TCR (fig. 4.11, 3^{rd} column). Apparently, CRV2 does not depend on CD8 for binding of peptide/HLA complexes. Nevertheless, CRV $steptamer^+$ $CD8^+$ T cells showed a more intense staining than their $CD8^-$ counterparts, which may be contributed to additional binding avidity provided by CD8 (also see MFIs of CRV $streptamer^+$ T cells in another experiment, fig. 4.14). Comparing CRV streptamer and Vβ stainings, as done for CRV1, was not possible for CRV2 because no commercial antibody specific for Vβ6 was available. Murinisation of CRV2 did not boost TCR expression, neither on $CD8^+$, nor on $CD8^-$ T cells (fig. 4.11, 4^{th} column). Thus, only TCRs that are weakly expressed and may suffer from TCR mis-pairing and/or TCR competition in αβ T cells (such as CRV1) appear to benefit from murinisation. Similar results were obtained using PBMCs from another donor (0102). Consequently, the disadvantage of introducing foreign peptide sequences into the TCRs constructs, which ultimately could cause rejection of TCR-engineered T cells after adoptive transfer, outweighs the potential gain in TCR expression by murinisation.

HLA class I-restricted TCRs, such as YSE and CRV2, could be successfully expressed on both $Vδ2^-$ T cells, which likely contain a large majority of αβ T cells, and $Vδ2^+$ T cells (fig. 4.15). CRV2 was more strongly expressed on CD3-stimulated $Vδ2^-$ T cells than on PAM-stimulated

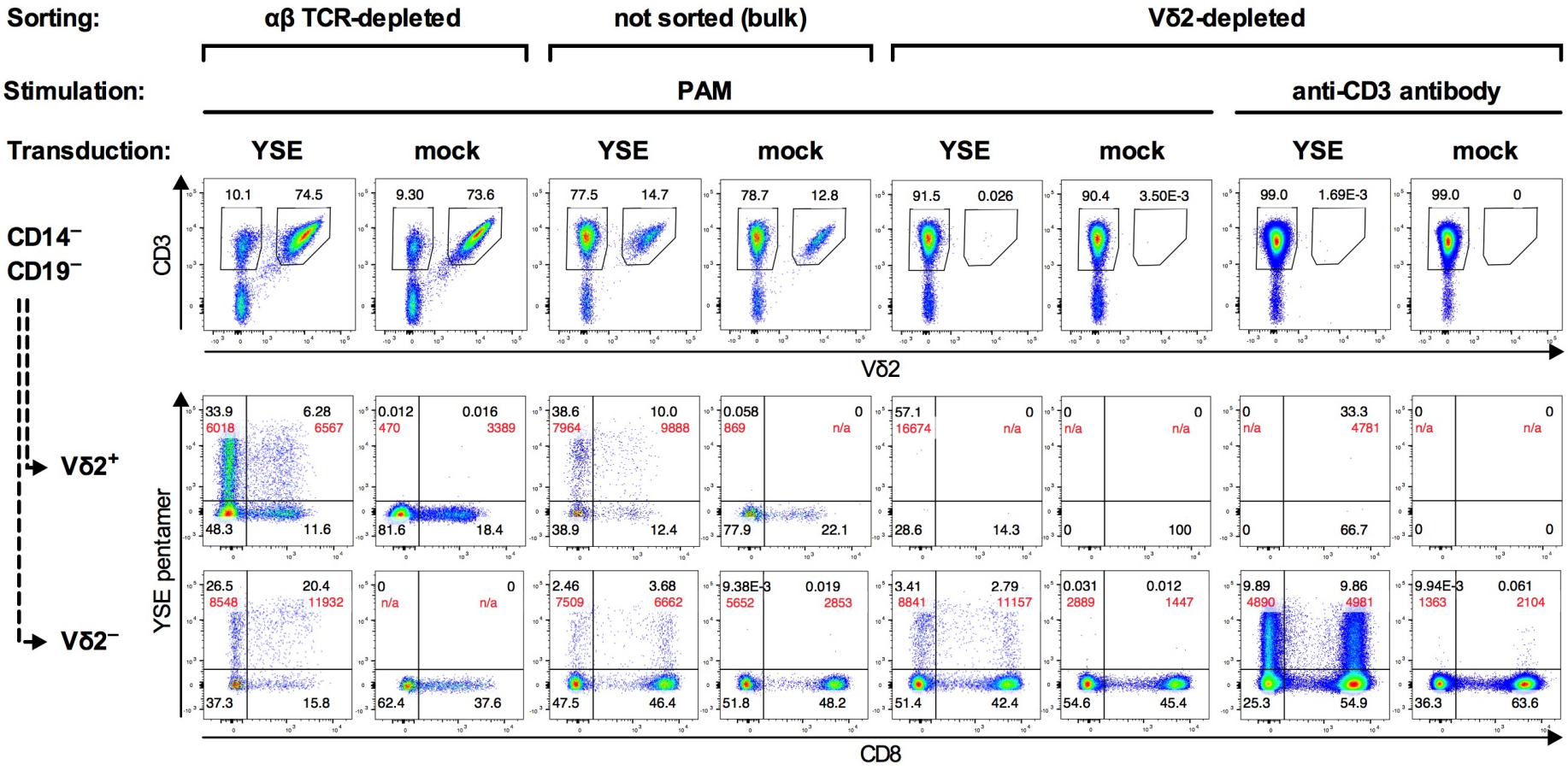


Figure 4.13. Effect of different immunomagnetic sorting on expression of YSE TCR prior to stimulation and retroviral transduction. αβ T cell-depleted, bulk or δ2 T cell-depleted PBMCs from donor 1107 had been stimulated with PAM and were transduced by retroviruses that either delivered the genes of YSE TCR or no genetic cargo ("mock"). For comparison, δ2 T cell-depleted PBMCs had been stimulated with anti-CD3 antibody and were transduced likewise. TCR expression was assessed on day 7 by YSE pentamers. Pseudocolour dot plots show the expression of CD3 and Vδ2 on $CD14^-$ $CD19^-$ cells (top row). Expression of YSE TCR was analysed by pentamer staining on $CD14^-$ $CD19^-$ $Vδ2^+$ T cells (centre row) and $CD14^-$ $CD19^-$ $Vδ2^-$ T cells (bottom row) and is displayed together with expression of CD8. MFI of CRV $streptamer^+$ populations are displayed in red ("n/a" = not applicable).

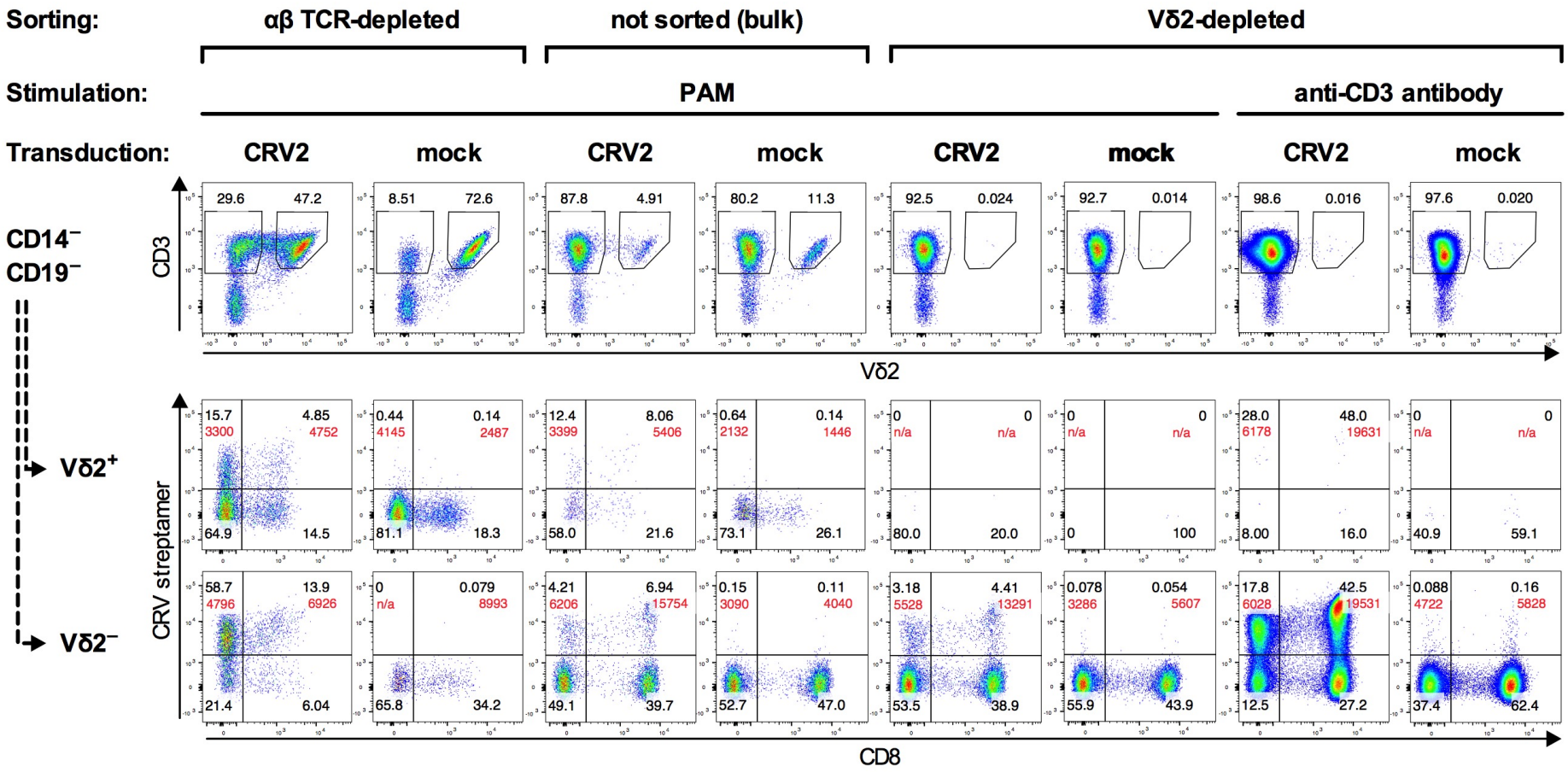


Figure 4.14. Effect of different immunomagnetic sorting on expression of CRV2 TCR prior to stimulation and retroviral transduction. αβ T cell-depleted, bulk or δ2 T cell-depleted PBMCs from donor 1107 had been stimulated with PAM and were transduced by retroviruses that either delivered the genes of CRV2 TCR or no genetic cargo ("mock"). For comparison, δ2 T cell-depleted PBMCs had been stimulated with anti-CD3 antibody and were transduced likewise. TCR expression was assessed on day 7 by CRV streptamers. NK cells that may bind to CRV streptamers via CD158b, although they have not been transduced, were excluded by gating on CD158b$^-$ cells. Pseudocolour dot plots show the expression of CD3 and Vδ2 on CD14$^-$ CD19$^-$ cells (top row). Expression of CRV2 TCR was analysed by streptamer staining on CD14$^-$ CD19$^-$ Vδ2$^+$ T cells (centre row) and CD14$^-$ CD19$^-$ Vδ2$^-$ T cells (bottom row) and is displayed together with expression of CD8. MFI of CRV streptamer$^+$ populations are displayed in red ("n/a" = not applicable).

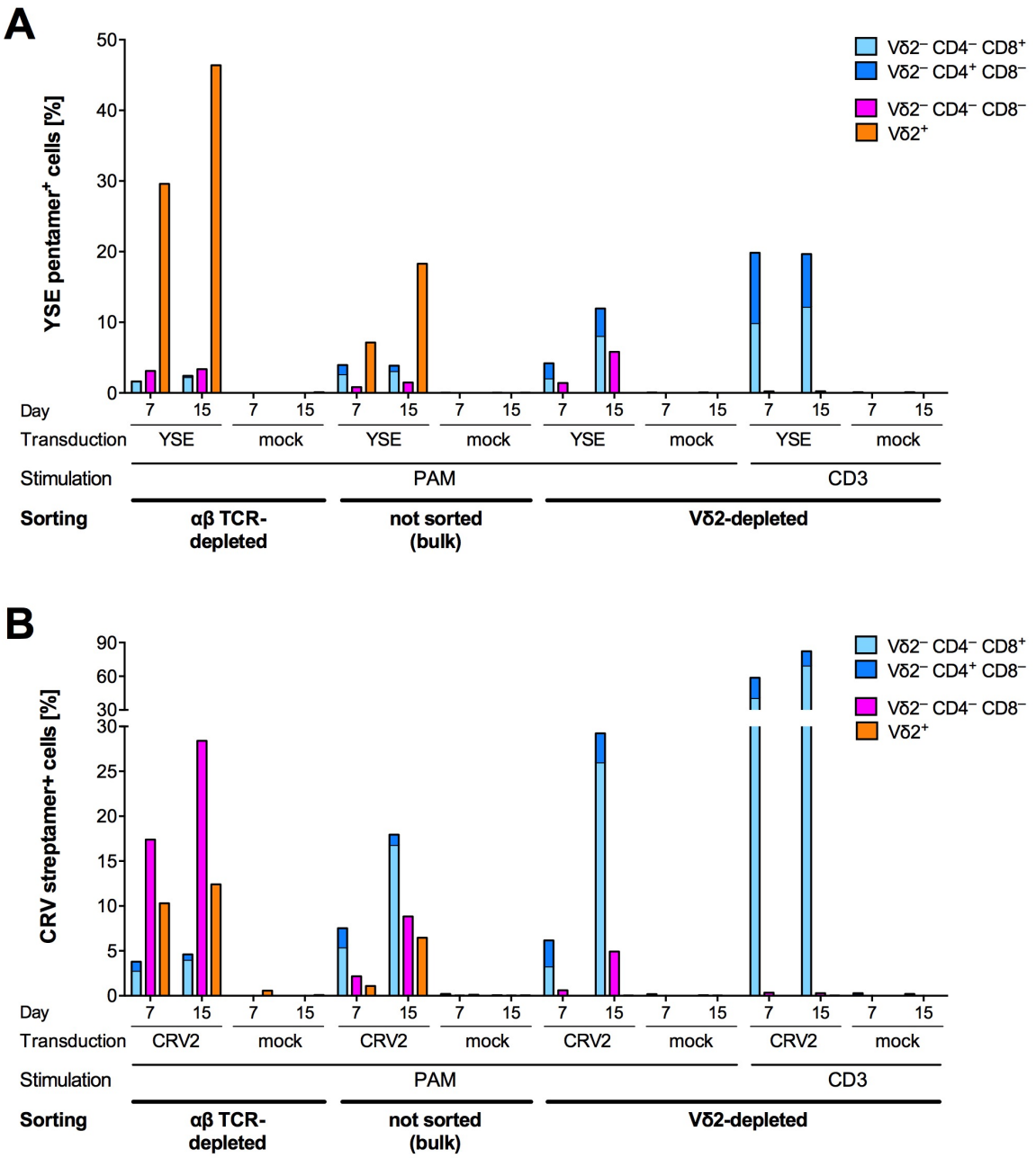


Figure 4.15. Effect of different immunomagnetic sorting and different stimulation on the expression of transgenic TCRs YSE (A) and CRV2 (B). αβ T cell-depleted, bulk or δ2 T cell-depleted PBMCs from donor 1107 had been stimulated with PAM and were transduced by retroviruses that either delivered the genes of YSE TCR, CRV2 TCR or no genetic cargo ("mock"). For comparison, δ2 T cell-depleted PBMCs had been stimulated with anti-CD3 antibody and were transduced likewise. On day 10, YSE-transgenic T cell lines were restimulated with an HLA-A*01:01-matched mini-LCL endogenously expressing pp65 (donor 0112) and CRV-transgenic T cell lines were restimulated with an HLA-C*07:02-matched mini-LCL endogenously expressing IE-1 (donor 1021). TCR expression was assessed on days 7 and 15 by YSE pentamers or CRV streptamers, respectively (see fig. 4.13 & 4.14 for pseudocolour dot plots from day 7). NK cells that may bind to CRV streptamers via CD158b, although they have not been transduced, were excluded by gating on CD158b$^-$ cells. Proportions of CD3$^+$ T cells that expressed the transgenic TCR among Vδ2$^-$ CD4$^-$ CD8$^+$ (bright blue), Vδ2$^-$ CD4$^+$ CD8$^-$ (dark blue), Vδ2$^-$ CD4$^-$ CD8$^-$ (pink) and Vδ2$^+$ (orange) cells were normalised to total CD14$^-$ CD19$^-$ cells.

Vδ2$^+$ T cells (61.0% of CD4$^+$ Vδ2$^-$ and 60.9% of CD8$^+$ Vδ2$^-$ vs. 20.6% of Vδ2$^+$ T cells were CRV streptamer$^+$, fig. 4.14), whereas the opposite held true for YSE (28.3% of CD4$^+$ and 15.9% of CD8$^+$ Vδ2$^-$ vs. 40.2% of Vδ2$^+$ T cells, fig. 4.13). This behaviour was observed in another donor each (1613 and 1223, respectively, data not shown). These data may indicate that CRV2 is not or less affected by TCR mis-pairing, as more CD4$^+$ Vδ2$^-$ T cells express functional CRV2 TCRs than Vδ2$^+$ T cells, the latter of which will not form mixed TCR dimers. If CRV2 would be subjected to TCR mis-pairing in αβ T cells, one would expect this ratio to be inverted. A staining with an anti-Vβ6 antibody could not be performed for CRV2 because no suitable antibody is commercially available, as noted before. For YSE TCR, stainings of the same cells using an anti-Vβ1 antibody have not been performed either, but in an independent experiment CD3-stimulated or PAM-stimulated bulk PBMCs of donor 1223 were transduced with YSE TCR and stained by both YSE pentamer and Vβ1 antibody (fig. 4.12): among CD3-stimulated PBMCs, CD4$^+$ and CD8$^+$ Vδ2$^-$ T cells showed less YSE pentamer$^+$ than Vβ1$^+$ proportions, whereas the proportion of YSE pentamer$^+$ Vδ2$^+$ T cells was higher than the one of Vβ1$^+$ Vδ2$^+$ T cells in PAM-stimulated bulk PBMCs. In both experiments described above, markedly more Vδ2$^+$ T cells could be stained with the YSE pentamer than Vδ2$^-$ T cells. Thus, the expression of YSE TCR may be dampened by TCR mis-pairing in αβ T cells.

Importantly, HLA class I-restricted αβ TCRs are not generally better expressed in CD8$^+$ Vδ2$^-$ T cells than in CD8$^-$ Vδ2$^-$ T cells or CD8$^-$ Vδ2$^+$ T cells, as shown for YSE and CRV2 TCRs above (fig. 4.13 & 4.14, respectively). Another reason for the low degree of dependence on co-expression of CD8 could be that these TCRs are quite strong TCRs concerning their competitiveness for CD3 proteins. Indeed, transgenic αβ TCRs seemed to suppress the endogenous TCR of δ2 T cells. This TCR competition was especially pronounced for CRV2, but also observed with YSE TCR (fig. 4.15).
Although depletion of αβ TCR$^+$ cells typically reduced their frequency to 0.1% in the negative fraction (fig. 4.19), almost 10% of CD3$^+$ Vδ2$^-$ cells were found four days after mock-transduction (fig. 4.13 & 4.14). This fraction could have been formed by other γδ T cells, such as δ1 T cells, or by reappearing αβ T cells that escaped depletion during immunomagnetic separation because they may not have expressed αβ TCRs at the time due to transient TCR down-regulation after activation. On the other hand, these cells with a CD3$^+$ Vδ2$^-$ phenotype may in fact be δ2 T cells that transiently down-regulated γδ TCRs as a result of stimulation by PAM. Arguing in favour of the latter, transduction of αβ TCR-depleted and PAM-stimulated PBMC cultures with a CRV2-encoding retrovirus further increased a fraction of the same phenotype to 29.6% CD3$^+$ Vδ2$^-$ cells. The majority of these cells was CD4$^-$ and CD8$^-$, but CRV streptamer$^+$ (fig. 4.14). This confirmed that (I) αβ T cell depletion was efficient (see section 4.4.1) and αβ T cells did not reappear in large numbers afterwards because most of them would strongly express either CD4 or CD8 (compared with low expression of CD4 or CD8 on a minority of δ2 T cells; exemplary stainings see fig. 4.23 and fig. 4.14, respectively), and that (II) transgenic expression of some αβ TCRs, such as CRV2, suppresses the endogenous γδ TCR thus converting δ2 T cells to CD3$^+$ Vδ2$^-$ cells (fig. 4.14).

In summary, the data shown above demonstrates that HCMV-specific, HLA class I-restricted

TCRs can be functionally expressed on both αβ and δ2 T cells, but the proportion of TCR-transgenic T cells and the intensity of TCR expression varies depending on the individual TCR that was transduced. Some TCRs (e.g. CRV2) are strongly expressed also in the absence of CD8 co-receptor, while others seem to rely on expression of the latter (e.g. CRV1, fig. 4.11). Murinisation can boost the expression of a "weak" TCR, such as CRV1, on $CD8^-$ T cells, but does not improve expression of a "strong" TCR, such as CRV2 (fig. 4.11). While some αβ TCRs did co-exist with Vγ9Vδ2 TCRs, others suppressed expression of γδ TCRs on the cell surface of δ2 T cells (fig. 4.15). Due to the high efficiency of αβ T cell depletion by immunomagnetic sorting (see section 4.4.1) and the competition of TCRs in δ2 T cells, $CD3^+$ $V\delta2^-$ cells of αβ TCR-depleted PBMCs likely were δ2 T cells nonetheless.

4.3.2 Recognition of HCMV-infected fibroblasts by TCR-transgenic T cells via HLA-C*07:02

HCMV aims to deceive and escape the immune system by different mechanisms. One of them targets the presentation of viral peptides by HLA complexes. Several proteins encoded in the US2-11 region of the HCMV genome interfere with antigen presentation on HLA class I (Ahn et al., 1997; Jones and Sun, 1997; Jones et al., 1996; Wiertz et al., 1996). Consequently, infected host cells present fewer HLA molecules loaded with viral peptides. While several T cell clones restricted by HLA-A or -B molecules were shown to suffer from the effects of these immunoevasins on presentation of viral peptides, T cell clones that were restricted by HLA-C*07:02 were able to recognise HCMV-infected fibroblasts despite the actions of the immunoevasins (Ameres et al., 2013). Thus, HLA-C*07:02 may be considered an immunoprivileged restriction for HCMV-specific T cells. This observation combined with the relative high allele frequency of HLA-C*07:02 across different human populations (e.g. 13.7% in Caucasians, Gragert et al., 2013, also see tab. 5.1), makes TCRs restricted by HLA-C*07:02 an interesting tool for adoptive T cell therapy of HCMV. In total, genes of nine TCRs specific for two different viral epitopes presented by HLA-C*07:02 were isolated and prepared for retroviral transfer, four TCRs specific for epitope CRV from IE-1 and five TCRs specific for epitope FRC from UL28 (tab. 4.2).

4.3.2.1 Recognition of HCMV-infected fibroblasts mediated by CRV (IE-$1_{309\text{-}317}$)-specific TCRs

The successful gene transfer and subsequent expression of HCMV-specific TCRs in αβ and δ2 T cells was shown in the previous section. Now, their capability of mediating the recognition of HCMV-infected target cells was tested. For HLA-C*07:02-restricted TCRs, HLA-matched MRC-5 fibroblasts were chosen as targets. By pre-treating the fibroblasts with IFN-γ for 72 hours before infection, their HLA expression was enhanced (Ameres et al., 2014), thus reinforcing T cell recognition (Ameres et al., 2013). 48 hours after infection with different HCMV strains, the MRC-5 fibroblasts were co-incubated with HCMV-specific T cell clones or TCR-transgenic T cell lines for 16–21 hours. Fibroblasts were infected with one of two wild type (WT) strains of HCMV, namely AD169 and Merlin, or the genetically modified virus strain CMV-Δall, which is derived from AD169 but lacks the four immunoevasins US2, US3, US6 and US11 (Besold et al., 2009, originally named RV-KB6). Mock-infected or peptide-pulsed target cells were used as negative or positive

controls, respectively. αβ T cells from PBMC donors 0112 and 1107 that had been transduced with different TCRs recognising the same epitope (CRV1-CRV4, specific for IE-$1_{309\text{-}317}$, tab. 4.2) secreted high levels of IFN-γ in response to MRC-5 fibroblasts if these were loaded with CRV peptide or infected with CMV-Δall (≫10,000 pg/ml, fig. 4.16 B). In contrast, mock-transduced αβ T cell lines from both donors released only little IFN-γ (<800 pg/ml), when incubated with the same target cells. Generally, fibroblasts that had not been infected or loaded with CRV peptide elicited low IFN-γ levels irrespective of effector cell type (<900 pg/ml). After encountering fibroblasts infected with one of the two WT strains AD169 or Merlin, the TCR-transgenic αβ T cell lines secreted moderate IFN-γ levels (<1,800 pg/ml), which surmounted IFN-γ levels of respective negative controls by no more than a factor of five. The two CRV-specific T cell clones 1010M #7 and F46M #36, however, did not only strongly recognise MRC-5 fibroblasts loaded with CRV peptide or infected with CMV-Δall (>28,000 pg/ml), but also secreted high levels of IFN-γ if fibroblasts had been infected with AD169 or Merlin (9,500–17,000 pg/ml, fig. 4.16 B). Intriguingly, the T cell clone F46M #36, from which the TCR CRV3 was derived, evoked a distinct response towards AD169- or Merlin-infected fibroblasts, while αβ T cell lines expressing CRV3 failed to do so. Although both effector cell types express the same TCR and at least partly provide CD8 co-receptor, TCR-transgenic T cell lines cannot mimic the antiviral effector function of the T cell clone. It seems that the presence of the four immunoevasins US2, US3, US6 and US11 in AD169 does not impair the two HLA-C*07:02-restricted CRV-specific T cell clones F46M #36 or 1010M #7, but prevents recognition of HCMV infection in fibroblasts by T cell lines expressing CRV-specific TCRs. Moreover, TCR-transgenic αβ T cells showed the same power in recognising fibroblasts infected with CMV-Δall than T cell clones did. These observations were confirmed in two other independent experiments involving T cell clones 1010M #7 and F46M #36, as well as TCR-transgenic T cell lines derived from PBMCs of donors 0102, 1107 and 1613 (data not shown). Thus, other reasons likely are responsible for this functional impairment of TCR-transgenic T cells.

In all the TCR constructs used in these experiments (except for CRV2α & CRV2β, tab. 2.8), the TCRα and TCRβ chains were encoded by a single gene, separated by a P2A element. After translation, 21 amino acids remain attached to the C terminus of the TCRβ chain and a single proline is attached to the N terminus of the TCRα chain (Donnelly et al., 2001, also see section 3.2.3). Therefore, it was tested whether the remaining P2A amino acid sequences negatively affected TCR pairing or signalling. For this purpose, TCRα and TCRβ chains of CRV2 were delivered by separate retroviruses into the same T cells refraining from use of P2A (CRV2α & CRV2β), which ensured that no additional amino acids were attached to the TCR chains, and that their amino acid sequence was exactly identical to their native sequence in the parental T cells. Furthermore, the effect of preferential TCR pairing was studied by employing a murinised variant of CRV2 (CRV2mu). In both cases, the TCR-transgenic αβ T cells recognised MRC-5 fibroblasts loaded with CRV peptide or infected with CMV-Δall to the same extent than TCR-transgenic αβ T cells that had been transduced with a retrovirus coding for the fully human CRV2β–P2A–CRV2α bicistronic TCR construct (CRV2, fig. 4.16 B). Neither CRV2mu nor the combination of CRV2α and CRV2β, however, could reconstitute the sensitivity for fibroblasts infected with one of the two WT strains AD169 or Merlin.

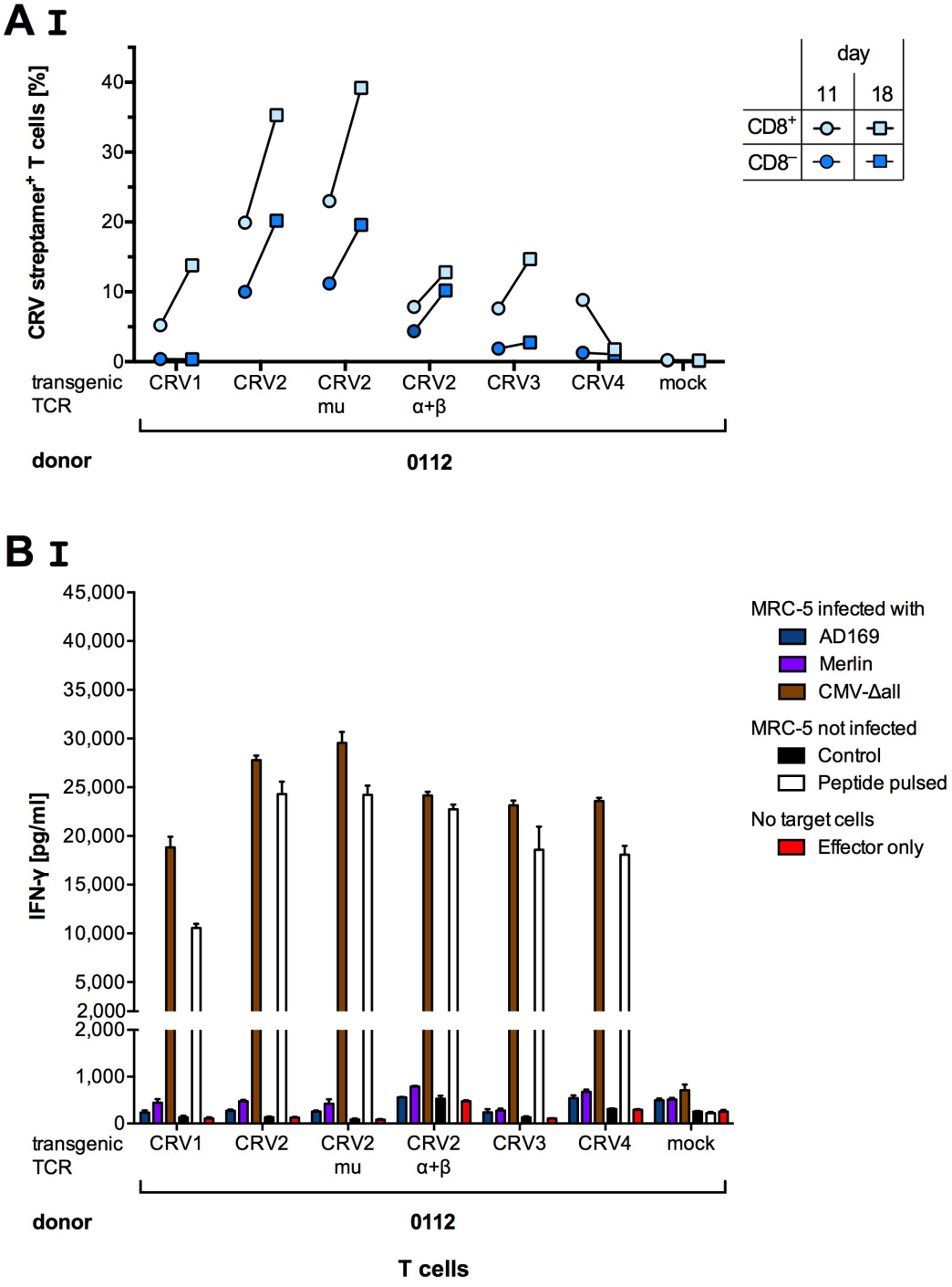


Figure 4.16. Expression of CRV TCRs by αβ T cells and their recognition of HCMV-infected MRC-5 fibroblasts. αβ TCR-enriched fractions from PBMCs of healthy donors 0112 (I) and 1107 (II) had been stimulated with anti-CD3 antibody and were transduced with different TCRs specific for CRV ($IE_{309\text{-}317}$, tab. 4.2). TCR-transgenic T cell lines were restimulated by autologous IE-1-expressing mini-LCLs on day 12. **A:** Expression of CRV-specific TCRs was assessed by CRV streptamers on day 11 and 18. Proportions of CRV streptamer$^+$ cells among CD8$^-$ and CD8$^+$ αβ T cells, both of which were pre-gated as CD14$^-$ CD19$^-$ CD3$^+$ Vδ2$^-$ cells, are shown for all transduced cell lines from donors 0112 and 1107.
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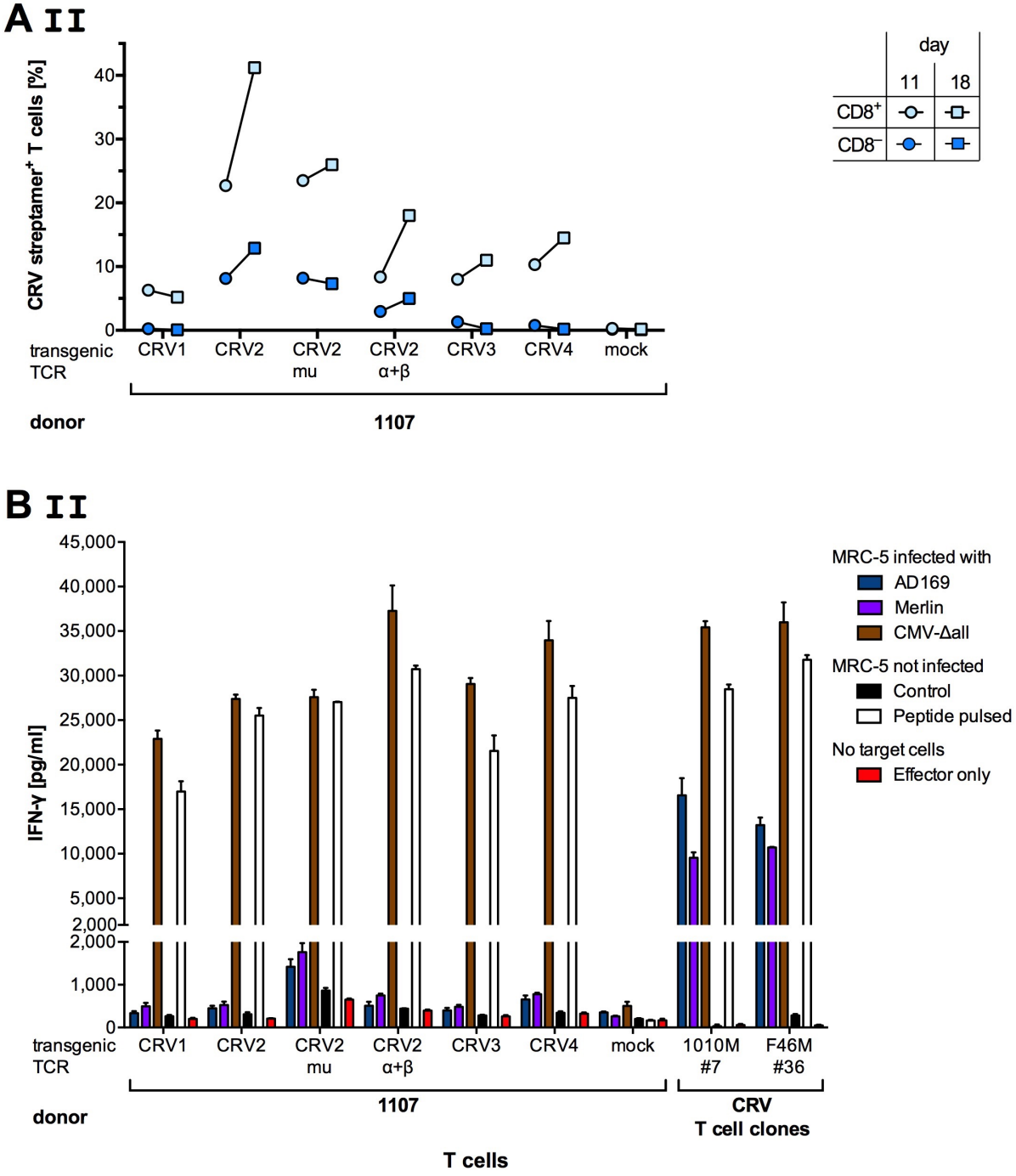


Figure 4.16. ***(continued from previous page)***
B: On day 14, MRC-5 fibroblasts were pre-treated with 300 U/ml IFN-γ for 72 hours. On day 17, they were infected with HCMV strain AD169 (blue bars), Merlin (purple bars) or CMV-Δall (brown bars) at an MOI of 5 for another 48 hours in the absence of IFN-γ. Non-infected target cells were either loaded with CRV peptide (white bars) or left untreated (black bars) to serve as positive or negative controls, respectively. On day 19, TCR-transgenic T cell lines or CRV-specific T cell clones were co-incubated overnight with target cells at an e:t ratio of 20,000:10,000 or 10,000:10,000, respectively. The graph shows the concentration of secreted IFN-γ in the supernatant as evaluated by ELISA. The mean and standard deviation of triplicate samples are shown.

In summary, each TCR specific for the epitope CRV (IE-$1_{309\text{-}317}$) enabled the TCR-transgenic T cells to recognise HCMV-infected MRC-5 fibroblasts (fig. 4.16 B). Although, the proportions of T cells expressing a functional CRV TCR varied from less than 5% to more than 40% depending on the T cell subset ($CD8^+$ or $CD8^-$) and the individual CRV-specific TCR (fig. 4.16 A), similar levels of IFN-γ were found to be secreted by these TCR-transgenic T cell lines in response to fibroblasts loaded with CRV peptide or infected with CMV-Δall (fig. 4.16 B). At the same time, none of the TCRs mediated unspecific reactivity. Thus, all CRV-specific TCRs are functional and specific for the epitope CRV. However, none of the TCRs was able to confer the same sensitivity for AD169-infected fibroblasts that was observed for CRV-specific T cell clones (Ameres, 2012, and data presented in this study) to transgenic T cells. This deficiency was unexpected but consistent for several independent experiments and for PBMCs derived from four different donors.

One of the plausible explanations of this unexpected effect could be found in the proportions of TCR-expressing T cells. While all cells of a T cell clone express the CRV-specific TCR, rarely more than 50% of the cells of TCR-transgenic T cell lines were found to express the transgenic TCR a few days after retroviral transduction. Moreover, the intensity of TCR expression may influence the sensitivity of the T cells, which may be key in binding to a limited amount of viral peptides presented on HLA molecules, especially if target cells are infected with a virus strain that expresses all immunoevasins. The CRV-specific T cell clones 1010M #7 (expressing an independent TCR) and F46M #36 (the source of the TCR CRV3), as well as TCR-transgenic T cell lines from donor 1107 expressing either CRV2 or CRV3 were stained with CRV steptamers and analysed by flow cytometry. The fraction of CRV $streptamer^+$ cells was larger for both T cell clones (≥99%) than for TCR-transgenic T cell lines that had been restimulated with autologous, IE-1-expressing mini-LCLs once, but nonetheless the fraction of $streptamer^+$ cells in these transgenic T cell lines was quite high (85.2% of CRV2-transduced and 54.8% of CRV3-transduced $CD3^+$ $Vδ2^-$ T cells, fig. 4.17). The intensity of TCR expression by a CRV2-transgenic T cell line (6,372 AU of $CD14^-$ $CD19^-$ $CD158b^-$ cells, fig. 4.17) was comparable to T cell clone F46M #36 (7,935 AU) as judged by mean fluorescence intensity (MFI) from CRV streptamer stainings. The T cell clone 1010M #7 showed a more intense TCR expression (21,809 AU), whereas the CRV3-transgenic T cell line showed a less intense TCR expression (1,989 AU). Further narrowing the focus on $CD3^+$ $Vδ2^-$ $CD8^+$ CRV $streptamer^+$ T cells, revealed that the CRV2-transgenic T cell line (10,008 AU) even outperformed the T cell clone F46M #36 (8,209 AU) concerning intensity of TCR expression. Similar data were obtained for CRV2mu-transgenic T cell lines in the same experiment (fig. 4.17).
The proportions of TCR-transgenic αβ T cells depicted in figure 4.16 A are substantially lower than those from this comparative analysis (fig. 4.17). Nevertheless, when TCR-transgenic bulk T cells were tested on HCMV-infected MRC-5 fibroblasts in the same manner than TCR-transgenic αβ T cells (fig. 4.16 B), the resulting IFN-γ patterns were almost identical (data not shown), confirming previous observations. The intensity of TCR expression on T cell clone F46M #36 was comparable to CRV2- or CRV2mu-transgenic T cells and the CRV $streptamer^+$ proportions of CRV2- or CRV2mu-transgenic T cell lines were only reduced by 14% or 18% in comparison to F46M #36, respectively (fig. 4.17). Thus, other factors than TCR expression may be more

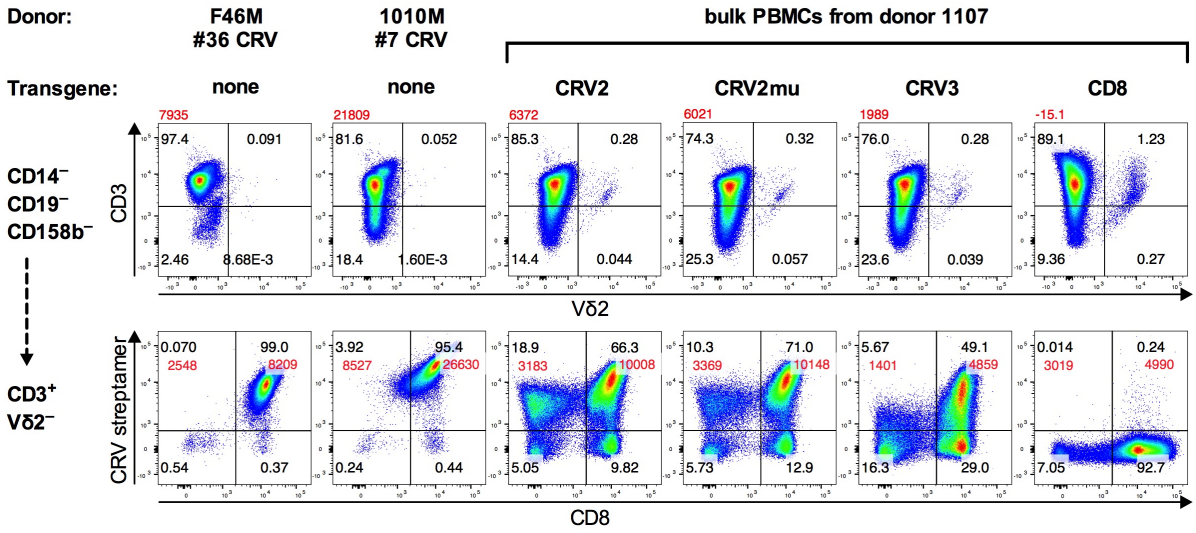


Figure 4.17. Peptide/HLA streptamer staining of CRV-specific αβ T cell clones or TCR-transgenic T cells transduced with a CRV-specific TCR. Bulk PBMCs from donor 1107 had been stimulated with anti-CD3 antibody and were transduced with one of the three TCRs CRV2, CRV2mu or CRV3 or with CD8 as a control transgene. Transduced PBMCs were restimulated with autologous, IE-1-expressing mini-LCLs on day 10. Six days later, T cell clones F46M #36 and 1010M #7 were thawed, stained with CRV streptamers and analysed by FACS simultaneously with the transduced T cell lines. Top row: Expression of CD3 and Vδ2 TCR chain among $CD14^-$ $CD19^-$ $CD158b^-$ cells. MFI of CRV streptamer staining is displayed in red for all cells on top of the graph. Bottom row: Staining of CRV-specific TCR and CD8 of $CD3^+$ $Vδ2^-$ T cells derived from $CD14^-$ $CD19^-$ $CD158b^-$ cells. MFI of CRV streptamer is shown in red for $CD8^-$ and $CD8^+$ subpopulations within the respective gate.

important in overcoming the lack of sensitivity of TCR-transgenic T cells towards infection with AD169.

4.3.2.2 Recognition of HCMV-infected fibroblasts mediated by FRC ($UL28_{327\text{-}335}$)-specific TCRs

Five more TCRs restricted by HLA-C*07:02, but specific for a different epitope, namely FRC ($UL28_{327\text{-}335}$), were transferred into T cells after CD3 stimulation of bulk PBMCs and evaluated similarly (FRC1, FRC6, FRC32, FRC86 & FRC93, see tab. 4.2). FRC1, FRC6, FRC32 and FRC93, but not FRC86, could be stained by FRC streptamers on TCR-transgenic T cells (fig. 4.18 A), indicating that four out of five TCRs were functionally expressed. FRC86's TCRβ chain could be stained with an anti-Vβ13.2 antibody to a similar extent than Vβ2 of FRC1 or Vβ3 of FRC6, FRC32 and FRC93 (fig. 4.18 A), indicating that transduction efficiency and TCRβ chain integrity were not corrupted. The sequence of FRC86's TCRα chain was reanalysed in order to find explanations for its potential lack of expression. It was found that the cloned and transduced version of FRC86's TCRα sequence differed from all available reference sequences of IMGT for TRAV13-2*01 (Scaviner and Lefranc, 2000b) in nucleotide positions (nt pos.) 43 and 68 resulting in amino acid exchanges at positions 15 and 23, respectively, of the full-length TCRα protein.

The Vα8 (= TRAV13) primer that was used until that time as one of the 34 Vα primers to

amplify fragments of TCRα cDNA by PCR (see section 3.2.2) contained an error: it harboured a guanosine (G) at nt pos. 12 of the primer's sequence (see tab. 2.9), but all TRAV13 alleles, for which the primer was designed, harbour a cytidine (C) at the corresponding nt pos. 43 (Scaviner and Lefranc, 2000b). Thus, amplification of the TCRα cDNA by PCR using the erroneous Vα8 primer had turned nucleotide C43 into G43 thereby turning amino acid L15 into V15, which lies within the leader peptide. This exchange potentially affected the function of the leader peptide. It is unlikely, however, that this exchange affected the leader peptide's cleavage because the cleavage site is positioned at some distance downstream between amino acid 21 and 22, and the recognition motif of the signal peptidase (Hiller et al., 2004) was probably not affected. The erroneous primer has long been in use in this (Schub, 2010) and collaborating research groups (Schuster, 2008) and apparently the error was not noticed before. This may serve as a reminder to be vigilant regarding such types of errors. At the time of *in silico* assembly of TCR constructs and commercial gene synthesis, this error went unnoticed and was thus incorporated in the cloned TCRα chain of FRC86. In order to avoid introduction of sequence errors in PCRs for TRAV13-1 or TRAV13-2 in the future, the Vα8 primer was corrected and re-synthesised ("Vα8corr").
The other difference between IMGT's reference sequences and the TCRα amplicon of FRC86 was an adenosine (A) at nt pos. 68 instead of a G, as found in the reference sequences of TRAV13-2*01 and TRAV13-2*02 (Scaviner and Lefranc, 2000b). The TCRα cDNA of T cell clone XLBC18 #86 was amplified again using the same Cα-specific primer 3'T-Cα, but a different Vα-specific primer (TRAV13-2, tab. 2.10) that binds more upstream (closer to the 5' end) than the primer Vα8 does. Sequencing of this amplicon confirmed that the unexpected nucleotide A68 was present in the original TCRα chain of the parental T cell clone. Thus, this T cell clone harbours an allele of TRAV13-2 that has not been previously described and that results in an Asn-to-Ser exchange at amino acid position 23. Unfortunately, when the TCR was assembled *in silico*, the nucleotide A68 was replaced by mistake with G68 as present in standard sequence. Consequently, amino acid N23 may have hindered successful TCR pairing. Although the function of FRC86 likely could have been restored by correcting both mistakes, the sequence of the TCRα chain of FRC86 was not corrected and FRC86's use was discontinued.

Of the remaining four FRC-specific TCRs FRC1, FRC6, FRC32 and FRC93, each TCR mediated a strong release of IFN-γ by TCR-transgenic T cells in response to MRC-5 fibroblasts that had been infected with CMV-Δall (4,300–6,000 pg/ml, fig. 4.18 B). None of the TCRs, however, was able to drive a noteworthy IFN-γ secretion by TCR-transgenic T cells after encountering AD169-infected MRC-5 fibroblasts (<80 pg/ml). As expected, non-infected fibroblasts were not recognised (<20 pg/ml), whereas FRC peptide-pulsed fibroblasts also caused a marked IFN-γ secretion by TCR-transgenic T cells (1,300–2,200 pg/ml). Fibroblasts that were infected with the HCMV strain TB40-BAC4 evoked intermediate IFN-γ levels by TCR-transgenic T cells (600–1,600 pg/ml). No FRC-specific T cell clones were available for comparison with TCR-transgenic T cells.
Like CRV-specific TCRs, FRC-specific TCRs also failed to confer sensitivity for AD169-infected fibroblasts to TCR-transgenic T cells. After testing 8 different HLA-C*07:02-restricted TCRs that responded perfectly well to CMV-Δall-infected and peptide-loaded fibroblasts, it is very unlikely that this deficiency is caused by errors contained in some of the TCR sequences. Ul-

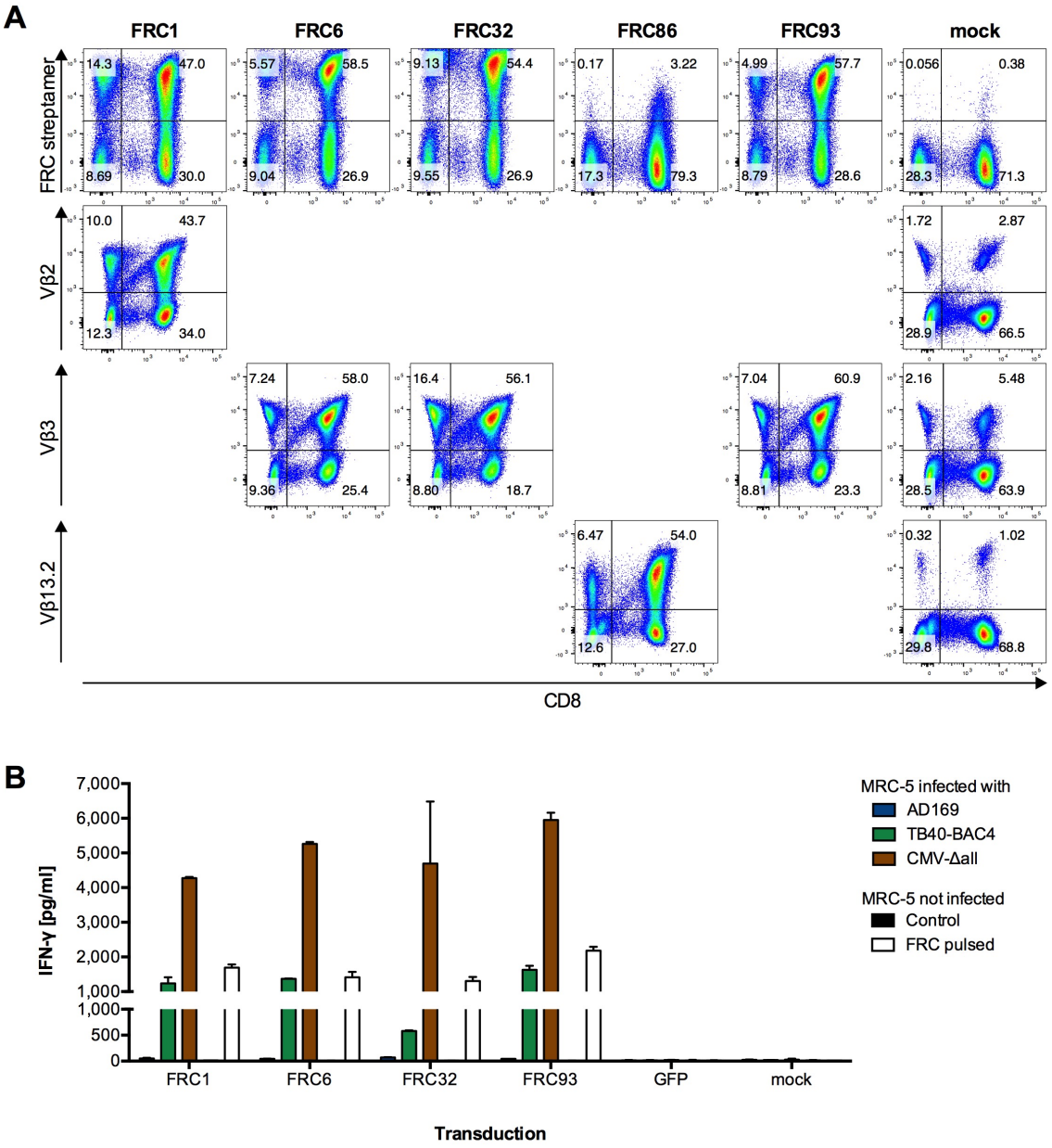


Figure 4.18. Expression of FRC-specific TCRs by T cells and their conveyed recognition of HCMV-infected MRC-5 fibroblasts. Bulk PBMCs from healthy donor 1107 had been stimulated with anti-CD3 antibody and were transduced with different retroviruses delivering genes of an FRC-specific TCR ($UL28_{327\text{-}335}$, tab. 4.2), GFP or no genetic cargo ("mock"). Transduced T cell lines were restimulated by autologous peptide-loaded mini-LCLs on day 11 and expression of FRC-specific TCRs was assessed by FRC streptamers and Vβ-specific antibodies on day 16. **A:** Pseudocolour dot plots show the proportions of CRV streptamer$^+$ (top row) or Vβ$^+$ (other rows) cells among CD8$^-$ and CD8$^+$ T cells, both of which were pre-gated as CD14$^-$ CD19$^-$ CD3$^+$ Vδ2$^-$ cells. **B:** On day 13, MRC-5 fibroblasts were pre-treated with 300 U/ml IFN-γ for 72 hours. On day 16, they were infected with HCMV strain AD169 (blue bars), TB40-BAC4 (green bars) or CMV-Δall (brown bars) at an MOI of 5 for another 48 hours in the absence of IFN-γ. Non-infected target cells were either loaded with FRC peptide (white bars) or left untreated (black bars) to serve as positive or negative controls, respectively. GFP- or mock-transduced T cell lines were used as control effector cells to monitor unspecific activation through allogeneic T cell stimulation. On day 18, transgenic T cell lines were co-incubated overnight with target cells at an e:t ratio of 20,000:10,000. The graph shows the concentration of secreted IFN-γ in the supernatant as evaluated by ELISA. The mean and standard deviation of duplicate samples are shown.

timately, the reason for this deficiency may lie in the nature of the effector cells that host the HCMV-specific TCR or their composition, but not the TCR sequences or their expression profiles.

4.3.2.3 Reasons for different sensitivity of HLA-C*07:02-restricted T cell clones and TCR-transgenic T cells towards infection with AD169

T cell clones were expanded from single cells and thereby free of other cell types that are usually contained in PBMCs. TCR-transgenic T cells, however, may encompass several other cell types that could interfere with the detection of HCMV-infected target cells by HCMV-specific T cells and subsequent antiviral actions. After immunomagnetic sorting or prolonged *in vitro* propagation, T cell lines that had been stimulated with anti-CD3 antibody prior to transduction were usually devoid of most other cell types, such as $CD14^+$ monocytes, $CD19^+$ B cells, $CD158b^+$ NK cells (each population <1% for all TCR-transgenic T cell lines shown in fig. 4.17 for example, data not shown). Regulatory T (T_{reg}) cells, on the other hand, may be conserved even throughout prolonged *in vitro* cultivation. During co-cultivation with HCMV-infected fibroblasts they may prevent antiviral effector functions. The amount of secreted IL-10 in the supernatant of CRV1- or CRV2-transgenic T cells from CD3-stimulated, bulk PBMCs of donors 0102 and 1107 co-incubated with HCMV-infected MRC-5 fibroblasts, however, was generally low ($\leq$110 pg/ml) and mock-transduced T cells produced similar levels of IL-10 (<65 pg/ml).
In order to evaluate the IFN-γ secretion by $CD8^+$ T cells alone, the same TCR-transgenic T cell lines, as well as an FRC1- and an FRC6-transgenic T cell line from CD3-stimulated, bulk PBMCs of donor 1107 were subjected to immunomagnetic sorting of $CD8^+$ cells. After sorting, all cell lines contained less than 0.7% $CD14^+$ or $CD19^+$ T cells and less than 3.8% $CD4^+$ T cells. In spite of distinct IFN-γ secretion in response to CMV-Δall-infected fibroblasts, TCR-transgenic $CD8^+$ T cells did not recognise AD169-infected fibroblasts any stronger than previously observed (data not shown). Thus, T_{reg} cells are unlikely to interfere with the recognition of infection by HCMV stain AD169.

If not by other cells contained in the TCR-transgenic T cell lines, the deficiency must be caused by something within the T cell subsets themselves. Since inhibitory effects of T_{reg} cells can be largely excluded, the differentiation status of T cell clones and TCR-transgenic T cell lines was analysed. As previously, $\alpha\beta$ T cells were grouped into naïve (N = $CCR7^+$ $CD45RA^+$), central memory (CM = $CCR7^+$ $CD45RA^-$), effector memory (EM = $CCR7^-$ $CD45RA^-$) and effector memory re-expressing CD45RA (EMRA = $CCR7^-$ $CD45RA^+$) subpopulations according to their differentiation status (N $\rightarrow$ CM $\rightarrow$ EM $\rightarrow$ EMRA, from early to late). Both CRV-specific T cell clones F46M #36 and 1010M #7 were almost exclusively comprised of T_{EM} cells ($\geq$97.3%), which is the expected phenotype for long-term T cell lines and clones. In contrast, CRV2-, CRV2mu- or CRV3-transgenic T cell lines that were derived from CD3-stimulated, bulk PBMCs (see fig. 4.17) encompassed only 74.1%, 72.6% or 77.6% $CD3^+$ $CD8^+$ $CD14^-$ $CD19^-$ T_{EM} cells, respectively. Their second largest subset was that of T_{CM} cells accounting for 20.0%, 18.1% or 14.6% of $CD3^+$ $CD8^+$ $CD14^-$ $CD19^-$ T cells, respectively. Very similar subset distributions were observed for $CD4^+$ T cells. Since T_{CM} cell can replenish the pool of T_{EM} (Sallusto et al., 2004), the analysed

TCR-transgenic T cells are likely not at a disadvantage concerning their differentiation status in comparison to the two CRV-specific T cell clones.
Several HLA-specific activating and inhibitory receptors are expressed on NK cells, but may also be expressed on subsets of T cells. These receptors may co-modulate T cell recognition and may be expressed at different levels on T cell clones in comparison to TCR-transgenic T cell lines. Relevant receptors include killer cell immunoglobulin-like receptors (KIRs), like KIR2DL2/L3 (CD158b), and killer lectin-like receptors (KLRs), like NKG2A and NKG2D. The inhibitory receptors KIR2DL2 and KIR2DL3 bind HLA-C group 1 (C1) molecules that harbour an asparagine at position 80 (Mandelboim et al., 1996), such as HLA-C*07 alleles. When analysed by FACS, less than 0.3% of the cells of CRV-specific T cell clone 1010M #7 and less than 0.1% of the cells of CRV2-, CRV2mu- or CRV3-transgenic T cell lines derived from CD3-stimulated, bulk PBMCs (pre-gated as $CD3^+$ $CD8^+$ $CD14^-$ $CD19^-$ T cells, also see fig. 4.17 and above) stained positive for CD158b. 2.8% of the cells of CRV-specific T cell clone F46M #36 expressed CD158b, but this did not interfere with recognition of AD169-infected fibroblasts as shown in figure 4.16 B II. NKG2A is expressed as a heterodimer together with CD94, binds to non-classical HLA class I molecule HLA-E and transmits an inhibitory signal to prevent cytotoxicity by the NKG2A-expressing cell (Borrego et al., 1998). NKG2A was expressed by less than 0.1% of the cells of CRV-specific T cell clones F46M #36 or 1010M #7 and by less than 0.3% of the cells of CRV2-, CRV2mu- or CRV3-transgenic T cell lines that were derived from CD3-stimulated, bulk PBMCs. NKG2D is expressed as a homodimer and binds to the stress-inducible ligands MHC class I chain-related protein A (MICA), MICB and UL16-binding proteins 1, 2, 3 and 4 (ULBP1-4), thereby transmitting an activating signal that promotes cytotoxicity by the NKG2D-expressing cell (Sutherland et al., 2001). The MFI of NKG2D expression on T cell clones F46M #36 and 1010M #7 was 1,003 and 483 AU, respectively. CRV2-, CRV2mu- or CRV3-transgenic T cell lines showed a similar intensity of NKG2D expression with MFIs of 760, 790 or 950 AU, respectively (pre-gated as $CD3^+$ $CD8^+$ $CD14^-$ $CD19^-$ T cells). Thus, NKG2A was not expressed by any of the T cells and NKG2D's expression was negligible both on CRV-specific T cell clones and TCR-transgenic T cell lines.
Taken together, phenotypic analysis could not resolve why both effector cell types behave differently in their recognition of AD169-infected fibroblasts though they are both restricted by HLA-C*07:02 and some even express the same TCR (CRV3 isolated from F46M #36). Consequently, the relative functional deficiency of TCR-transgenic T cell lines equipped with an HLA-C*07:02-restricted TCR to detect and react to AD169 infection could not be explained so far and requires further investigation. HLA-C*07:02 remains an interesting restriction to target in HCMV-specific T cell therapies, since the superiority over other HLA-A and -B alleles was reported convincingly regarding recognition by naturally occurring HCMV-specific T cells from the memory repertoire (Ameres et al., 2013).

4.4 Characterisation of HCMV-specific TCRs restricted by HLA class II

The importance of a functional HCMV-specific T_H cell response has been reported by many studies (Gamadia et al., 2003; Lilleri et al., 2008; Rentenaar et al., 2000) and is especially emphasised in immunocompromised patients, such as transplant recipients (Gabanti et al., 2014;

Walter et al., 1995) and HIV$^+$ individuals (Komanduri et al., 1998). HCMV-specific CD4$^+$ T_H cells exert direct antiviral effector functions (Einsele et al., 2002; Jackson et al., 2017) and maintain HCMV-specific CD8$^+$ CTL responses (Gabanti et al., 2014; Walter et al., 1995). Due to the broadly acknowledged importance of HCMV-specific CD4$^+$ T_H cells in the control of HCMV reactivation (Einsele et al., 2002; Gabanti et al., 2014; Gamadia et al., 2003; Sellar and Peggs, 2012; Sester et al., 2001), this study aimed to characterise HLA class II-restricted TCRs that were isolated from such cells in order to establish them for application in adoptive T cell therapy. For this purpose, a set of 13 HLA class II-restricted TCRs specific for various epitopes of either pp65 or IE-1 were studied (tab. 4.2).

4.4.1 Immunomagnetic separation of PBMCs before stimulation with pamidronate and subsequent TCR transfer

Some αβ T cells were rendered susceptible to retroviral transduction of GFP due to the stimulation of bulk PBMCs with PAM as apparent by the emergence of GFP-expressing CD3$^+$ Vδ2$^-$ T cells (fig. 4.9 D, right graph). This simultaneous, non-intended transduction of αβ T cells did also occur with TCR-encoding retroviruses (fig. 4.13 & 4.14, see results for bulk PBMCs). Strikingly, even after experimental depletion of δ2 T cells, PAM stimulation facilitated retroviral transduction of Vδ2$^-$ T cells that were CD4$^+$ or CD8$^+$ (see TCR expression of Vδ2$^-$ cells among Vδ2-depleted PBMCs, fig. 4.15). When TCR-transgenic, unsorted PBMCs, which had been stimulated with PAM prior to transduction, were restimulated by antigen-expressing mini-LCLs, both TCR-transgenic αβ and TCR-transgenic δ2 T cells expanded and showed increased expres-

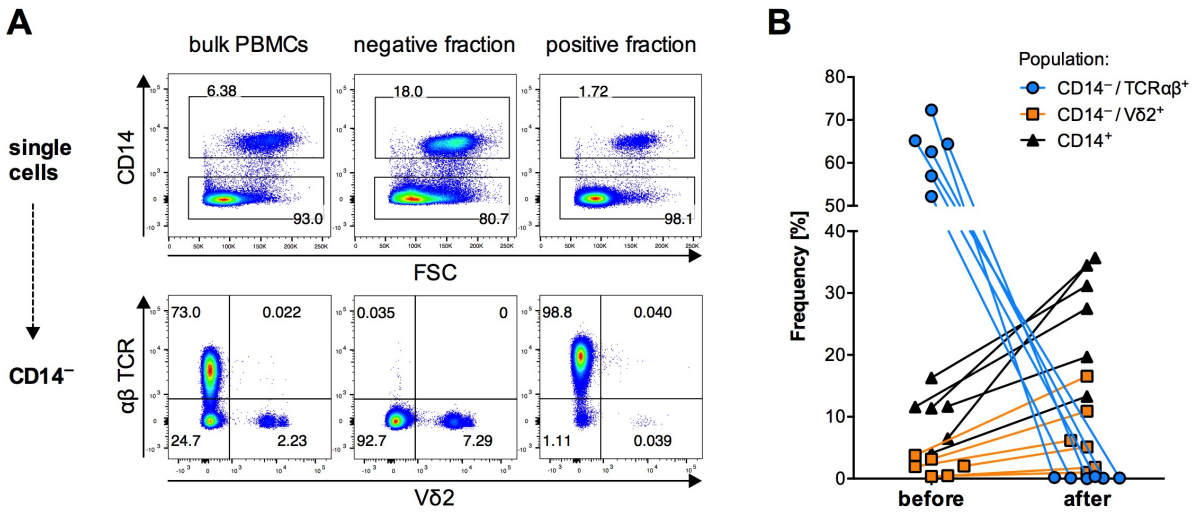


Figure 4.19. Composition of cell populations before and after immunomagnetic separation of αβ TCR$^+$ T cells. A: Proportion of CD14$^+$ monocytes (top row) among single cells (after exclusion of duplicates, etc.) and the proportion of αβ TCR$^+$ Vδ2$^-$ or αβ TCR$^-$ Vδ2$^+$ T cells (bottom row) among CD14$^-$ cells from donor 1107 before sorting (left column) or after sorting, which resulted in an αβ T cell-depleted (centre column) and an αβ T cell-enriched fraction (right column), are displayed. **B:** Analysis of the three cell populations in six different donors (0112, 0506, 1004, 1013, 1107, 1613). The displayed proportions of αβ TCR$^+$ T cells (blue circles) and Vδ2$^+$ T cells (orange squares) resulted from multiplication of the frequency of CD14$^-$ cells with the frequency of each of the two subsets among CD14$^-$ cells in order to achieve comparability to CD14$^+$ monocytes (black triangles).

sion of the transgenic TCR (fig. 4.15, see results for bulk PBMCs, day 7 vs. 15).
So bulk PBMCs that had been stimulated with PAM before retroviral transduction of an HCMV-specific TCR contained TCR-transgenic αβ T cells and TCR-transgenic δ2 T cells. Consequently, it was difficult to distinguish the contribution of these two subsets to HCMV-specific function. Thus, αβ T cells were depleted by magnetic activated cell sorting (MACS) with an APC-labelled, αβ TCR-specific antibody and anti-APC MicroBeads (see section 3.4.1) in order to assess the potency of TCR-transgenic δ2 T cells in the absence of contribution by TCR-transgenic αβ T cells. Two fractions were obtained by this procedure: an (αβ TCR-) positive fraction containing at least 90% αβ T cells (94% αβ T cells among live, single cells on average, evaluated in five donors) and an (αβ TCR-) negative fraction encompassing the majority of $CD14^+$ monocytes, γδ T cells (fig. 4.19 A), $CD19^+$ B cells, and NK cells. Before immunomagnetic separation, αβ T cells predominated bulk PBMCs (50–70 %), but afterwards less than 0.35% αβ T cells could be detected in the αβ TCR-depleted fraction (median of 0.10% among six donors, fig. 4.19 B).
αβ TCR^+ fractions were stimulated with anti-CD3 antibody prior to retroviral transduction to produce TCR-transgenic αβ T cells. αβ TCR^- fractions were treated with PAM before transduction to obtain TCR-transgenic δ2 T cells. While αβ T cells do not require the presence of additional immune cells to be activated by anti-CD3 antibodies, δ2 T cells rely on cell-cell interactions with other cells, like monocytes, to be activated by PAM-induced phosphoantigens (Miyagawa et al., 2001). As expected, $CD14^+$ monocytes were enriched in the αβ TCR^- fractions after αβ T cell depletion (fig. 4.19) providing the basis for specific stimulation of δ2 T cells by PAM treatment of this fraction.

4.4.2 Expression of HLA class II-restricted, HCMV-specific TCRs on αβ and δ2 T cells

Consistent with the previously established protocol for retroviral transduction (sections 3.5.1 & 4.2), the genes of HLA class II-restricted TCRs were transferred into αβ T cells and δ2 T cells using the αβ TCR-enriched fraction and the αβ TCR-depleted fraction after immunomagnetic separation, respectively. The same sorting procedure was used in all experiments described in sections 4.4.2 and 4.4.5. Despite the presence of other T cells, such as Vδ1 T cells in αβ TCR-depleted fractions, the T cell lines that were established by PAM stimulation from these fractions were dominated by δ2 T cells, and therefore will be called "δ2 T cell lines" in the following. The surface expression of transgenic αβ TCRs was assessed by staining with HLA tetramers and/or antibodies followed by flow cytometric analysis. Peptide/HLA class II tetramers (section 2.8) were available for TCRs recognising the epitopes QPFM ($pp65_{261\text{-}275}$), YRIQ ($pp65_{369\text{-}387}$), AGIL ($pp65_{489\text{-}503}$) and EFFT ($IE\text{-}1_{213\text{-}225}$, tab. 4.2), all of them restricted by HLA-DR molecules. The surface expression of all other TCRs on transgenic T cell lines was more circumstantially assessed by staining with antibodies specific for the Vβ domain of each TCR (tab. 2.14). The TCRs YRIQ18, YRIQ36, RRKM18 and EFFT50 (tab. 4.2) could not be stained by any anti-Vβ antibody because no antibody specific for Vβ6 (= TRBV7) was available for purchase. The expression of these TCRs could be monitored indirectly on δ2 T cells by staining with an anti-αβ TCR antibody (Clone: IP26, tab. 2.14). Consequently, the expression of these four TCRs could not be assessed by antibody staining on αβ T cells, since they already expressed endogenous αβ TCRs. However, the expression of these TCRs could be assessed by

peptide/HLA class II tetramers, except for RRKM18.

The pp65-specific TCR **KYQE16** was never transferred into any T cells within the work program of this thesis, due to the lack of an available HLA-DQB1*05:01$^+$ PBMC donor from which moDCs could be derived for testing TCR-transgenic effector T cells. As a result, successful expression and function of KYQE16 remains to be shown.
As mentioned in section 4.1.2, the putative HLA restriction of the CD4$^+$ T cell clone F46P #40 MSIY (HLA-DRB1*15:01, Wiesner, 2005) could not be verified using a TCR-transgenic T cell line. The MSIY/HLA-DRB1*15:01 tetramer failed to stain TCR-transgenic T cell lines several days after their transduction with a retrovirus that encoded the **MSIY40** TCR (data not shown). This could potentially be due to deficient tetramers or errors within the TCR sequences of MSIY40 that prevented its expression. However, the latter is not likely because approximately 20% of αβ TCR-depleted PBMCs (i.e. a δ2 T cell line) from donor 1004 that had been transduced with MSIY40 and CD4 stained positive for αβ TCR and Vβ3, which is the specific V region encompassed in MSIY40's TCRβ chain. Since TCRα and TCRβ chains of MSIY40 are not expected to pair with TCRγ and TCRδ chains in δ2 T cells, this indicates successful pairing of both chains of MSIY40 on the surface of transduced cells. Concerning the quality of the tetramer, its manufacturer Immunaware had reported sub-optimal folding for MSIY/HLA-DRB1*15:01 monomers in the delivery notes (60%, see section 2.8). Thus, a deficiency in MSIY/HLA-DRB1*15:01 tetramers is likely to have caused the failure in staining MSIY40-transgenic T cells. This raises the question whether the putative HLA-restriction DRB1*15:01 is correct (already discussed in section 4.1.2) or whether DRB1*15:01 is an allotype that is more difficult to correctly refold *in vitro* in the presence of peptide. This would not be too surprising, since production of good-quality HLA class II tetramers is technically challenging (Braendstrup et al., 2013). As a result, the efficacy of MSIY40 in recognition of HCMV-infected target cells remains to be elucidated.
Functional expression of MSIY40 was verified indirectly by TCR-transgenic αβ T cells from donor 0112 that strongly recognised HLA-matched pp65-expressing mini-LCLs from donor F46 (10,000 pg IFN-γ per ml), but produced much less IFN-γ in response to control mini-LCLs from the same donor (1,000 pg/ml, fig. 4.5). This kind of specific recognition of pp65 was observed for another set of HLA-matched mini-LCLs (donor F65), further supporting the integrity of MSIY40's TCR sequences.

In contrast, the genes of multiple other HCMV-specific, HLA class II-restricted TCRs could be transferred into αβ as well as δ2 T cells, and expression of the respective TCRs could be detected on the cell surface. The TCRβ chains of **QPFM17, EPDV21, SVMK6 and SVMK27** (tab. 4.2) were similarly well expressed by CD4$^+$ as well as CD4$^-$ (mainly CD8$^+$) αβ T cells (fig. 4.20, 2nd column). The proportion of cells expressing the particular TCRβ chain and the intensity of expression were slightly increased on CD4$^+$ αβ T cells compared with their CD4$^-$ counterparts for all TCRs. Except for EPDV21, each Vβ staining showed a high percentage of αβ T cells expressing the TCRβ chain of the particular transgenic TCR (fig. 4.20, 2nd column, 32.3–41.5 % after subtraction of the proportion of Vβ$^+$ cells of respective mock controls, which was in a range of 0.5% to 5.0%). The comparatively small proportion of anti-Vβ5.2-stained αβ T cells was most likely not due to inefficient retroviral transduction of EPDV21, since more than 30% of CD14$^-$

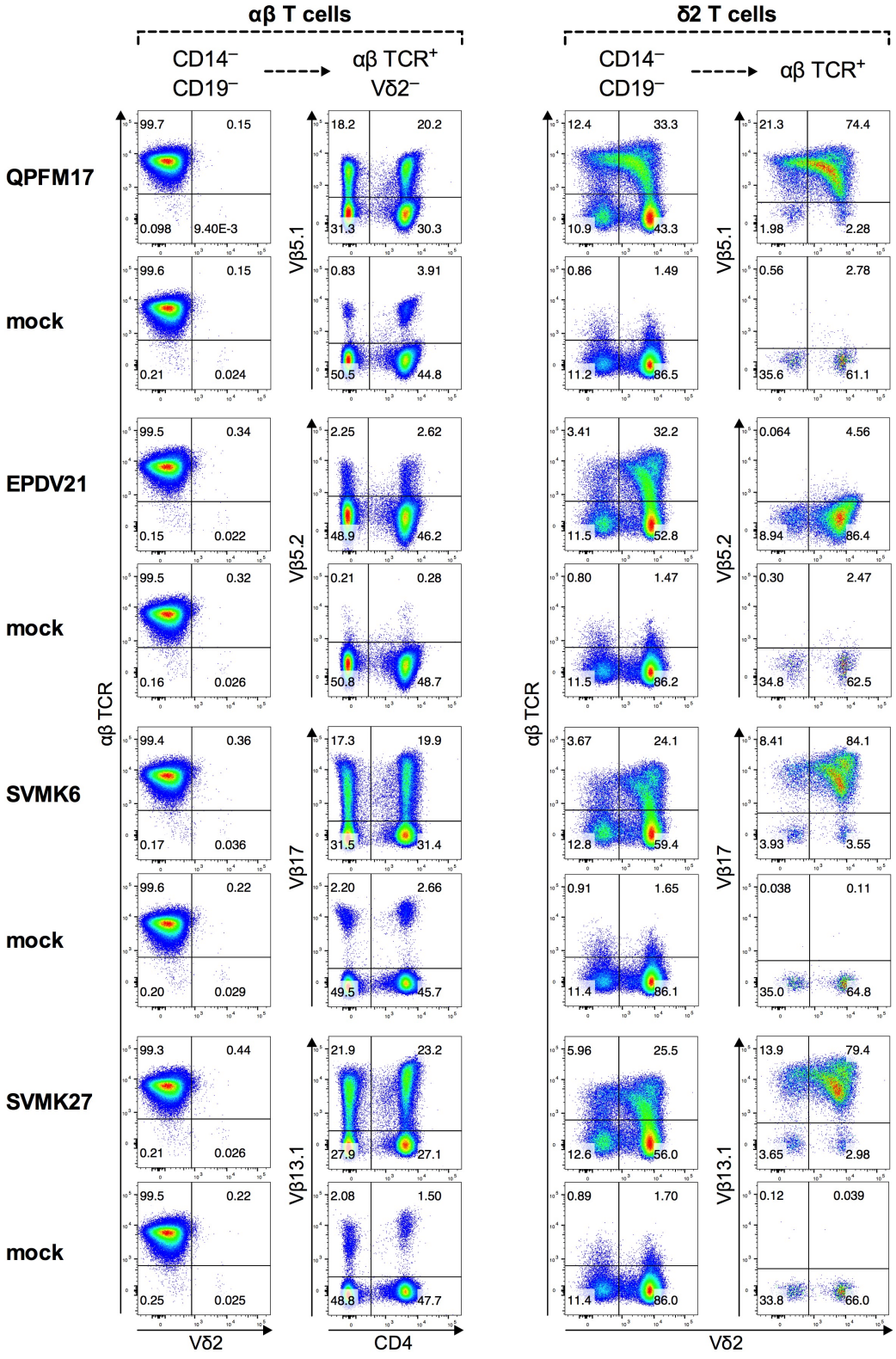


Figure 4.20. Expression of HLA class II-restricted TCRs QPFM17, EPDV21, SVMK6 or SVMK27 by αβ and δ2 T cells. αβ TCR-enriched ("αβ T cells") or αβ TCR-depleted ("δ2 T cells") PBMCs were stimulated with anti-CD3 antibody or PAM, respectively, before transduction. Mock-transduced (using retroviruses devoid of genetic cargo) T cells served as negative control. Six days after transduction, T cell lines were stained with antibodies specific for CD4, CD14, CD19, αβ TCR, Vδ2 TCR chain and various variable domains of TCRβ chains and analysed by flow cytometry. All cells were pre-gated as live, $CD14^-$ and $CD19^-$. Representative pseudocolour dot plots from one (0506) out of two donors (the other being 1613) are shown.

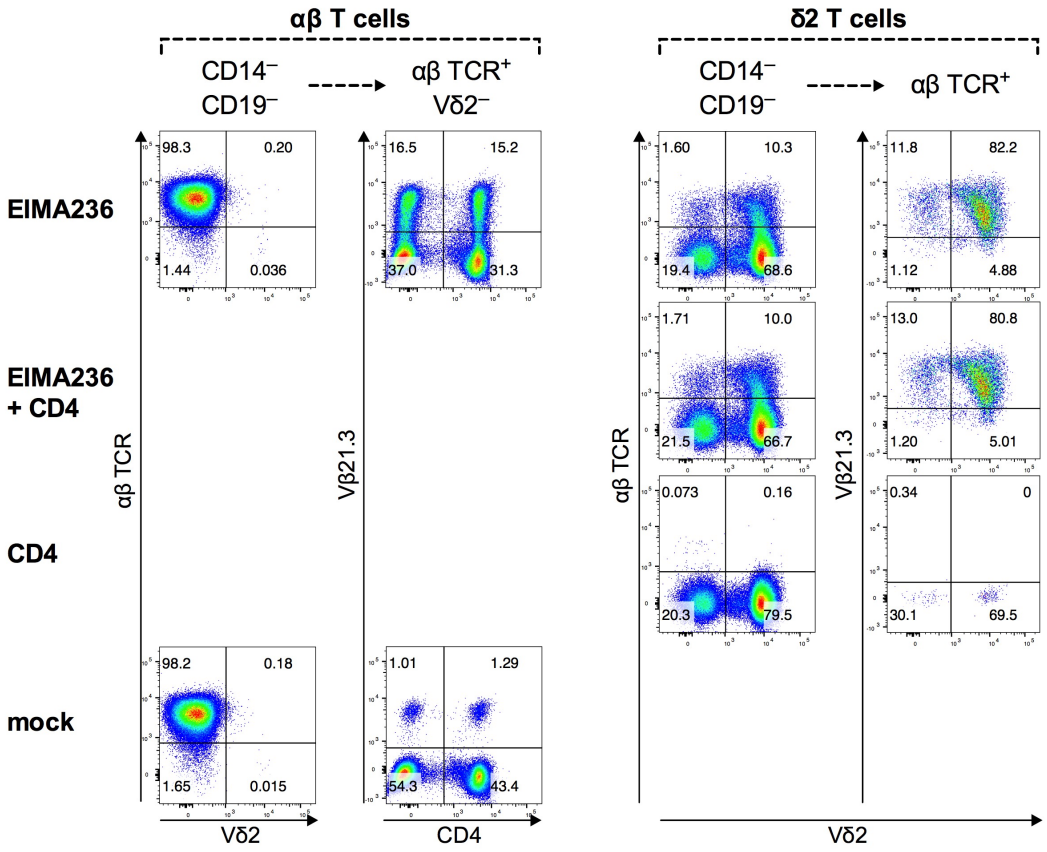


Figure 4.21. Expression of HLA class II-restricted TCR EIMA236 by αβ and δ2 T cells. αβ TCR-enriched ("αβ T cells") or αβ TCR-depleted ("δ2 T cells") PBMCs were stimulated with anti-CD3 antibody or PAM, respectively, before transduction. Mock-transduced (using retroviruses devoid of genetic cargo) or CD4-transduced T cells served as negative control. T cell lines were stained with antibodies specific for CD4, CD14, CD19, αβ TCR, Vδ2 TCR chain and Vβ21.3 TCR variable domain six days after transduction and analysed by flow cytometry. All cells were pre-gated as live, $CD14^-$ and $CD19^-$. Representative pseudocolour dot plots from one (1107) out of two donors (the other being 1013) are shown.

$CD19^-$ cells of a δ2 T cell line transduced with the same retroviral vector stained positive for αβ TCR, while their Vβ5.2 staining intensity was also low, even lower than for TCR-transduced αβ T cells (fig. 4.20, 3^{rd} vs. 4^{th} column). Due to unknown reasons, the staining with anti-Vβ5.2 antibody repeatedly underestimated expression of Vβ5.2 TCR chain by EPDV21-transgenic T cells. Probably, the Vβ5.2-specific antibody competes with the αβ TCR-specific antibody for binding to EPDV21's TCRβ chain due to steric inhibition and tends to lose this competition. Subsequent stainings of EPDV21-transgenic δ2 T cells were performed without Vβ5.2-specific antibody because of the limited quality of staining with this antibody.

Similar to αβ T cells, a large proportion of δ2 T cells expressed the αβ TCRs QPFM17, EPDV21, SVMK6 or SVMK27 several days after retroviral transduction (25.2–43.4 % αβ TCR^+ cells among $CD14^-$ $CD19^-$ cells after subtraction of the proportion of αβ TCR^+ cells of corresponding parallel mock controls, fig. 4.20, 3^{rd} column). More than 90% of αβ TCR^+ T cells stained positive for the respective Vβ, except for EPDV21 (fig. 4.20, 4^{th} column) as discussed. In a small fraction of cells transduced with EPDV21, SVMK6 or SVMK27, αβ TCRs, but not the Vδ2 TCR chain, could

be stained (3.4, 3.7 or 6.0% of CD14$^-$ CD19$^-$ cells, respectively, fig. 4.20, 3rd column). Since the corresponding mock controls had much smaller proportions of such δ2$^-$, αβ$^+$ T cells, it is likely that the majority of these αβ$^+$ T cells is the transduced cultures that expressed the transgenic TCRs. As observed with HLA class I-restricted TCRs before (section 4.3.1), the expression of a transgenic αβ TCR converts a fraction of δ2 T cells into Vδ2$^-$ αβ TCR$^+$ T cells. QPFM17, however, showed at least twice the proportion of Vδ2$^-$ αβ TCR$^+$ T cells (12.4%, fig. 4.20, 3rd column), which indicates that some αβ TCRs, such as QPFM17, more strongly dominate over the endogenous γδ TCR and markedly reduce surface expression of the latter. Strong competition has also been observed with some HLA class I-restricted TCRs, such as CRV2 (section 4.3.1). A similar set of data for QPFM17, EPDV21, SVMK6 and SVMK27 was obtained using transgenic αβ T cell lines from donor 1613 (data not shown). Unfortunately, QPFM/HLA-DRB1*13:02 tetramers had failed to stain the parental T cell clone F46P #17 QPFM even at high concentration of 90 nM, which is why no tetramer stainings were performed using QPFM17-transgenic T cell lines. Similar to MSIY/HLA-DRB1*15:01 monomers, Immunaware had also reported suboptimal folding for QPFM/HLA-DRB1*13:02 monomer in the delivery notes (70%, see section 2.8), which may cause the malfunction of QPFM/HLA-DRB1*13:02 tetramers.
Judging by the data obtained with Vβ-specific antibodies, the expression of transgenic, HLA class II-restricted TCRs did not differ much between αβ and δ2 T cells, but the latter offered a valuable insight into the inherent strength of each individual TCR in competition for surface expression. However, the intensity of the particular Vβ expression by CD4$^+$ αβ T cells appeared to be slightly higher than that of CD4$^-$ αβ T cells and that of δ2 T cells, most of which are CD4$^-$ by default (Groh et al., 1989), for the TCRs QPFM17, SVMK6 and SVMK27 (fig. 4.20).

The TCRs **AGIL19, EFFT22, EFFT50 and RRKM18** were expressed by a large proportion of δ2 T cells from donor 0112 five days after retroviral transduction (40, 32, 27 and 32% αβ TCR$^+$ cells among CD14$^-$ CD19$^-$ cells, respectively, fig. 4.23). In other experiments, a similar proportion of αβ TCR$^+$ δ2 T cells was observed in a second donor (35%, donor 0506), as well as smaller fractions in two other donors (21 and 11% for donors 1013 and 1107, respectively) after transduction with RRKM18-encoding retrovirus. Transfer of EFFT22's TCR genes into δ2 T cells generated a marked proportion of Vδ2$^-$ αβ TCR$^+$ T cells (15.1% of CD14$^-$ CD19$^-$ cells, fig. 4.23). In contrast, AGIL19, EFFT50 and RRKM18 showed fewer Vδ2$^-$ αβ TCR$^+$ T cells (9.1, 5.8 and 6.6%, respectively, fig. 4.23). Thus, EFFT22 may compete exceptionally strongly with the endogenous TCR of δ2 T cells for surface expression, as observed for other TCRs, like QPFM17 and CRV2.
Efficient transduction and surface expression of the TCRs AGIL19, EFFT22 and EFFT50 on δ2 T cells was demonstrated by staining with specific peptide/HLA tetramers (fig. 4.24). RRKM18 could not be stained with tetramers because specific HLA-DRB3*02:02 monomers produced by Immunaware (Copenhagen, Denmark) failed to refold correctly in presence of KRKM peptide (no. 124 of the IE-1 library, tab. 2.11; section 2.8), and were therefore not provided by the manufacturer. Potent suppression of endogenous γδ TCR expression on δ2 T cells that were transduced with EFFT22 TCR was observed for four different donors in two independent experiments (0506, 1107, 1613 & 0112, of which representative data is displayed for 0112 in fig. 4.24). Thus, EFFT22 may be considered a "strong" TCR due to its extraordinary dominant

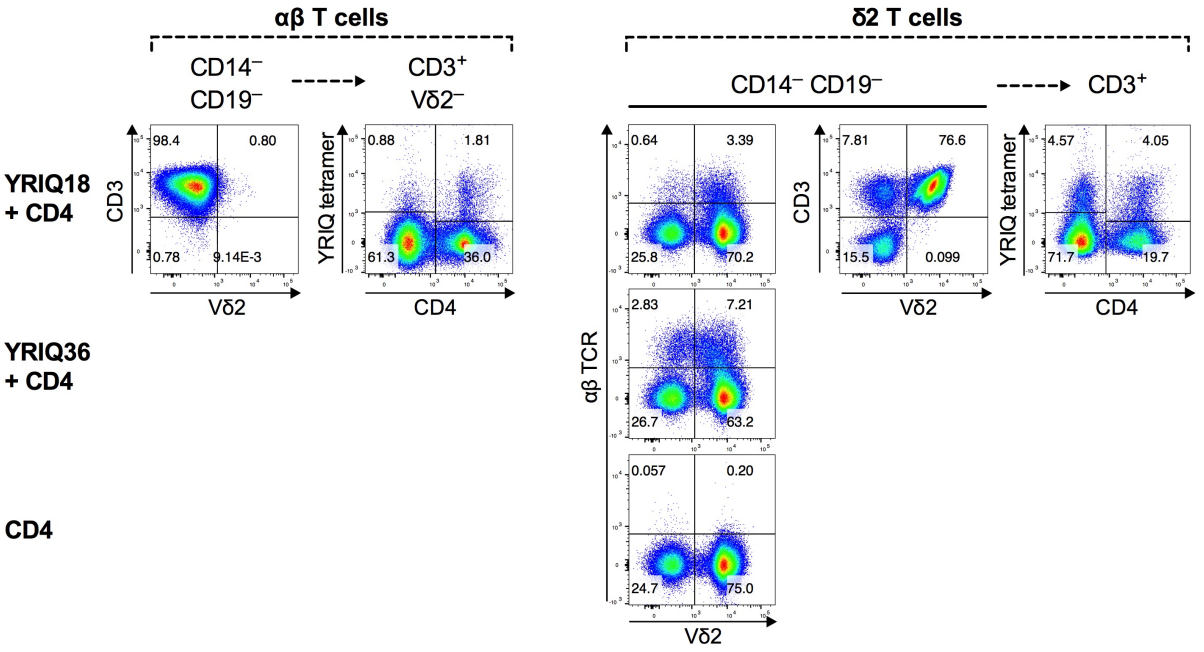


Figure 4.22. Expression of HLA class II-restricted TCRs YRIQ18 or YRIQ36 by αβ and δ2 T cells. αβ TCR-enriched ("αβ T cells") or αβ TCR-depleted ("δ2 T cells") PBMCs from donor 1107 were stimulated with anti-CD3 antibody or PAM, respectively, before co-transduction with TCR and CD4 (ratio 2:1). Antibodies specific for αβ TCR and Vδ2 TCR chain were used to evaluate expression of YRIQ18 or YRIQ36 on δ2 T cell lines six days after transduction by flow cytometry. δ2 T cells transduced with CD4 alone served as negative control. Seven days after transduction, YRIQ/HLA-DRB1*13:02 tetramers were used to assess expression of YRIQ18 by αβ and δ2 T cell lines. No negative control was stained simultaneously. Pseudocolour dot plots of live, $CD14^-$ and $CD19^-$ cells or their descendants are shown as indicated above the graphs.

surface expression in δ2 T cells in comparison to other αβ TCRs, such as AGIL19 or EFFT50. In general, transduction and subsequent expression of EFFT22 in δ2 T cells was very efficient: 29 to 44% of live, $CD14^-$, $CD19^-$ cells of δ2 T cell lines from four donors stained positive for EFFT/HLA-DRB5*01:01 tetramers five or six days after transduction with EFFT22. Similarly, roughly 30% of $CD4^+$ and $CD8^+$ αβ T cells each could be stained with EFFT tetramers six days after transducing αβ T cells from two donors (0506 & 1613) with EFFT22. In contrast to TCR EFFT22, the TCRs AGIL19 and EFFT50 were largely expressed in T cells that maintained their expression of Vδ2 (fig. 4.24). Thus, bi-specific T cells were generated. It remains to be tested whether this dual TCR expression pattern gives rise to T cell reactivity behaviours that may be particularly desirable for immunotherapeutic application.

Overall, AGIL19 was highly expressed by transgenic δ2-expressing T cells: 40, 33, 24 or 19% of live, $CD14^-$, $CD19^-$ cells of δ2 T cell lines from four donors stained positive for AGIL/HLA-DRB3*02:02 tetramers five or six days after transduction with AGIL19-encoding retrovirus (donor 0112, 0506, 1107 or 1613, respectively, of which representative data is displayed for 0112 in fig. 4.24). In contrast, only 8 or 12% of $CD4^+$ αβ T cells transduced with AGIL19 could be stained with AGIL tetramer (donor 0506 or 1613, respectively). $Tetramer^+$ $CD8^+$ αβ T cells could not be evaluated due to fluorochrome interferences.

YRIQ18 and YRIQ36 were successfully expressed, but on a comparatively smaller fraction of δ2 T cells after retroviral co-transduction of CD4 and the respective TCR (4 and 10% αβ TCR^+ cells among $CD14^-$ $CD19^-$ cells, respectively, fig. 4.22, 3rd column). Staining the CD4- and YRIQ18-transgenic δ2 T cell line with YRIQ/HLA-DRB1*13:02 tetramers six days after transduction revealed twice the number of cells that expressed YRIQ18 than staining with αβ TCR-specific antibody had (fig. 4.22, 5th column). In this experiment, an αβ T cell line from the same donor was transduced with CD4- and YRIQ18-encoding retroviruses, too. The proportion of αβ T cells that could be positively stained with YRIQ/HLA-DRB1*13:02 tetramers six days after transduction was similarly low (3%, fig. 4.22, 2nd column). Since the YRIQ tetramer staining was not controlled by staining cells that had been transduced with CD4-encoding retrovirus, but not with the TCR, the results of the YRIQ tetramer staining have to be taken with caution at this point.
Staining δ2 T cells after retroviral co-transduction of CD4 and YRIQ18 or YRIQ36 with αβ TCR-specific antibody showed that the proportion of δ2 T cells that expressed YRIQ36 was more than twice as large as the proportion of δ2 T cells that expressed YRIQ18 (fig. 4.22, 3rd column). Additionally, the αβ TCR-specific antibody stained YRIQ36-transgenic δ2 T cells more intensively than YRIQ18-transgenic δ2 T cells. This may indicate that YRIQ36 is generally better expressed in transgenic T cells than YRIQ18 after retroviral transduction. Unfortunately, expression of YRIQ36 was not assessed by tetramer staining, but might potentially yield better results than YRIQ18 due to the stronger expression of YRIQ36 as evaluated by staining of αβ TCR surface expression.
No Vβ-specific antibodies were available for YRIQ18 and YRIQ36 to directly compare expression of corresponding TCRβ chains on αβ and δ2 T cells with the previously described stainings. But the large proportion of AGIL19- or QPFM17-transgenic T cells showed that susceptibility of the receiving T cells from this particular donor to retroviral transduction was not generally low: When δ2 T cells from the same donor were transduced with AGIL19- or QPFM17-encoding retrovirus, 36 or 32% of $CD14^-$ $CD19^-$ cells (excluding αβ TCR^- $Vδ2^-$ cells) expressed the TCRβ chain of the respective TCR (Vβ2 or Vβ5.1, respectively; data not shown). Similarly, 45 or 44% of αβ T cells expressed the TCRβ chain of TCR AGIL19 or QPFM17 after respective retroviral transduction. Thus, the low proportion of TCR-transgenic T cells from this donor after transduction with YRIQ18- or YRIQ36-encoding retrovirus (fig. 4.22) was likely caused by other factors, such as poor quality of retroviruses or FACS staining.

In order to discuss the expression of the IE-1-specific TCR **EIMA236** on αβ and δ2 T cells, its function has to be addressed prematurely because EIMA236-transgenic T cells later turned out to be unable to recognise HLA-matched, IE-1-expressing mini-LCLs (data not shown). Since this failure in target cell recognition may have been caused by errors contained in the TCR sequences, cDNA of both TCR chains that had been isolated from the parental T cell clone F64M #236 was amplified via PCR a second time. The TCRα sequence was reproduced using the primers Vα3 and 3'T-Cα as before (tab. 2.9) and the sequence did not show any error. In contrast, re-sequencing of TCRβ using the newly designed primer TRBV11-2*01_forward (tab. 2.10) and CP1 (tab. 2.9) clearly showed an `A` at nucleotide position (nt pos.) 164, which was occupied by a `G` in previous sequencing data. This had resulted in a Gln (nt pos. 163-165: `CAG`)-to-Arg (`CGG`)

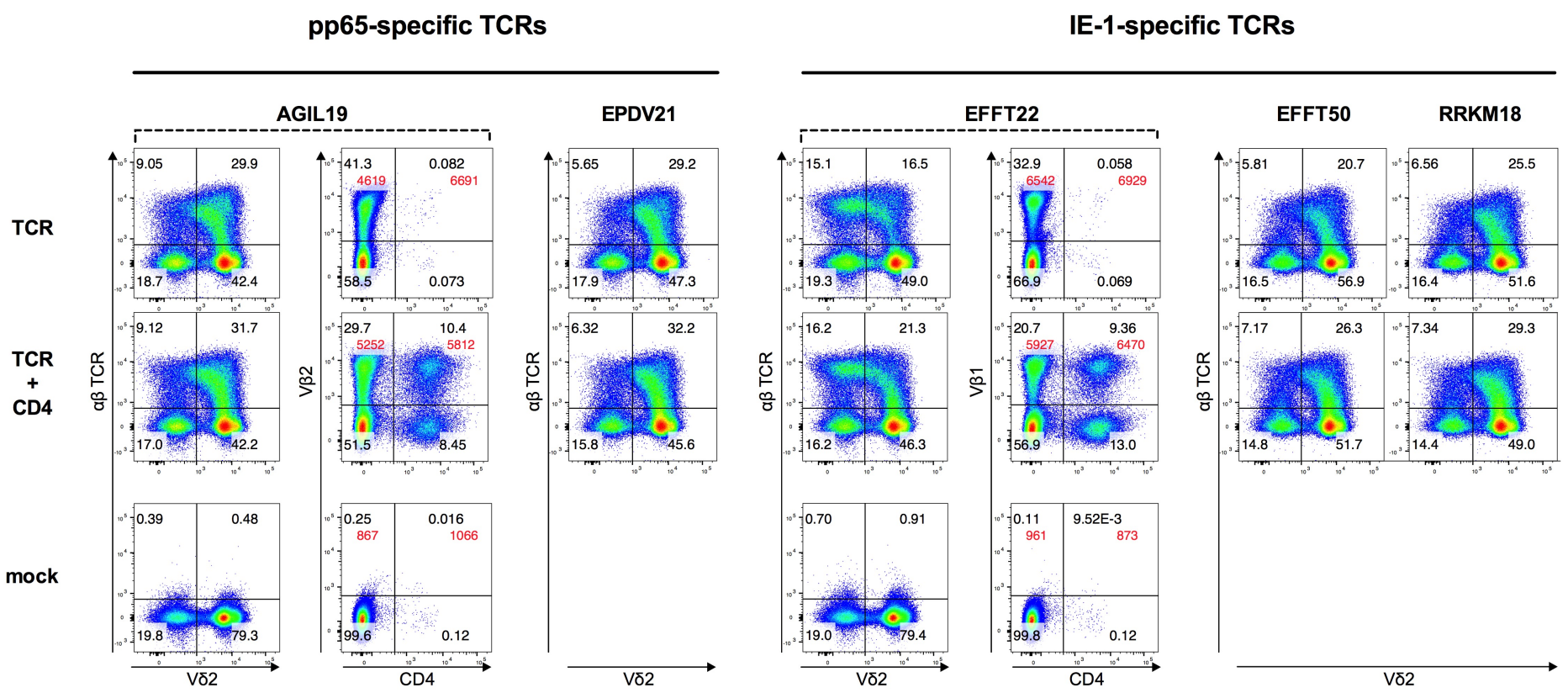


Figure 4.23. Effect of CD4 co-transduction on the expression of HLA class II-restricted TCRs AGIL19, EFFT22, EFFT50, EPDV21 and RRKM18 on δ2 T cells assessed with antibodies specific for αβ TCR, Vβ1 or Vβ2 TCR chain. αβ TCR-depleted PBMCs were stimulated with PAM before retroviral transduction with TCR alone, CD4 alone or both in combination. Mock-transduced (using retroviruses devoid of genetic cargo) T cells served as negative control. The resulting transgenic δ2 T cell lines (the same as in fig. 4.24) were stained with antibodies specific for CD4, CD14, CD19, αβ TCR, Vδ2 TCR chain and Vβ1 or Vβ2 TCR chains five days after transduction and analysed by flow cytometry. Data of all plots displayed was pre-gated on live, $CD14^-$ and $CD19^-$ cells. Pseudocolour dot plots from donor 0112 are shown. Black numbers indicate the percentage of cells in each quadrant, red numbers indicate the mean fluorescence intensity (MFI) of the cells in the quadrant along the y-axis (αβ TCR or Vβ staining).

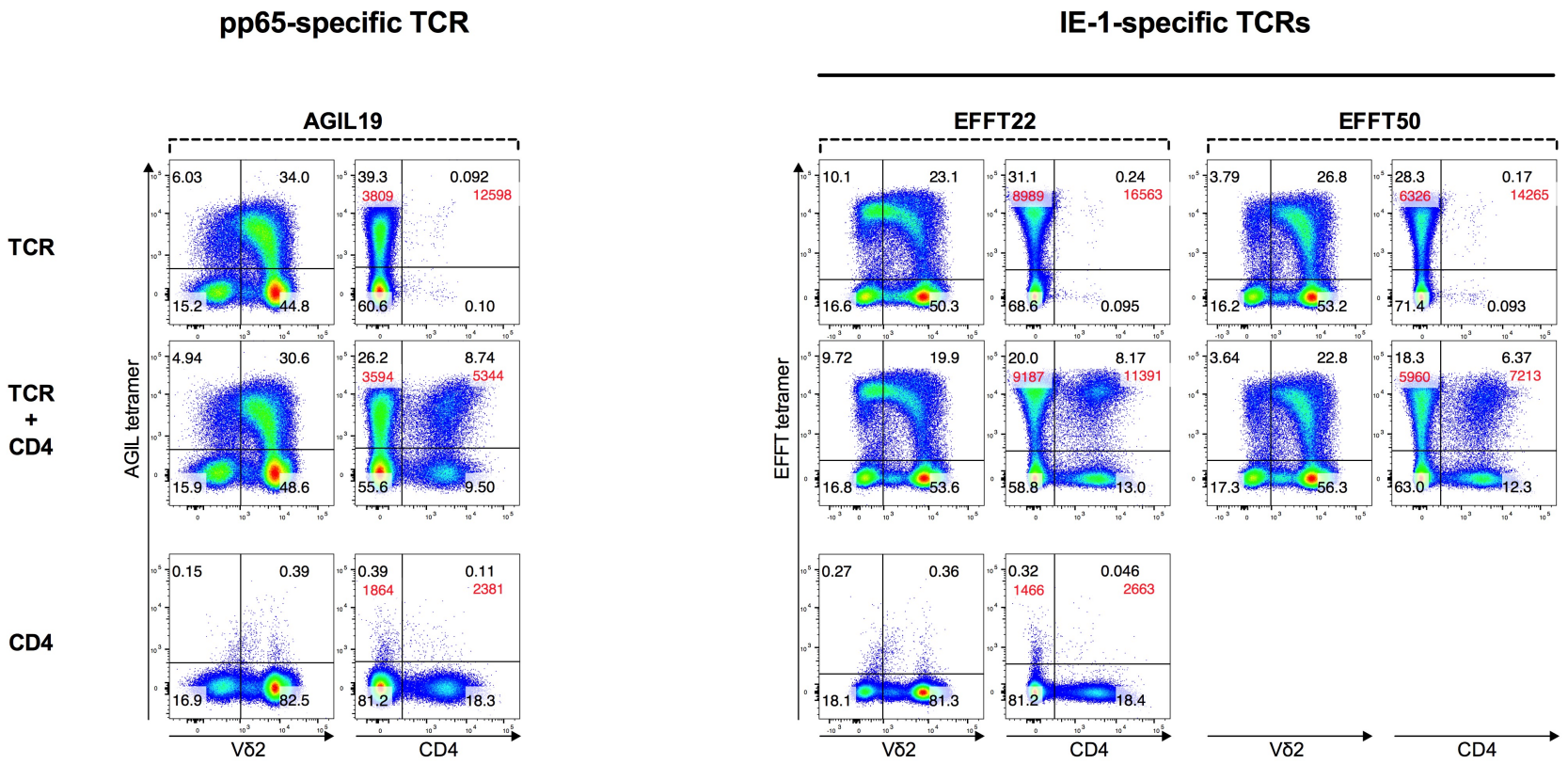


Figure 4.24. Effect of CD4 co-transduction on the expression of HLA class II-restricted TCRs AGIL19, EFFT22 and EFFT50 on δ2 T cells assessed with specific peptide/HLA tetramers. αβ TCR-depleted PBMCs were stimulated with PAM before retroviral transduction with TCR alone, CD4 alone or both in combination. Mock-transduced (using retroviruses devoid of genetic cargo) T cells served as negative control. The resulting transgenic δ2 T cell lines (the same as in fig. 4.23) were stained with antibodies specific for CD4, CD14, CD19 and Vδ2 TCR chain and with AGIL/HLA-DRB3*02:02 or EFFT/HLA-DRB5*01:01 tetramers five days after transduction and analysed by flow cytometry. Data of all plots displayed was pre-gated on live, $CD14^-$ and $CD19^-$ cells. Representative pseudocolour dot plots from one (0112) out of two donors (1107) are shown as pseudocolour dot plots. Black numbers indicate the percentage of cells in each quadrant, red numbers indicate the mean fluorescence intensity (MFI) of the cells in the quadrant along the y-axis (tetramer staining).

Table 4.3. Summary of expression of HCMV-specific, HLA class II-restricted TCRs by αβ and δ2 T cell lines. Surface expression of TCRs on transgenic T cell lines was assessed by staining with Vβ-specific antibodies, except for TCRs YRIQ18, YRIQ36, RRKM18 and EFFT50 because no commercial Vβ6-specific antibody was available. The expression of αβ TCRs could also be monitored on δ2 T cells, but not αβ T cells, by staining with an anti-αβ TCR antibody. Additionally, peptide/HLA tetramers were available for TCRs specific for the epitopes QPFM ($pp65_{261\text{-}275}$), YRIQ ($pp65_{369\text{-}387}$), AGIL ($pp65_{489\text{-}503}$) and EFFT ($IE\text{-}1_{213\text{-}225}$). The number of different experiments during which TCRs were transduced and successfully expressed on αβ and δ2 T cells are listed in the 3rd and 4th column, respectively. The performance of each of these reagents in T cell staining was qualitatively evaluated and categorised as described below. Some tests could not be performed due to reagents that were not available ("n.a.") and some of the conditions were not tested ("n.t.") experimentally.

HCMV antigen	TCR	Times of confirmed expression in T cells		Vβ staining		Tetramer staining	
		αβ	δ2	Vβ	quality	HLA	quality
pp65	QPFM17	3	3	5.1	good	DRB1*13:02	failed
	YRIQ18	n.t.	2	6.4	n.a.	DRB1*13:02	poor
	YRIQ36	n.t.	2	6.4	n.a.	DRB1*13:02	n.t.
	MSIY40	n.t.	1	3	good	DRB1*15:01	failed
	AGIL19	3	5	2	good	DRB3*03:01	good
	EPDV21	2	3	5.2	poor	DQB1*05:01	n.a.
	KYQE16	n.t.	n.t.	20	n.t.	DQB1*03:01	n.a.
IE-1	EIMA236	3	2	21.3	good	DRB1*13:01	n.a.
	RRKM18	n.t.	4	6.1	n.a.	DRB3*02:02	failed
	SVMK6	2	1	17	good	DPB1*03:01	n.a.
	SVMK27	2	1	13.1	good	DPB1*03:01	n.a.
	EFFT22	4	7	1	good	DRB5*01:01	very good
	EFFT50	2	n.t.	6.5	n.a.	DRB5*01:01	very good

exchange during *in silico* TCR construct assembly that was not detected before gene synthesis of TCRs. The DNA base error was likely introduced by the degenerated TCRβ-specific primer VP2 (tab. 2.9): VP2 binds to TCRβ cDNA from nt pos. 149 to 168 (starting to count at the first base of the start codon) and positions an inosine (`I`) opposite of nt pos. 164, which has been shown to potentially generate `A`→`G` transition (Yasui et al., 2008). In order to obtain a flawless TCRβ sequence of EIMA236, the TCRβ chain was corrected and synthesised anew.
The TCRβ chain of corrected EIMA236 TCR could be detected on 15 or 12% of δ2 T cells and 9 or 15% of αβ T cells from donor 1013 or 1107, respectively (data for donor 1107 depicted in fig. 4.21, 2nd and 3rd column). While the corrected EIMA236 TCR conveyed sensitivity for peptide-loaded moDCs to TCR-transgenic αβ T cells, HCMV-infected moDCs and antigen-expressing mini-LCLs were not recognised despite matching HLAs (data not shown). Therefore, EIMA236 transduction was discontinued and other, more immediately promising TCRs were studied in greater detail, instead.

Table 4.3 shows a summary of all data gathered on the expression of HCMV-specific, HLA class II-restricted TCRs in αβ and δ2 T cell lines. Leaving the TCR KYQE16 aside, which was never transferred into any T cell line, 8 out of 12 TCRs could be stained with a Vβ-

specific antibody that confirmed the TCR gene transfer and at least the expression of the respective TCRβ chain by transduced T cells. 3 out of 12 TCRs were stained well either by AGIL/HLA-DRB3*03:01 tetramers or by EFFT/HLA-DRB5*01:01 tetramers thereby confirming that α and β chains of the transferred HCMV-specific TCR had paired, were present on the cell surface and could interact with their cognate antigen (i.e. peptide/HLA complexes). In contrast, KRKM/HLA-DRB3*02:02 tetramers could not be produced successfully, QPFM/HLA-DRB1*13:02 tetramers and MSIY/HLA-DRB1*15:01 tetramers failed to stain specific T cells, and YRIQ/HLA-DRB1*13:02 tetramers provided poor staining quality. Despite the lack of a Vβ-specific antibody and/or a specific peptide/HLA tetramer, the expression of each of the αβ TCRs could be stained with an αβ TCR-specific antibody after the TCR genes had been transferred into δ2 T cells. Through this method or one of the two methods mentioned before, the surface expression of 12 out of 13 HCMV-specific, HLA class II-restricted TCRs could be confirmed. While KYQE16 was never transferred and remains to be evaluated, YRIQ18 and EIMA236 were the only two TCRs that were only expressed by a small proportion of T cells after retroviral transduction. The remaining 10 HCMV-specific, HLA class II-restricted TCRs were typically expressed on 20 to 40% of T cells four to seven days after retroviral transduction.

4.4.2.1 Role of CD4 in transgenic expression of HLA class II-restricted, HCMV-specific TCRs

In order to determine whether expression of HLA class II-restricted TCRs depended on co-expression of CD4, δ2 T cells were transduced with TCR alone, co-receptor alone or both in combination. Retroviral vectors were produced by HEK293T cells that had been transfected with 1.0 µg of TCR plasmid DNA, 0.5 µg of CD4 plasmid DNA or the combination of both, in addition to the necessary gag/pol and env plasmid DNA (1.0 µg each; plasmids are listed and described in tab. 2.7 & 2.8, the general method is described in section 3.5.1). Thus, the genes of TCR and CD4 were likely delivered at an approximate ratio of 2 to 1 into δ2 T cells that were transfected with the combined retroviral vectors. Co-production of retroviruses coding for TCR and CD4 in the same culture may not only generate single retroviral particles, but may result in retroviral aggregates as well. Thus, susceptible T cells may simultaneously receive both kinds of transgenes, TCR and CD4, with a higher likelihood than expected from a random distribution.

Comparing TCR-transgenic αβ T cells from donor 1013 or 1107 after transduction with an EIMA263-encoding retrovirus, $CD4^+$ and $CD4^-$ (i.e. mainly $CD8^+$) αβ T cells expressed similar proportions of Vβ21.3, which is the variable domain of the TCRβ chain of EIMA236 (about 9 or 15% $V\beta21.3^+$ cells among $CD14^-$ $CD19^-$ αβ TCR^+ $V\delta2^-$ T cells, respectively, after subtraction of the proportion of $V\beta21.3^+$ cells of associated mock controls, fig. 4.21, 2nd column). About 12% of δ2 T cells, most of which are $CD4^-$ by default, expressed the αβ TCR EIMA236 irrespective of CD4 co-transduction (fig. 4.21, 3rd column). Thus, EIMA236 did not appear to depend on CD4 for surface expression on $CD4^-$ T cells.

After simultaneously transducing CD4 and TCR chains, the proportions of $CD4^-$ δ2 T cells expressing one of the TCRs AGIL19, EFFT22 or EFFT50 were 32, 25 or 23%, while 48, 39 or 34%

of CD4$^+$ δ2 T cells expressed the respective TCR (as assessed by tetramer staining pre-gating on live CD14$^-$ CD19$^-$ cells, fig. 4.24). Similar results were obtained for AGIL19 and EFFT22 by staining the particular TCRβ chain with a Vβ2- or Vβ1-specific antibody, respectively (fig. 4.23). δ2 T cells that were positively stained by peptide/HLA tetramers or a corresponding Vβ-specific antibody also expressed proportionally more CD4 than δ2 T cells that could not be stained by either of the two reagents (fig. 4.24 & 4.23). Thus, T cells expressing the TCR transgene were overrepresented among T cells expressing the CD4 transgene and vice versa, although this overrepresentation was moderate, and all TCRs could be expressed and stained in CD4$^-$ T cells as well. This overrepresentation may be due to a subset of δ2 T cells that was more susceptible to retroviral transduction than other δ2 T cells and that received both the CD4 gene and the TCR genes. Moreover, aggregates of retroviral vectors, which would deliver both types of transgenes to susceptible δ2 T cells may contribute to this observation, too. Since the efficacy of retroviral transfer of TCR and CD4 genes may not be independent, subset distributions may not be sufficient to show biological cooperation between TCR and CD4 expression. Therefore, the mean fluorescence intensity (MFI) of TCR expression on δ2 T cells either expressing the transgenic TCR alone or in combination with CD4 was compared by tetramer and Vβ stainings. CD4$^+$ δ2 T cells yielded a 1.2- to 1.5-fold increased MFI of AGIL or EFFT tetramer$^+$ cells than CD4$^-$ δ2 T cells (fig. 4.24, centre row). Likewise, the MFIs of Vβ2 or Vβ1 were 1.1-fold increased on CD4$^+$ δ2 T cells transduced with AGIL19 or EFFT22, respectively (fig. 4.23, centre row). The increased tetramer staining intensity of CD4$^+$ δ2 T cells may be due to increased TCR expression owing to the presence of CD4 co-receptor, but may also be caused by enhanced avidity of the tetramer-TCR interaction through presence of CD4 co-receptor. In contrast, the stainings using Vβ2- or Vβ1-specific antibodies are not likely to be affected by co-expression of CD4 and showed only a slightly increased intensity on CD4$^+$ δ2 T cells, which argues against a critical role of CD4 in the expression of TCRs AGIL19, EFFT22 or EFFT50. Importantly, the MFIs of tetramer and Vβ stainings of δ2 T cells transduced with TCR alone did not differ greatly from the MFIs of T cells that were transduced with TCR and CD4 simultaneously (fig. 4.24 and 4.23, top vs. centre row).

Altogether, the TCRs AGIL19, EFFT22 and EFFT50 did not depend on CD4 for functional expression on the cell surface judging from tetramer stainings, although there was some increase in MFI and relative number of positive cells if CD4 was present. Also EDPV21 and RRKM18 were solidly expressed even in the absence of CD4 co-transduction as assessed with a αβ TCR-specific antibody (fig. 4.23). The expression of TCRβ chains of QPFM17, EIMA236, SVMK6 or SVMK27 on TCR-transgenic CD4$^+$ αβ T cells was comparable to CD4$^-$ analogues (assessed by staining with Vβ-specific antibodies, fig. 4.20 & 4.21). As the absence of CD4 did not have a profound impact on expression of nine HLA class II-restricted TCRs, co-transduction of CD4 was not regularly implemented and the expression and function of the remaining HLA class II-restricted TCRs were analysed without CD4 co-transduction. Whether CD4 affects the function of HLA class II-restricted TCRs will be discussed in section 4.4.5.

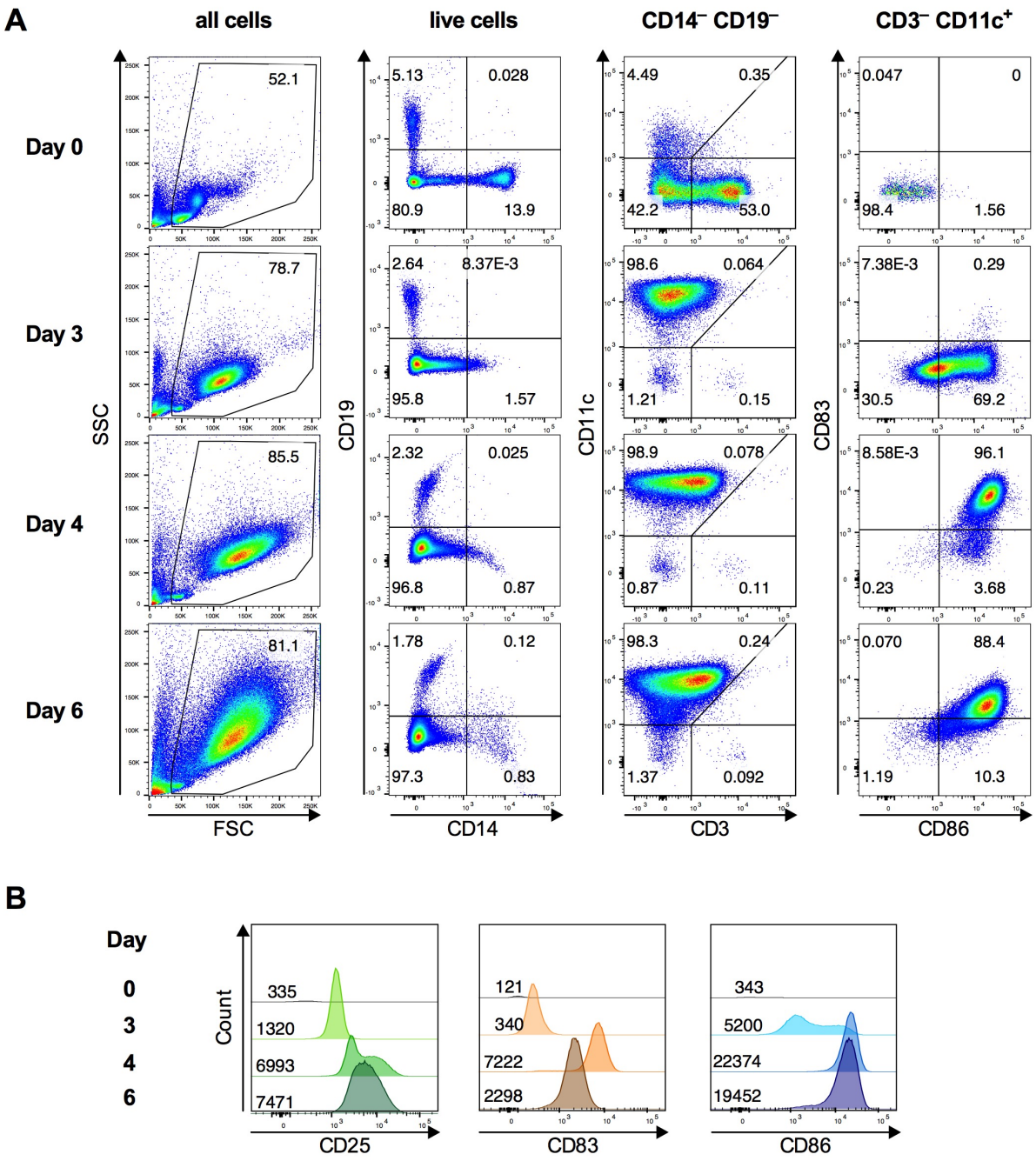


Figure 4.25. FACS analysis of monocyte-derived dendritic cells (moDCs) during their generation and maturation. Bulk PBMCs were incubated at 37 °C for ≥2.5 hours to allow plastic adherence of monocytes (day 0). Non-adherent cells were washed off and remaining cells were stimulated with GM-CSF and IL-4 to generate immature moDCs until day 3. After another 24 hours in the presence of a maturation cocktail containing IL-1β, IL-6, TNF-α and PGE_2, moDCs were matured (day 4). Subsequently, mature moDCs were either infected with HCMV for 48 hours or not infected to serve as control targets. Representative data of non-infected DCs from one donor (1613) out of 7 donors is shown. **A:** Pseudocolour dot plots show the gating and phenotype of analysed DCs (1st column: SSC vs. FSC; 2nd column: CD19 vs. CD14; 3rd column: CD11c vs. CD3; 4th column: CD83 vs. CD86; CD25 staining is not shown, but was always assessed simultaneously). **B:** Overlaid histograms show the staining intensity of CD25 (green), CD83 (orange) or CD86 (blue) expression by $CD14^-$ $CD19^-$ $CD3^-$ $CD11c^+$ DCs at the indicated time points of analysis. MFI of the same population and time point are indicated for the respective marker within each graph. On day 0, DC numbers and MFIs of the selected markers were low causing small curves that are barely visible in the graphs.

4.4.3 Monocyte-derived dendritic cells as target cells for HLA class II-restricted T cells

In order to evaluate the recognition of HCMV infection by HLA class II-restricted T cells, target cells were required that expressed sufficient levels of HLA class II molecules and that could be infected with fibroblast-adapted strains of HCMV. While fibroblasts can be easily infected, they generally do not express HLA class II molecules (Pober et al., 1983; Stevanovic et al., 2013). In contrast, mature monocyte-derived dendritic cells (moDCs) fulfil both requirements (Sénéchal et al., 2004) and were thus selected as target cells. moDCs were always freshly prepared using PBMCs from independent healthy adults (tab. 2.2), which were HLA-matched with the HLA restriction of the T cell clone or TCR-transgenic T cell lines to be tested. Isolated PBMCs were rested for at least 2.5 hours to let the monocytes adhere to the plastic surface. After washing off all non-adherent cells, medium containing GM-CSF and IL-4 was added for three days to drive differentiation of monocytes into immature moDCs. Mature moDCs were generated by adding a maturation cocktail (IL-1β, IL-6, TNF-α, PGE_2) for 24 hours, which corresponds to the classical maturation cocktail published by Jonuleit et al. (2000). Next, moDCs were either infected with HCMV for 48 hours or mock-treated to serve as controls. The composition of cultured cells and the activation status of moDCs were monitored throughout the procedure by staining with antibodies (tab. 2.14) prior to flow cytometric analysis.
DCs isolated from peripheral blood on day 0 had a low expression (measured as MFI) of the activation markers CD25 and CD83 or the co-stimulatory molecule CD86 (fig. 4.25). Immature moDCs (day 3 of the protocol) showed no or dim expression of CD25 and CD83, but showed intermediate levels of CD86 expression. After 24 hours of maturation, all three markers were expressed at high levels, which decreased following subsequent infection with HCMV or control incubation in case of CD83, but CD25 and CD86 remained approximately constant. Taken together, moDCs could be generated at a satisfactory purity (>90% of live cells) with a mature phenotype that was mostly retained even after 48 hours of cultivation in the absence of cytokine supplementation. This phenotype was also conserved when moDCs were infected with HCMV (data not shown).

4.4.4 Recognition of HCMV-infected moDCs by $CD4^+$ αβ T cell clones

Prior to testing TCR-transgenic T cell lines, the recognition of HCMV-infected moDCs by $CD4^+$ T cells was evaluated in order to verify suitability of moDCs as target cells. These tests were performed with $CD4^+$ T cell clones from which the TCRs shown in table 4.2 had been isolated. Frozen aliquots were available for T cell clones F46P #17 QPFM, F46P #45 AGIL, 1220M #22 EFFT, F65M #18 RRKM and 1021M #6 SVMK, which were thawed and immediately co-incubated overnight with moDCs that had been infected with different HCMV strains. moDCs were infected with an MOI of 5, in accordance with previous work by colleagues Ameres et al. (2013). moDCs that had not been infected served as negative controls (mock-treated) or positive controls (peptide loaded). Since DCs are professional APCs, they do not only present endogenous peptides on HLA class I molecules, but also take up and process extracellular material, such as cellular debris or protein aggregates, and present the resulting peptide fragments on HLA class II molecules. In these experiments, it was important to distinguish antigen presented after *bona fide* infection of DCs from antigen presented after uptake of defective viruses or other inactive

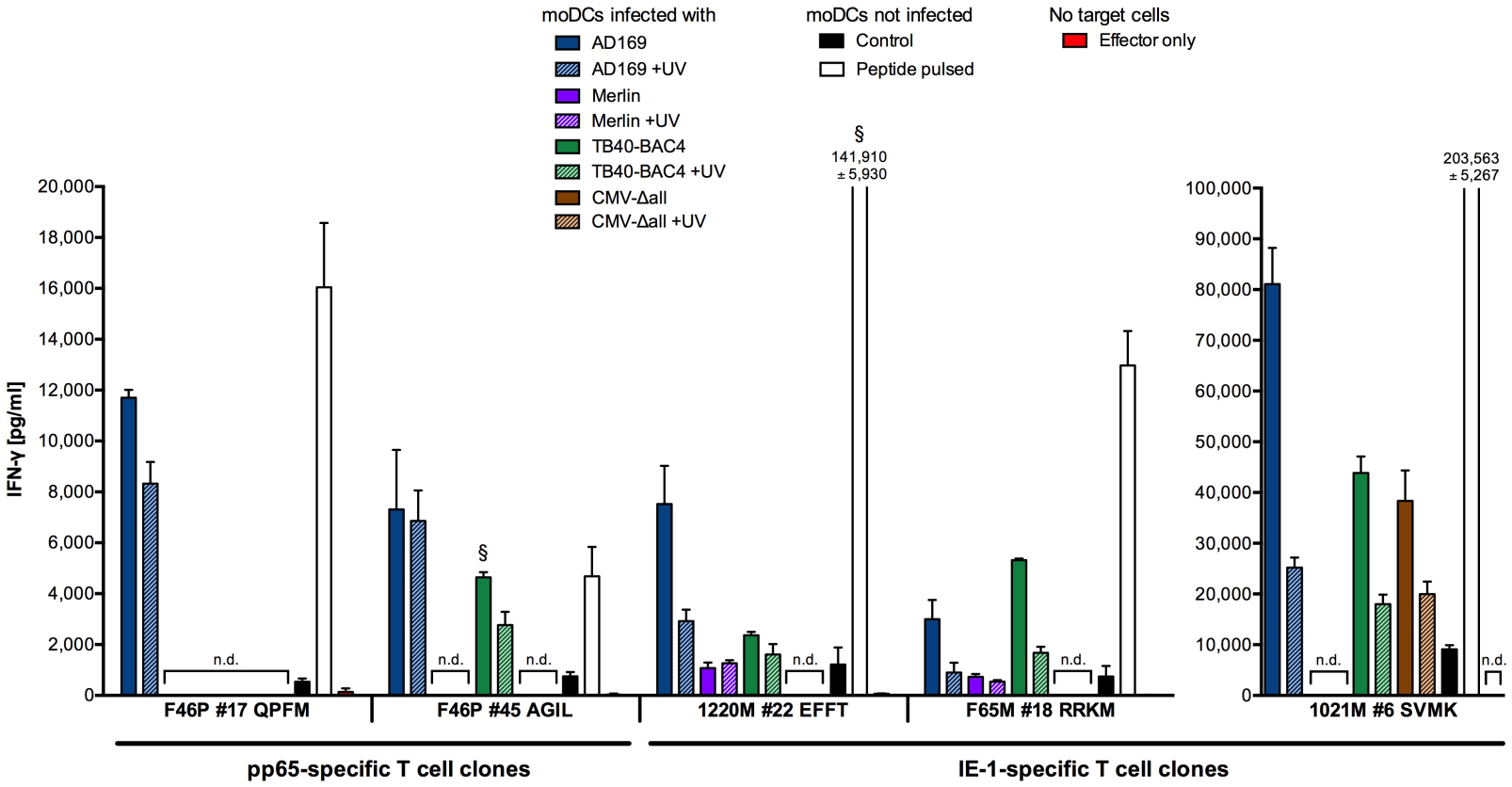


Figure 4.26. Recognition of HCMV-infected monocyte-derived dendritic cells (moDCs) by HLA class II-restricted T cell clones. The $CD4^+$ αβ T cell clones F46P #17 QPFM, F46P #45 AGIL, 1220M #22 EFFT, F65M #18 RRKM and 1021M #6 SVMK were co-incubated overnight with target cells at an e:t ratio of 20,000:10,000 (#22 & #18) or 10,000:10,000 (#17, #45 & #6) cells per well. Mature moDCs from donors 1022 (for F46P #17) or 1613 (for all other T cell clones) had been infected with HCMV strain AD169 (solid blue bars), Merlin (solid purple bars), TB40-BAC4 (solid green bars), CMV-Δall (solid brown bars) at an MOI of 5 for 48 hours in the absence of cytokine supplementation or had been treated correspondingly with UV-inactivated HCMV inoculum (hatched bars in corresponding colours). Non-infected target cells were either loaded with corresponding antigenic peptides (white bars) or left untreated (black bars) to serve as positive or negative controls, respectively. The graphs show concentration of secreted IFN-γ in the supernatant as evaluated by ELISA. The mean and standard deviation of triplicate samples are shown, except for bars marked with "§", for which duplicates were analysed. Due to scarcity of effector or target cells some combinations could not be tested ("n.d." = not determined). Representative data is shown for F46P #45 AGIL (results for 1 out of 2 moDC donors), 1220M #22 EFFT, and F65M #18 RRKM (results for 1 out of 3 moDC donors, each). HLA restrictions of the T cell clones are listed in table 4.2.

material in the virus preparation. HCMV particles contain various proteins, but in different quantities: While pp65 (UL83) is copiously produced during the HCMV lytic cycle and is one of the most abundant proteins in HCMV particles, IE-1 (UL123) protein has not been identified as a component of virions (Büscher et al., 2015; Varnum et al., 2004) or at very low levels (Reyda et al., 2014). Therefore, the hypothesis to be tested was that pp65 would be taken up and presented to $CD4^+$ T cells from HCMV preparations (inactive viruses, virus-like particles, debris), whereas IE-1 presentation would require *bona fide* infection of moDCs. In order to distinguish these scenarios, moDCs were either treated with infectious (i.e. non-irradiated) or UV-inactivated HCMV inoculum. UV irradiation damages viral DNA and renders it defective for transcription thereby preventing *de novo* synthesis of viral proteins in cells that have taken up virions (Borysiewicz et al., 1988). This will allow to distinguish the effects of infection from those of mere uptake of antigenic material.

In accordance with this hypothesis, the pp65-specific T cell clones F46P # 17 QPFM and F46P #45 AGIL secreted a similar amount of IFN-γ in response to moDCs that were treated with infectious AD169 virus than to moDCs that were treated with UV-inactivated virus (fig. 4.26). In contrast, the IE-1-specific T cell clones 1220M #22 EFFT, F65M #18 RRKM and 1021M #6 SVMK produced at least 2.5-fold more IFN-γ after co-incubation with AD169-infected moDCs than with moDCs that had been treated with UV-inactivated AD169 virus. Moreover, F65M #18 RRKM and 1021M #6 SVMK produced at least 2.4-fold more IFN-γ in response to moDCs infected with HCMV strain TB40-BAC4 than in response to moDCs treated with UV-irradiated TB40-BAC4. 1220M #22 EFFT did not react accordingly, but secreted low IFN-γ levels indicating no or weak recognition of TB40-BAC4 infection. F46P #45 AGIL showed better recognition of moDCs infected with TB40-BAC4 in comparison to UV-irradiated TB40-BAC4 (factor 1.7), but could not match the selective recognition of infectious virus by IE-1-specific T cell clones.
Infection with HCMV strain Merlin failed to elicit any IFN-γ secretion above background levels by the two IE-1-specific T cell clones 1220M #22 EFFT and F65M #18 RRKM (fig. 4.26). Recognition of moDCs infected with the genetically modified virus strain CMV-Δall, which is derived from AD169 but lacks the four immunoevasins US2, US3, US6 and US11 (Besold et al., 2009), was not stronger than that of AD169 for IE-1-specific 1021M #6 SVMK (fig. 4.26) or F65M #18 RRKM (data not shown), but weaker or comparable, respectively. This indicated that these four immunoevasins did not negatively affect presentation of viral peptides on HLA class II molecules of DCs in contrast to their strong impact on peptide presentation on HLA class I molecules. Infection with CMV-Δall and corresponding reactions of TCR-transgenic T cell lines will be addressed in the following paragraphs.
This experiment provided evidence that newly synthesised IE-1 was presented to $CD4^+$ T cells after *bona fide* infection of moDCs, whereas presentation of pp65 was predominantly due to uptake of non-infectious HCMV material.

4.4.5 Recognition of HCMV-infected moDCs by T cell lines that express an HCMV-specific, HLA class II-restricted TCR

4.4.5.1 Recognition by pp65-specific TCR-transgenic T cells

In the next step, the reactivity of TCR-transgenic, HLA class II-restricted T cells against HCMV-infected moDCs was tested. Similar to their parental T cell clones (fig. 4.26), the pp65-specific, HLA-DR-restricted TCRs AGIL19, QPFM17, YRIQ18, and YRIQ36 mediated distinct and comparable IFN-γ secretion by transgenic T cell lines in response to moDCs treated with infectious HCMV or UV-inactivated virus (fig. 4.27). Each TCR conveyed comparable recognition of

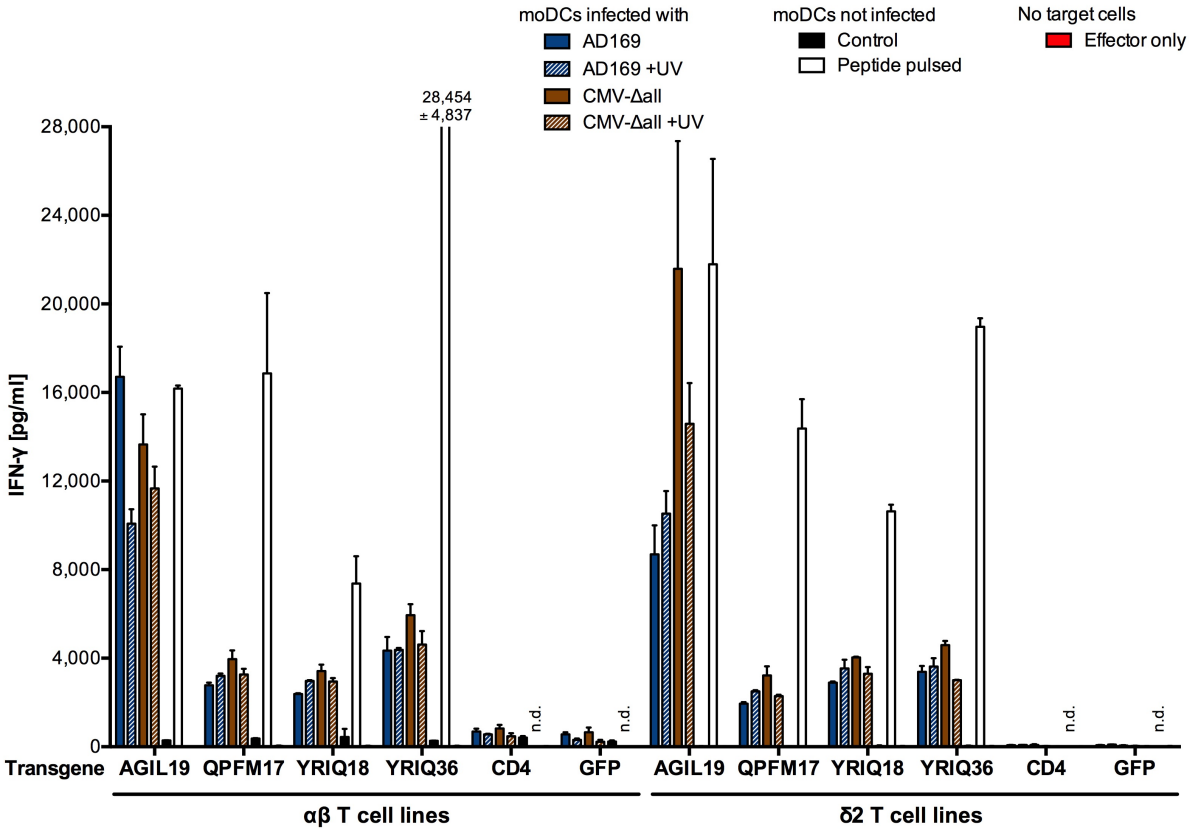


Figure 4.27. Recognition of HCMV-infected moDCs by αβ and δ2 T cell lines that express pp65-specific, HLA class II-restricted TCRs. After immunomagnetic separation, the αβ TCR^+ and the αβ TCR^- fraction from donor 1107 had been stimulated with anti-CD3 antibody or PAM, respectively, before they were transduced with AGIL19, QPFM17, YRIQ18 or YRIQ36 and CD4 co-receptor at a ratio of 2 to 1. CD4 or GFP alone were used as transgene controls and transduced likewise. 7 days after transduction, effector cells were co-incubated overnight with differently treated moDCs at an e:t ratio of 20,000:20,000 cells per well. Mature moDCs from donor 1022 had previously been infected with HCMV strain AD169 (solid blue bars) or CMV-Δall (solid brown bars) at an MOI of 5 for 48 hours in the absence of cytokine supplementation or had been treated correspondingly with UV-inactivated HCMV inoculum (hatched bars in corresponding colours). Non-infected target cells were either loaded with corresponding antigenic peptides (white bars) or left untreated (black bars) to serve as positive or negative controls, respectively. The graph shows the concentration of IFN-γ in the supernatant as evaluated by ELISA. The mean and standard deviation of duplicate samples are shown. Some conditions were not tested ("n.d." = not determined). See table 4.2 for HLA restrictions of the TCRs.

moDCs to αβ T cells and to δ2 T cells, regardless of the HCMV virus strain used for infection of moDCs (AD169 vs. CMV-Δall). Remarkably, AGIL19 caused roughly 2- to 3-fold higher IFN-γ levels than the other three pp65-specific TCRs QPFM17, YRIQ18 and YRIQ36 in response to moDC infection with AD169 and CMV-Δall, irrespective of TCR-transgenic T cell subpopulation (αβ vs. δ2). Since the two TCRs AGIL19 and QPFM17 were very similarly expressed (45% or 44% Vβ2$^+$ among CD14$^-$ CD19$^-$ αβ TCR$^+$ Vδ2$^-$ T cells and 36% or 32% Vβ2$^+$ among CD14$^-$ CD19$^-$ δ2 T cells after exclusion of αβ TCR$^-$ Vδ2$^-$ cells, respectively), other properties must be responsible for the higher activity of TCR AGIL19. The HLA type of donor 1022, whose PBMCs were used for the generation of moDCs for this experiment (fig. 4.27) may provide an explanation: this donor carried two HLA alleles (HLA-DRB3*02:02 and 3*03:01) both of which probably presented the AGIL peptide to TCR AGIL19, but only one allele (HLA-DRB1*13:02) able to present YRIQ or QPFM peptides to their respective TCRs. Several other factors, such as differences in inherent strength of each TCR, as well as differences in peptide processing and peptide/HLA stability, may also come into play, but were too difficult to measure at this point. The two pp65-specific TCRs YRIQ18 and YRIQ36 conveyed IFN-γ secretion patterns to TCR-transgenic T cells that were very similar to QPFM17 (fig. 4.27), all of which are restricted by HLA-DRB1*13:01 and 1*13:02 (tab. 4.2). This is surprising because the detectable proportion of αβ and δ2 T cells that expressed YRIQ18 or YRIQ36 was very low (fig. 4.22) and markedly smaller than the proportion of T cells expressing other TCRs, like QPFM17 (see section 4.4.2), as evaluated by staining with αβ TCR-specific antibody and YRIQ tetramers just one day before the assay shown in figure 4.27. Possibly, the proportion of T cells expressing YRIQ18 or YRIQ36 was higher than both staining methods indicated.

In the experiment depicted in figure 4.27, δ2 T cells were of similar efficacy as their αβ T cell counterparts in recognising HCMV-infected moDCs through one of the four tested pp65-specific TCRs and in secreting robust levels of IFN-γ as a result of the recognition. TCR-transgenic δ2 T cell lines secreted between 0.5-fold and 1.6-fold (median, 0.8-fold) as much IFN-γ as αβ T cell lines confronted with the same targets. However, δ2 T cells displayed reduced non-HCMV-specific background reactivity to the HLA-matched moDCs, which was visible in two different ways. First, if transduced with HCMV-specific TCRs, δ2 T cell reactivity against moDCs that were not treated with HCMV was lower than in the equivalent condition with αβ T cells. Second, in the control condition, where δ2 T cells were not transduced with a TCR but with CD4 or GFP alone, their reactivity against moDCs was lower than the reactivity of αβ T cells in the corresponding condition – independent of the pre-treatment of the moDCs. Thus, this experiment suggests that αβ and δ2 T cells are similarly capable of mediating transgenic TCR-mediated reactivity to HCMV-infected cells, but δ2 T cells have lower background reactivity. This may be explained by the fact that T cells and moDCs were HLA-matched, but not autologous, and there may have been allo-HLA-specific reactivity by αβ T cells. Such reactivity is not expected for δ2 T cells due to their conserved, donor-independent native specificity.

4.4.5.2 Recognition by IE-1-specific TCR-transgenic T cells

Next, T cells of the two different types were transduced with IE-1-specific, HLA class II-restricted TCRs, and reactivity against HCMV-treated moDCs was studied. moDCs were treated with viruses AD169, TB40-BAC4, or CMV-Δall, each with or without UV inactivation. αβ T cells and δ2 T cells were transduced with three IE-1-specific TCRs: RRKM18, SVMK6, and SVMK27. For each TCR, there was clear evidence for HCMV-specific recognition of infected moDCs (fig. 4.28). Total reactivity against infected moDCs was generally higher for αβ than for δ2 T cells. However, αβ T cells showed a clearly higher background reactivity against uninfected moDCs, whereas such background was completely absent in δ2 T cells. For each of the three HCMV variant viruses, recognition was lower when the virus had been inactivated by UV irradiation, in

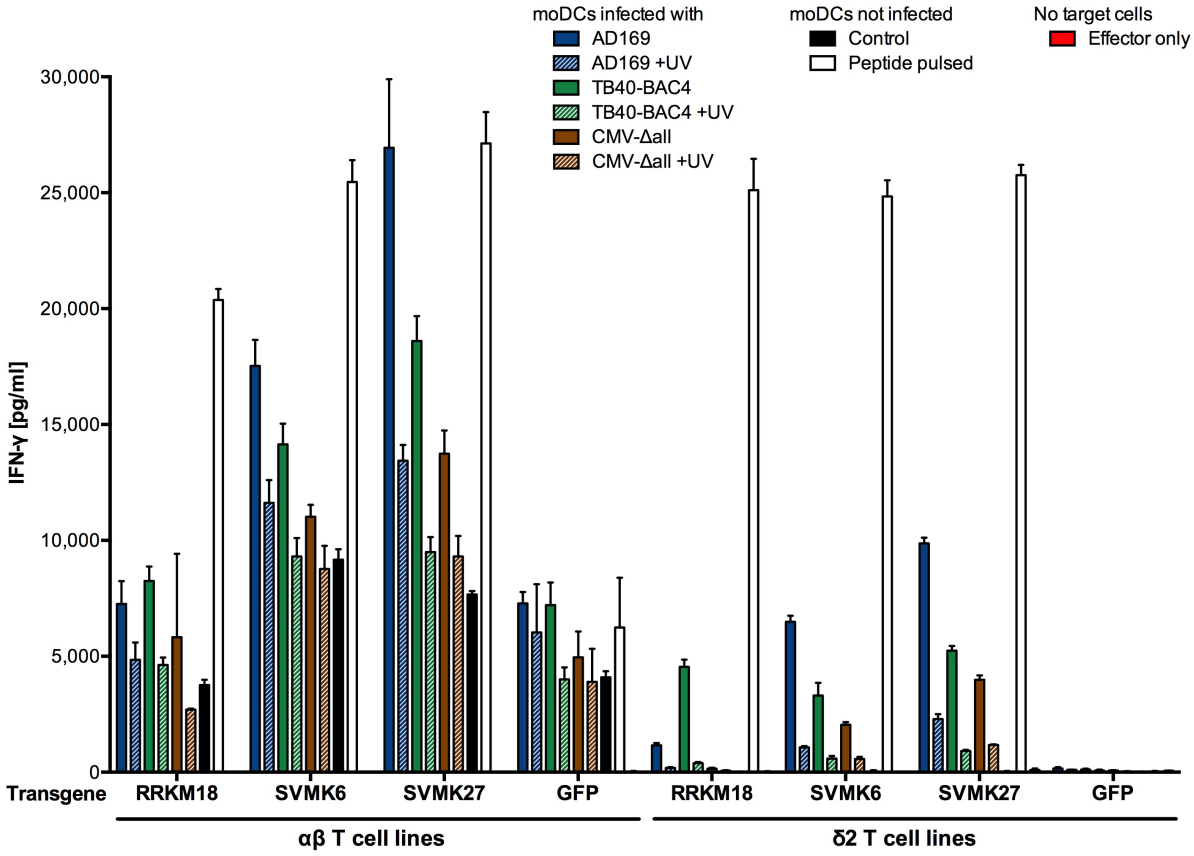


Figure 4.28. Recognition of HCMV-infected moDCs by αβ and δ2 T cell lines that express IE-1-specific, HLA class II-restricted TCRs. After immunomagnetic separation, the αβ TCR^+ and the αβ TCR^- fraction from donor 0506 had been stimulated with anti-CD3 antibody or PAM, respectively, before they were transduced with RRKM18, SVMK6 or SVMK27. GFP alone was used as transgene control and transduced likewise. See figure 4.20 for FACS analysis of SVMK6- and SVMK27-transgenic T cells. 7 days after transduction, effector cells were co-incubated overnight with differently treated moDCs at an e:t ratio of 20,000:10,000. Mature moDCs from donor 1613 had previously been infected with HCMV strain AD169 (solid blue bars), TB40-BAC4 (solid green bars) or CMV-Δall (solid brown bars) at an MOI of 5 for 48 hours in the absence of cytokine supplementation or had been treated correspondingly with UV-inactivated HCMV inoculum (hatched bars in corresponding colours). Non-infected target cells were either loaded with corresponding antigenic peptides (white bars) or left untreated (black bars) to serve as positive or negative controls, respectively. The graph shows concentration of secreted IFN-γ in the supernatant as evaluated by ELISA. The mean and standard deviation of triplicate samples are shown.

accordance with the hypothesis that endogenously expressed IE-1 after *bona fide* HCMV infection of moDCs was an important source of HLA class II-presented antigen. Consequently, a direct comparison of the recognition of HCMV-infected target cells between TCR-transgenic δ2 and αβ T cell populations in this particular case was hampered by the relatively large background reactivity of αβ T cells. In a second experiment (not shown here), similar unspecific activation of TCR-transgenic αβ T cells was observed: αβ T cells from donor 1013 that had been transduced with EFFT22- or RRKM18-encoding retrovirus or "empty" (mock) retrovirus secreted large amounts of IFN-γ in response to control moDCs from donors 0308 and 1613 (6,400–14,000 pg/ml and 2,100–3,900 pg/ml, respectively). The issue of unspecific reactivity of TCR-transgenic αβ T cells is further discussed in the paragraphs below taking all available results into account.

4.4.5.3 Overview of experiments with IE-1-specific and pp65-specific TCRs

The data shown in figures 4.27 and 4.28 depict results from two out of six independent experiments that were performed evaluating the recognition of HCMV-infected moDCs by T cells that had been transduced with retroviruses encoding HCMV-specific, HLA class II-restricted TCRs. Figures 4.29 and 4.30 summarise all six experiments in the form of secreted IFN-γ levels. Figure 4.29 shows experiments with αβ and δ2 T cells that expressed one of the pp65-specific TCRs AGIL19, QPFM17, YRIQ18, YRIQ36 and EPDV21 (see tab. 4.2 for details on the TCRs). In most of these experiments, the same TCR-transgenic T cells were tested on moDCs from two different HLA-matched donors; in the remainder of the experiments, only one HLA-matched moDC donor was used. Each data set depicted in figures 4.29 and 4.30 was obtained with a different combination of TCR-transgenic T cells and moDCs. Some of the TCR-transgenic T cell lines that were used in these experiments had also been transduced with CD4 and some of the transgenic T cell lines had been restimulated by HLA-matched, antigen-expressing mini-LCLs (see section 3.5.1) prior to co-cultivation with HCMV-infected moDCs. Data sets were excluded if the amount of secreted IFN-γ in response to control moDCs exceeded the amount of IFN-γ secreted in response to any kind of moDCs that were infected with HCMV (occurred once using δ2 T cells and four times using αβ T cells transduced with either RRKM18 or EFFT22).

AGIL19 differed from other pp65-specific, HLA class II-restricted TCRs in the extraordinary high levels of IFN-γ secretion by TCR-transgenic αβ and δ2 T cell lines after encountering AD169-infected or CMV-Δall-infected target cells (fig. 4.29). In δ2 T cells, TCR AGIL19 was also tested against HCMV strain TB40-BAC4. In this situation, there was clear virus-specific reactivity, but amounts of cytokine were clearly lower than against AD169 or its derivative CMV-Δall (fig. 4.29). The amino acid sequences of the AGIL epitope ($pp65_{489\text{-}503}$) and of 40 amino acids up- or downstream do not differ between AD169 (Bradley et al., 2009, GenBank accession number FJ527563) and TB40-BAC4 (Sinzger et al., 2008b, GenBank accession number EF999921). Thus, other reasons are likely responsible for AGIL19's differential sensitivity to HCMV virus strains AD169 and TB40-BAC4.

TCR **EPDV21** was clearly HCMV-specific in δ2 T cells, but amounts of IFN-γ were lower than for the other pp65-specific TCRs. EPDV21 was the only HLA-DQ-restricted TCR, whereas the

others were HLA-DR-restricted. The TCR donor and the two moDC donors were all matched for HLA-DQB1*03:01, but were not precisely matched for any HLA-DQA1 allele. This may explain the observed relatively low reactivity. While the TCR donor (1304) was homozygous for DQA1*05:01, the moDC donors (1613 and 0308) had other alleles of DQA1*05, and both additionally had DQA1*01:02. The situation is relatively complex, since mini-LCL experiments had previously shown that DQB-matched, DQA-mismatched, pp65-expressing mini-LCLs were sometimes even more strongly recognised by this TCR than the autologous, pp65-expressing mini-LCL (fig. 4.4 B). There was only one experiment with this TCR transduced into αβ T cells (fig. 4.29), but its significance was limited by the high background reactivity to uninfected moDCs. Cumulatively, the data suggests that TCR EPDV21 is functional with HLA-DQ heterodimers that contain DQB1*03:01 and one of a range of different DQA chains, but more precise analyses will be needed to establish its exact pattern of HLA restriction.
The pp65-specific TCRs **YRIQ18, YRIQ36, and QPFM17** showed consistent specific recognition of all three virus strains in αβ and in δ2 T cells, and this recognition was largely independent of UV irradiation, consistent with the aforementioned hypothesis on pp65 presentation. The only exception was one experiment with QPFM17 in αβ T cells that had a high background to uninfected moDCs, probably due to alloreactivity of donor T cells; this experiment could not be evaluated.

An analogous overview of experiments with IE-1-specific, HLA class II-restricted TCRs is shown in figure 4.30. These TCRs were EFFT22, EFFT50, RRKM18, SVMK6 and SVMK27 (see tab. 4.2 for details). There was clear evidence that all five TCRs mediated recognition of HCMV-infected cells by both types of TCR-transduced T cell lines, αβ and δ2. All TCRs recognised moDCs infected with "live" virus better than UV-inactivated virus, suggesting that IE-1 epitopes are at least partially derived from newly transcribed IE-1 in the viral replication cycle taking place in infected DCs.
There were certain differences between the three epitope specificities regarding their recognition of different HCMV strains. These differences seemed most pronounced for the TCRs targeting the epitope **EFFT**: such TCRs recognised AD169-infected targets much more strongly than TB40-BAC4-infected moDCs (fig. 4.30). However, the amino acid sequence of the EFFT epitope (IE-$1_{213\text{-}225}$) does not differ between AD169 and TB40/E (fig. 4.31 B), and the nearest difference in the surroundings of the epitope is a Met-Ile polymorphism at position 205, which is rather unlikely to influence processing or presentation of the IE-$1_{213\text{-}225}$ epitope (AD169 has Met, while TB40/E has Ile, fig. 4.31 B). Ultimately, the reason for these differences in recognition of HCMV strains remains to be elucidated.

RRKM18 was the only TCR that mediated preferential recognition of TB40-BAC4 over AD169 (fig. 4.30). The RRKM epitope differs in two amino acids between these HCMV strains. Therefore, potential differences in recognition of IE-1-derived peptides representing these HCMV strains were investigated using RRKM18-transgenic αβ T cells (fig. 4.31). These experiments showed that the TCR RRKM18 was about 10-fold more sensitive to IE-1 peptide no. 124, which is similar to TB40-BAC4's sequence, than to peptide no. 51, which represents AD169's sequence (fig. 4.31). The parental T cell clone F65M #18 RRKM was tested on the same peptide-loaded

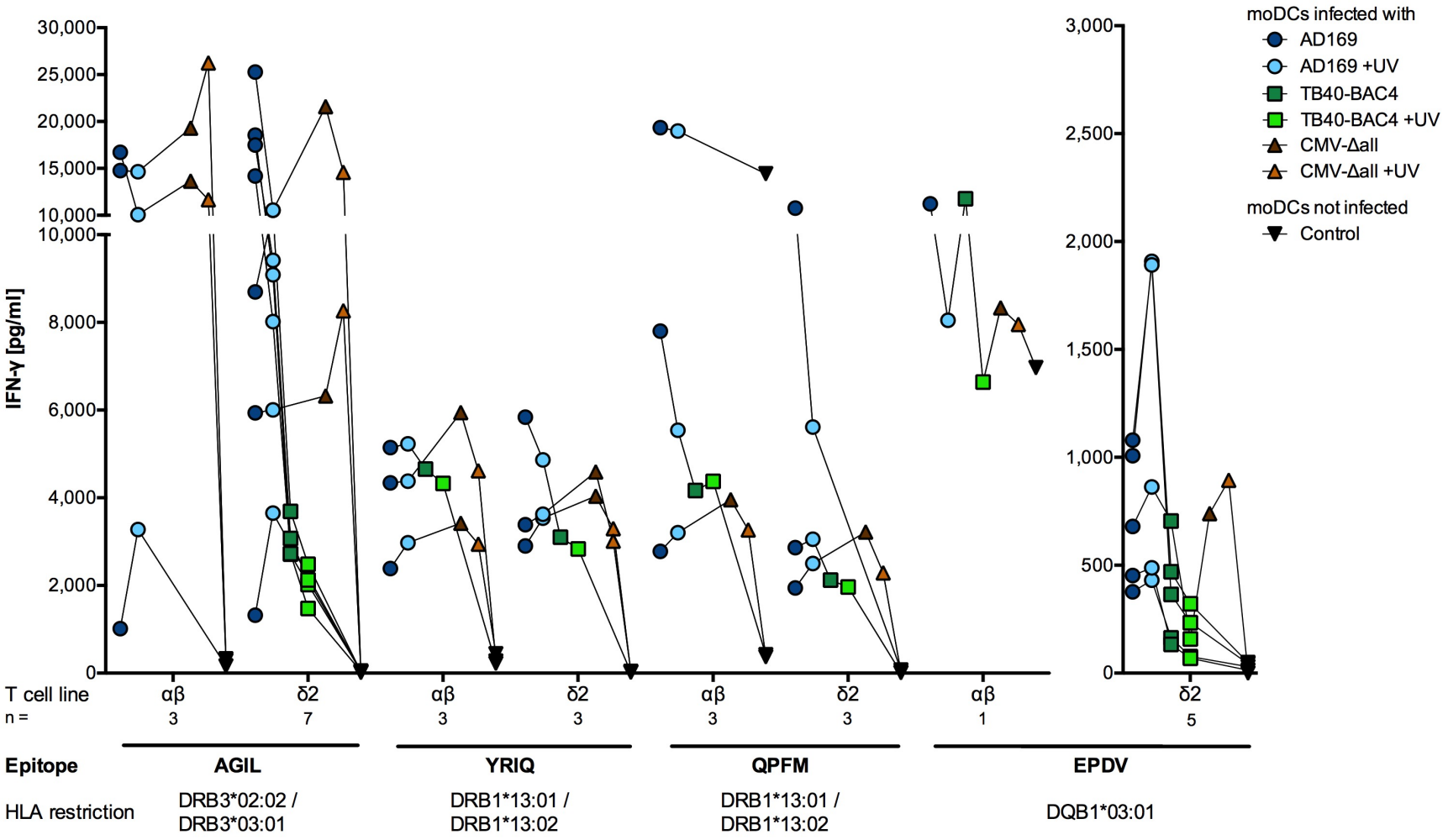


Figure 4.29. Summary of data on recognition of HCMV-infected moDCs by αβ and δ2 T cell lines that express pp65-specific, HLA class II-restricted αβ TCRs. After immunomagnetic separation, αβ TCR^+ and αβ TCR^- fractions from donors 0112, 0506 or 1107 had been stimulated with anti-CD3 antibody or PAM, respectively, before they were transduced with a TCR specific for one of the epitopes shown in the graph. Seven or eight days after transduction or restimulation, effector cells were co-incubated overnight with moDCs from an HLA-matched donor (0308, 1022, 1201, 1613 or 1918). Mature moDCs had previously been infected with HCMV strain AD169 (blue), TB40-BAC4 (green) or CMV-Δall (brown). Viruses were used untreated (dark colours) or were treated with UV irradiation (light colours). Virus was applied at an MOI of 5 for 48 hours in the absence of cytokine supplementation. Non-infected target cells served as negative control (black). The graph shows mean concentration of secreted IFN-γ in the supernatant as evaluated by ELISA. Data points with the same T cell line and the same moDC culture obtained in the same experiment are connected with lines. Results for the two TCRs YRIQ18 (one experiment) and YRIQ36 (two experiments) are shown together.

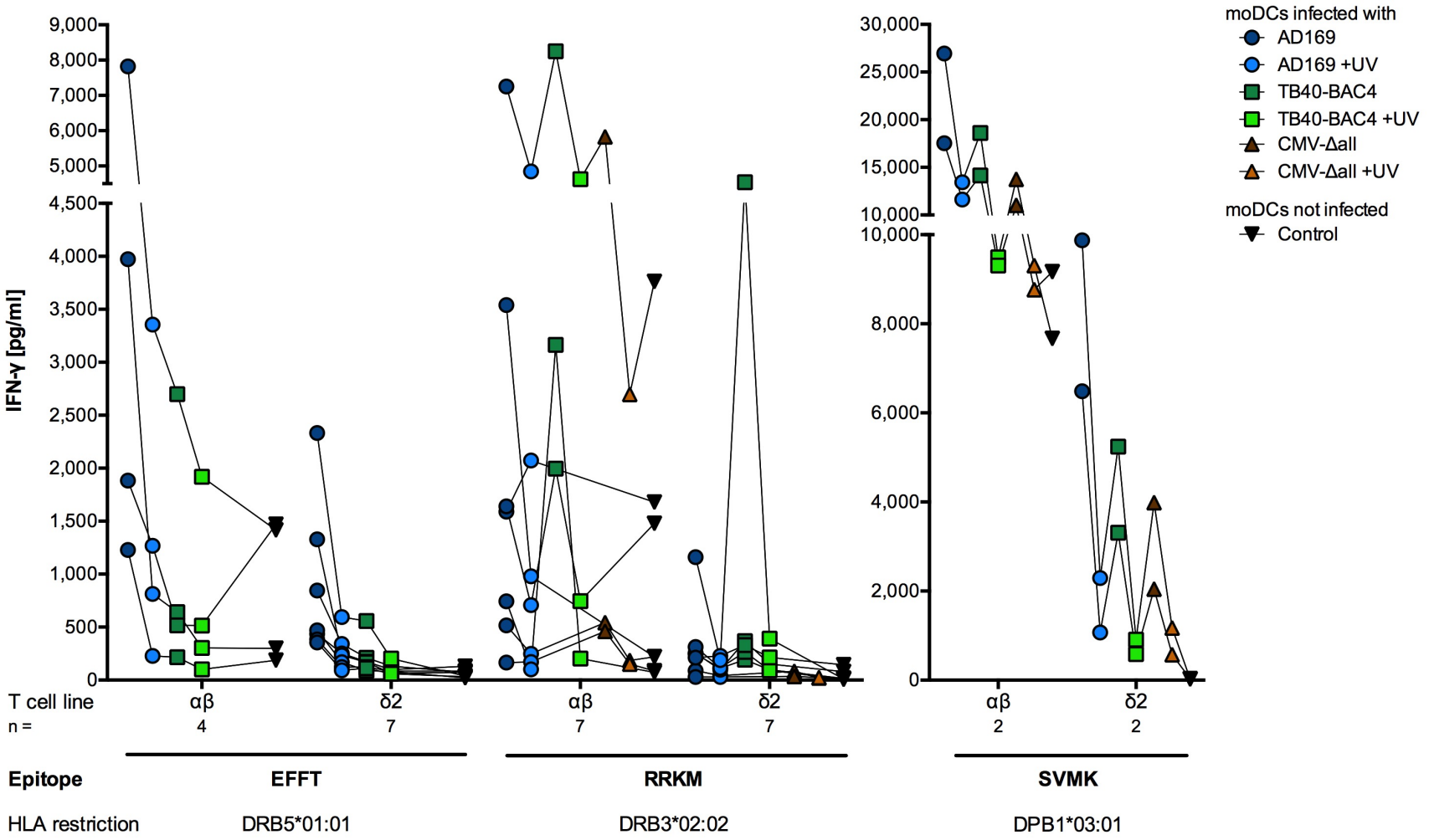
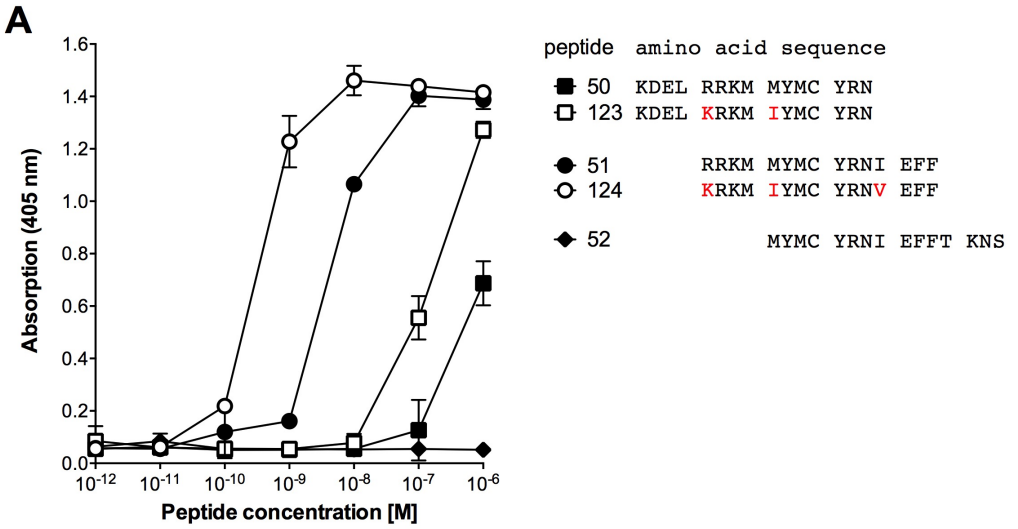


Figure 4.30. Summary of data on recognition of HCMV-infected moDCs by αβ and δ2 T cell lines that express IE-1-specific, HLA class II-restricted αβ TCRs. After immunomagnetic separation, αβ TCR^+ and αβ TCR^- fractions from donors 0112, 0506 or 1107 had been stimulated with anti-CD3 antibody or PAM, respectively, before they were transduced with a TCR specific for one of the epitopes shown in the graph. Seven or eight days after transduction or restimulation, effector cells were co-incubated overnight with moDCs from an HLA-matched donor (0308, 1022, 1201, 1613 or 1918). Mature moDCs had previously been infected with HCMV strain AD169 (blue), TB40-BAC4 (green) or CMV-Δall (brown). Viruses were used untreated (dark colours) or were treated with UV irradiation (light colours). Virus was applied at an MOI of 5 for 48 hours in the absence of cytokine supplementation. Non-infected target cells served as negative control (black). The graph shows mean concentration of secreted IFN-γ in the supernatant as evaluated by ELISA. Data points with the same T cell line and the same moDC culture obtained in the same experiment are connected with lines. TCRs tested were EFFT22 (nine experiments), EFFT50 (two experiments with αβ T cells only; shown together with EFFT22 as "EFFT"); RRKM18; SVMK6 and SVMK27 (one experiment each; shown together as "SVMK").

target cells in this experiment. F65M #18 RRKM showed the same hierarchy of recognition as the TCR-transduced cells (peptide no. 124 was about 10-fold more sensitively recognised than peptide no. 51) with the distinction that the original T cell clone was about 10-fold more sensitive to each peptide than the TCR-transduced cells (data not shown). IE-1 peptide no. 51 is identical to the amino acid sequence of IE-$1_{201\text{-}215}$ from HCMV strain AD169, which is RRKM MYMC YRNI EFF. Peptide no 124 differs from the former in three amino acids (KRKM IYMC YRNV EFF) and is found in IE-1 protein from HCMV strain Davis (fig. 4.31 B). HCMV strain TB40/E has the sequence KRKM IYMC YRNI EFF in this region. Thus, it covers only two (R201K, M205I) of the three amino acid exchanges that distinguish AD169 from Davis. Unfortunately, a peptide precisely corresponding to TB40-BAC4 was not available at the time of the experiment. It is nontheless likely that exchanges R201K and M205I contributed to superior recognition of the epitope, because peptides partially overlapping the RRKM epitope that are truncated immediately before the position of the third polymorphism (I212V) were also better recognised in the TB40-BAC4/Davis variant than in the AD169 variant (fig. 4.31 A).

The function of the HLA-DP-restricted TCRs **SVMK6 and SVMK27** was tested in one experiment that included, for each of the two TCRs, an αβ and a δ2 T cell line transduced with the TCR (fig. 4.30). Both TCRs recognised the three virus strains tested (AD169, TB40-BAC4, and the AD169 derivative CMV-Δall). The response was somewhat stronger against AD169 than against TB40-BAC4. SVMK6 conveyed similar sensitivity for HCMV-infected target cells to TCR-transgenic T cell lines than SVMK27. Among the IE-1-specific TCRs, SVMK6 and SVMK27 produced the strongest response towards target cells infected with AD169, TB40-BAC4 or CMV-Δall regardless of the T cell type that received the transgenic TCR (αβ vs. δ2 T cells). Thus, SVMK-specific TCRs are promising candidates for an application in adoptive T cell therapy of HCMV, but require verification of this result in independent experiments.

Looking at the overall picture, TCR-transgenic δ2 T cells were as effective as αβ T cells in recognising HCMV infection when they had been transduced with retrovirus encoding a pp65-specific, HLA class II-restricted TCR (fig. 4.29). If they expressed an IE-1-specific TCR after retroviral transduction, instead, TCR-transgenic δ2 T cells responded less intensely (both in specific signal and in background) than αβ T cells judging by the amount of secreted IFN-γ after target recognition (fig. 4.30). δ2 T cells consistently showed very low levels of unspecific reactivity as apparent by IFN-γ secretion in response to allogeneic control moDCs: With TCR-transgenic δ2 T cells from donors 0112, 0506 and 1107 the median background level of IFN-γ in response to control moDCs observed in 34 occasions involving moDCs from five different allogeneic donors (0308, 1022, 1201, 1613 & 1918) was 31 ± 33 pg/ml (±SD, maximum 150 pg/ml, fig. 4.29 & 4.30). In contrast, αβ T cells produced 10-fold elevated levels of IFN-γ on average with a few strong outliers (72–14,000 pg/ml, median 370 pg/ml) as a result of engaging control moDCs in 23 different occasions. This strikingly illustrates the very low unspecific reactivity of δ2 T cells in an allogeneic context, in which αβ T cells most often secreted markedly more IFN-γ after encountering HCMV-infected moDCs and sometimes even showed strong unspecific reactivity (see fig. 4.28 for exemplary data). In an allogeneic setting, such as often the case in SOT or HSCT, unspecific reactivity strictly needs to be avoided. Thus, depending on the context of



B

HCMV strain	Accession no. (GenBank)		Amino acid sequence	
AD169	NC_001347	191	AKARAKKDEL **RRKMMYMCYR NIEFF**TKNSA FPKTTNGCSQ	240
Towne	AY446869	191	AKARAKKDEL RRKMMYMCYR NI*EFFTKNSA FPKTT*NGCSQ	240
strain W	AY446865	191	AKARAKKDEL RRKMMYMCYR NIEFFTKNSA FPKTTNGCSQ	240
Merlin	AY446894	191	AKARAKKDEL RRKMMYMCYR NIEFFTKNSA FPKTTNGCSQ	240
TB40/E	AAR31361	191	AKARAKKDEL KRKMIYMCYR NIEFFTKNSA FPKTTNGCSQ	240
Toledo	AY446871	191	AKARAKKDEL KRKMMYMCYR NVEFFTKNSA FPKTTNGCSQ	240
Davis	AY446868	191	AKARAKKDEL KRKMIYMCYR NVEFFTKNSA FPKTTNGCSQ	240
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Figure 4.31. HCMV strain specificity of the IE-1-specific, HLA class II-restricted TCR RRKM18. A: Peptide titration of RRKM18-transgenic αβ T cells. HLA-matched control mini-LCLs from donor F65 were loaded with IE-1 peptides no. 50, 51, 52, 123 or 124 from the 15-mer IE-1 peptide library (tab. 2.11) at concentrations ranging from 10^{-12} to 10^{-6} M. Peptide-loaded mini-LCLs were co-incubated overnight with an αβ T cell line from donor 1107 that had been transduced with RRKM18-encoding retrovirus 13 days before the assay and had been restimulated with antigen-expressing mini-LCL from donor 1021 six days before the assay. The graph shows absorption at 405 nm wavelength as readout of an ELISA that evaluated concentration of IFN-γ in the co-culture supernatant. The mean and standard deviation of triplicate samples are shown. **B:** Amino acids 191 to 240 of IE-1 protein from different HCMV strains. Sequences were obtained from NCBI's GenBank (https://www.ncbi.nlm.nih.gov/genbank/, accession numbers as depicted in 2nd column) and translated into protein. Amino acid sequence of peptide no. 50 from the IE-1 library used in this study is underlined and depicted in bold letters. Differences in IE-1 sequence from AD169 are highlighted in red and indicated by a dot in the last line. The EFFT epitope (IE-$1_{213\text{-}225}$) is underlined and depicted in italic letters.

future clinical application (such as whether the T cell donor will be the patient, an HLA-matched donor or an HLA-mismatched donor), TCR-transgenic δ2 T cells may qualify as preferable if the intensity of both wanted and unwanted reactivities is taken into account.

4.4.5.4 Recognition of CMV-Δall in comparison to AD169

HCMV devoid of the immunoevasins US2, US3, US6 and US11 (**CMV-Δall**) elicited mildly higher IFN-γ secretion by QPFM17-, YRIQ18- or YRIQ36-transgenic αβ or δ2 T cells than

AD169 (1.4- to 1.7-fold, fig. 4.27). AGIL19 in combination with CD4 caused 0.8-fold decreased or 2.5-fold increased IFN-γ release when expressed by αβ or δ2 T cells, respectively. In this experiment, the same αβ and δ2 effector T cells transduced with AGIL19 and CD4 were also tested on moDCs from another donor, releasing 1.3- and 1.1-fold more IFN-γ in response to CMV-Δall- than AD169-infected moDCs (donor 1918, data not shown). Intriguingly, the opposite tendency was observed for IE-1-specific TCRs: on average, αβ or δ2 T cells expressing SVMK6, SVMK27 or RRKM18 released half as much IFN-γ in response to CMV-Δall than to AD169 infection (0.1- to 2.8-fold, median 0.5, one or two experiments per TCR and T cell population using SVMK- or RRKM-specific TCRs, respectively, fig. 4.28 and data not shown). Overall, the HLA-DR-restricted TCRs AGIL19, QPFM17, YRIQ18 and YRIQ36 as well as the HLA-DP-restricted TCRs SVMK6 and SVMK27 seem to be considerably less affected by the effect of immunoevasins US2, US3, US6 and US11 on peptide presentation (fig. 4.27 & 4.28, respectively) than most HLA class I-restricted T cell clones and HLA-C*07:02-restricted TCRs (see section 4.3.2). This finding is in agreement with demonstrations that US2 and US11 do not target HLA class I (Rehm et al., 2002), contradicting earlier research (Tomazin et al., 1999). The presented results support clinical application of HCMV-specific, HLA class II-restricted TCRs because HCMV does not seem capable in DCs to prevent presentation of viral peptides by HLA class II molecules. An overview of all data collected on recognition of moDCs that were challenged with CMV-Δall is provided in figures 4.29 & 4.30.

4.4.5.5 Role of CD4 co-receptor for function of HLA class II-restricted TCRs in transgenic T cells

While TCR expression had not particularly depended on the presence of **CD4 co-receptor** in transduced T cells (section 4.4.2), the importance of CD4 co-expression for the function of HLA class II-restricted TCRs remained to be elucidated. In order to evaluate the effect of CD4 on recognition of HCMV-infected moDCs by TCR-transgenic T cells, δ2 T cells from two donors (0112 & 1107) were transduced with one of the two pp65-specific TCRs AGIL19 or EPDV21 or one of the two IE-1-specific TCRs EFFT22 or RRKM18 alone or in combination with CD4 co-receptor. The expression of CD4, if applicable, and each individual TCR are shown in figures 4.23 and 4.24 for donor 0112. Two days after the FACS analysis, these TCR-transgenic δ2 T cells were co-incubated overnight with mature moDCs that had previously been infected with AD169 or TB40-BAC4 for 48 hours. In general, co-transduction of CD4 had only a very minor effect (0.4- to 1.4-fold IFN-γ secretion when CD4 was co-transduced, median 1.2, fig. 4.32). The TCR EFFT22 was an exception: IFN-γ secretion in response to active HCMV preparations was, unexpectedly, reduced when CD4 was co-transduced. Similar results were obtained with the same T cell lines from donor 0112 when tested on moDCs from a different donor (0308, data not shown). Since EFFT22 is the only TCR among the four discussed in this paragraph that quite strongly suppressed expression of endogenous γδ TCRs in δ2 T cells (see fig. 4.23 and 4.24), EFFT22 may be less dependent on CD4 co-expression due to its inherent strength of TCR expression.

In a replication of this experiment, the recognition of HCMV-infected moDCs by EFFT22-transgenic δ2 T cell lines from another donor (1107) was tested (data not shown). In this case,

δ2 T cells that had been transduced with retroviruses coding for EFFT22 and CD4 produced almost identical levels of IFN-γ in response to HCMV-infected moDCs from donor 1613 than EFFT22-transduced δ2 T cells. In summary, CD4 co-transduction had no or negligible effect on the recognition of HCMV-infected moDCs. This somewhat unexpected result suggests CD4 independence of the HLA class II-restricted TCRs studied here. This will simplify clinical development, since it appears that CD4 co-transduction is not necessary for generation of effective

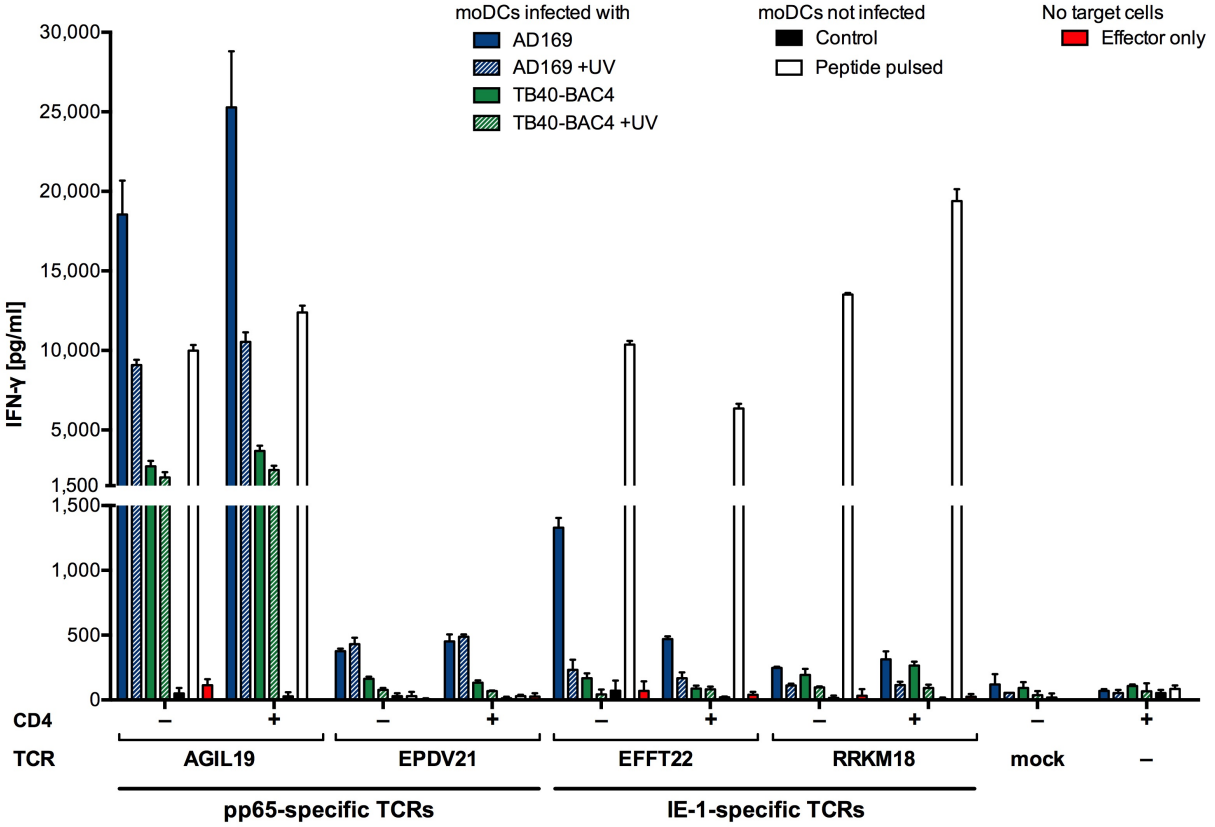

Figure 4.32. Effect of CD4 co-transduction on the recognition of HCMV-infected moDCs by δ2 T cell lines that express HLA class II-restricted TCRs. After immunomagnetic separation, the αβ TCR^- fraction had been stimulated with PAM before the cells were transduced with AGIL19, EPDV21, EFFT22 or RRKM18. δ2 T cells were transduced with TCR alone or together with CD4 co-receptor at a ratio of 2 to 1 of retroviral vector plasmid transfected into packaging cells. CD4 transduction alone and empty retrovirus delivering no transgene ("mock") were used as controls. See figures 4.23 & 4.24 for FACS analysis of TCR-transgenic T cells. 7 days after transduction, effector cells were co-incubated overnight with differently treated moDCs at an e:t ratio of 20,000:10,000. Mature moDCs had previously been infected with HCMV strain AD169 (solid blue bars) or TB40-BAC4 (solid green bars) at an MOI of 5 for 48 hours in the absence of cytokine supplementation or had been treated correspondingly with UV-inactivated HCMV inoculum (hatched bars in corresponding colours). Non-infected target cells were either loaded with corresponding antigenic peptides (white bars) or left untreated (black bars) to serve as positive or negative controls, respectively. For EPDV21, target cells were loaded with the pp65 peptide no. 57 (KVYL) instead of peptide no. 45 (EPDV, tab. 2.12) in this experiment because the correct epitope of EPDV21 was identified later (see section 3.4.4); this explains the missing positive control signal. The graph shows concentration of secreted IFN-γ in the supernatant as evaluated by ELISA. The mean and standard deviation of triplicate samples are shown. Representative results for one (0112) out of two δ2 T cell donors (the other was 1107; not shown) and one (1613) out of two moDC donors (the other was 0308; not shown) are shown.

HLA class II-restricted TCR-transgenic T cells specific for HCMV.

4.4.5.6 Differences in target cell recognition depending on the HCMV antigen: pp65 vs. IE-1

Since pp65, but not IE-1, is contained in large quantities in HCMV virions (Büscher et al., 2015; Reyda et al., 2014), it can be hypothesised that IE-1-specific T cells will preferentially respond to moDCs which have been functionally infected and subsequently produce IE-1 protein. In contrast, pp65-specific T cells will respond to any scavenging moDC, which has acquired viral proteins through ingestion of inactivated virions, cellular debris and other viral protein-containing material. Corroborating this hypothesis, UV irradiation greatly reduced recognition of HCMV-infection by IE-1-specific, but not pp65-specific, TCR-transgenic δ2 T cells (see exemplary data in fig. 4.27, 4.28 & 4.32 and summary in fig. 4.29 & 4.30).
For a more quantitative description of this effect, the ratio of secreted IFN-γ in response to moDCs treated with infectious HCMV to secreted IFN-γ in response to moDCs treated with UV-inactivated virus will here be designated active/inactive virus recognition ratio or, for short, A/I ratio. Indeed, pp65- and IE-1-specific TCRs functionally differed from each other in their A/I ratio. For AD169 and pp65-specific TCRs AGIL19 and EPDV21, the A/I ratio was 0.9 to 2.4 (median 1.5, fig. 4.32); for TB40-BAC4 and these two TCRs, it was 1.3 to 2.1 (median 1.7). In accordance with the hypothesis, the A/I ratio was higher for the IE-1-specific TCRs EFFT22 and RRKM18, both with AD169 (2.2 to 5.7, median 2.8) and with TB40-BAC4 (1.1 to 3.9, median 2.4, fig. 4.32). A similar behaviour was observed earlier for HLA class II-restricted $CD4^+$ T cell clones (fig. 4.26).

To obtain a better overview, all experiments were collectively evaluated for their active/inactive virus recognition (A/I) ratio (fig. 4.33 & 4.34). The general picture emerging from this analysis is somewhat complex, but it appears to broadly support the view that pp65-specific T cell lines reacted with similar strength to both types of target cells (A/I ratio ≈ 1) in most experiments, whereas most IE-1-specific T cell lines reacted at least two times stronger to target cells that had been treated with infectious AD169 than target cells that had been treated with UV-inactivated AD169 (A/I ratio ≥ 2, fig. 4.33 A).
In general, TCR-transgenic αβ and δ2 T cell lines provided very comparable A/I ratios when transduced with the same TCR, except for certain experiments with AGIL-, EPDV- and SVMK-specific TCRs (fig. 4.33A & C). With some exceptions, relatively similar patterns were obtained for viruses AD169 (fig. 4.33 A) and TB40-BAC4 (fig. 4.33 C).
The IFN-γ levels that were secreted by EFFT22- or EFFT50-transgenic T cell lines in response to moDCs treated with infectious TB40-BAC4 were rather low (fig. 4.30), as discussed earlier in section 4.4.5. Consequently, A/I ratios of EFFT22- or EFFT50-transgenic T cell lines for TB40-BAC4 were markedly lower in comparison to AD169 (fig. 4.33 C vs. A, respectively).

Some A/I ratios of TCR-transgenic αβ cell lines, as observed with TCRs SVMK6 and SVMK27 for example, were presumably influenced by high background levels of IFN-γ secretion in response to allogeneic moDCs irrespective of their treatment with HCMV (fig. 4.29 & 4.30). In order

to eliminate this confounding factor, background-corrected A/I ratios were calculated using the following formula:

$$\text{corrected A/I ratio} = \frac{\text{IFN-}\gamma\text{(HCMV)} - \text{IFN-}\gamma\text{(Control)}}{\text{IFN-}\gamma\text{(HCMV +UV)} - \text{IFN-}\gamma\text{(Control)}} \quad (3)$$

In accordance with the previous observation that TCR-transgenic δ2 T cell lines generally showed low unspecific activation by control moDCs (judging by IFN-γ secretion, fig. 4.29 & 4.30), the correction did not affect most of their A/I ratios, regardless of HCMV virus strain (AD169 or TB40-BAC4, fig. 4.33 A vs. B or C vs. D), except for the combination of EFFT-specific T cells and TB40-BAC4, where an elevation of some A/I values through correction was observed. In contrast, TCR-transgenic αβ T cells were more strongly affected by the correction. In order to avoid generating excessive values by the correction (values approaching infinity or reaching into the negative), ratios were manually set to a predefined value in two instances: (I) If the amount of IFN-γ secreted in response to control moDCs was higher than the one secreted in response to moDCs treated with UV-inactivated HCMV, the corrected A/I ratio was set to 200; (II) If the amount of IFN-γ secreted in response to control moDCs was higher than the one secreted in response to moDCs treated with infectious HCMV, no detectable specific response to HCMV-infected cells was assumed, and for purposes of graphical representation and non-parametric analysis the corrected A/I ratio was set to 0.1.
Remarkably, A/I ratios obtained with TCR-transgenic αβ T cell lines expressing pp65-specific TCRs were hardly affected by the correction, while most A/I ratios for αβ T cells obtained with EPDV21 or any IE-1-specific TCR were (fig. 4.33). Among all pp65-specific TCRs, the amount of IFN-γ secreted by TCR-transgenic αβ T cell lines in response to control moDCs ranged from 165 to 14,000 pg/ml (median $350 \pm 4{,}700$ pg/ml, fig. 4.29). If the two highest IFN-γ levels that had been obtained with EPDV21 (7,000 pg/ml) and with QPFM17 (14,000 pg/ml) were excluded, the strongest response to control moDCs was 440 pg/ml (median±SD = 310 ± 95 pg/ml). Combined with the high levels of IFN-γ that the same cells generally secreted in response to HCMV-infected moDCs (fig. 4.29), the correction did hardly affect the A/I ratios of αβ T cell lines expressing any pp65-specific TCR, except for EPDV21 (fig. 4.33). IE-1-specific TCRs, however, mediated generally lower IFN-γ levels after TCR-transgenic αβ T cell lines had encountered HCMV-infected moDCs, while causing relatively high reactivity of these cells to control moDCs (72–9,200 pg/ml, median±SD = $1{,}400 \pm 3{,}000$ pg/ml, fig. 4.30). As a result, the correction affected A/I ratios of αβ T cell lines expressing an IE-1-specific TCR more strongly than those expressing a pp65-specific TCR, regardless of HCMV virus strain (AD169 or TB40-BAC4, fig. 4.33).

In order to cumulatively test whether pp65-specific TCRs differed from IE-1-specific TCRs, all A/I values for a given HCMV strain (AD169 or TB40-BAC4) and a given type of transduced T cells (αβ or δ2) were pooled in two groups according to the HCMV antigen and subjected to statistical analysis (Mann-Whitney test). The medians of all A/I ratios for AD169 of αβ and δ2 T cell lines that expressed a pp65-specific TCR were 1.00 and 0.93, respectively (fig. 4.34 A & B). The corresponding median A/I values for IE-1-specific TCRs were 2.08 and 2.76, which demonstrated that IE-1 recognition, but not pp65 recognition, was overall dependent on active infection. Regardless of the TCR-transgenic T cell type (αβ vs. δ2), the difference between pp65

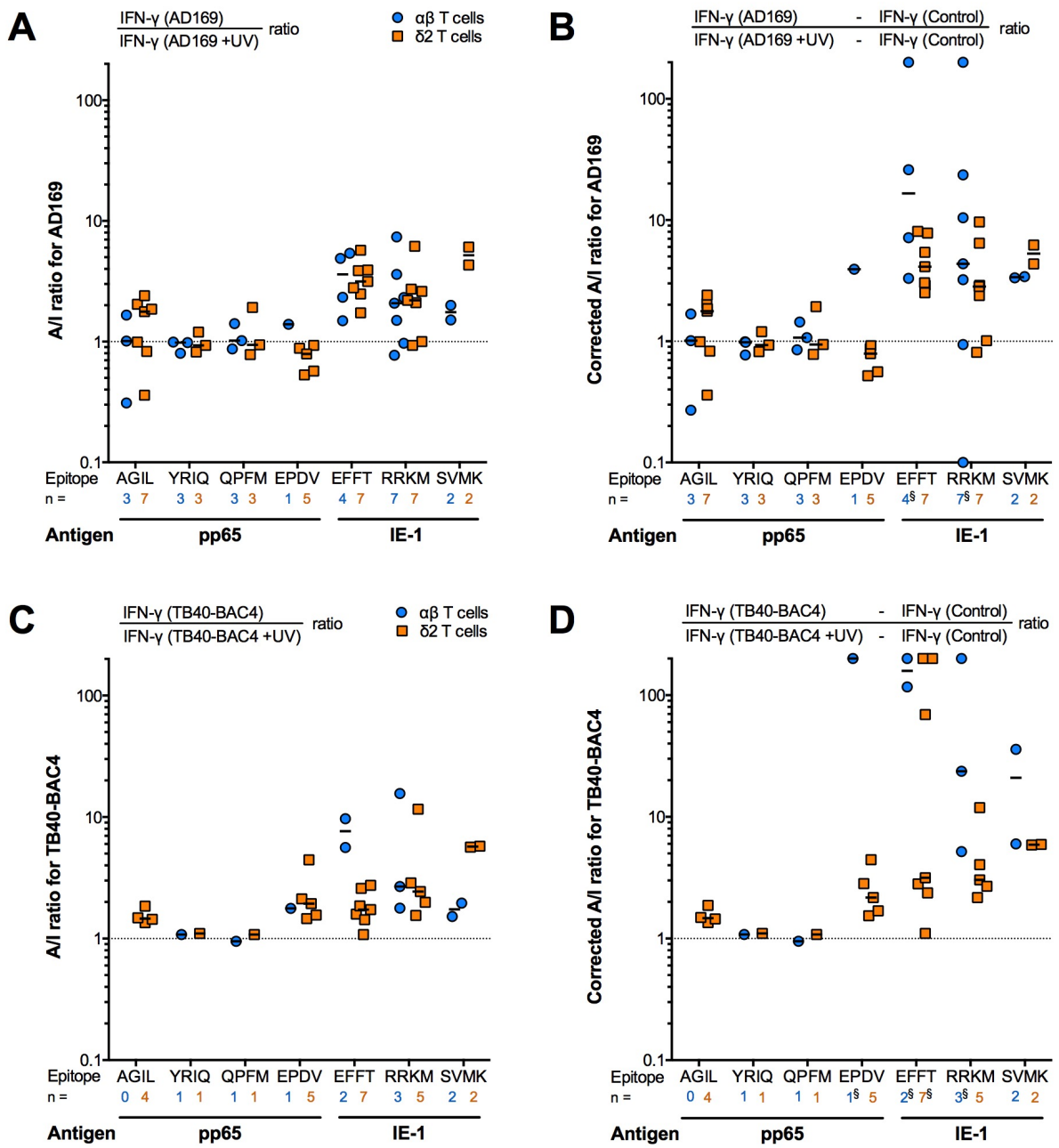

Figure 4.33. Active/inactive virus recognition (A/I) ratios of TCR-transgenic αβ (blue circles) **and δ2** (orange squares) **T cell lines for AD169** (graphs A & B) **and TB40-BAC4** (graphs C & D). Every dot marks the ratio of IFN-γ secreted by a TCR-transgenic T cell line in response to moDCs treated with infectious HCMV to IFN-γ secreted by identical effector cells in response to moDCs treated with UV-inactivated virus in the same experiment ("A/I ratio", graphs A & C). For the corrected A/I ratio (graphs B & D), "background" IFN-γ secreted in response to control moDCs was subtracted from IFN-γ secreted in response to moDCs that had been treated with infectious HCMV or UV-inactivated virus, before calculating the ratio. Corresponding formulas are displayed on top of each of the graphs. The resulting ratios for TCRs that recognise the same epitope (tab. 4.2, e.g. YRIQ18 & YRIQ36) were pooled into groups (e.g. YRIQ). The bar marks the median of each group. For groups marked with "§" one of the two adjustments has been applied for at least one data point: (I) If the amount of IFN-γ secreted in response to control moDCs was higher than the one secreted in response to moDCs treated with UV-inactivated HCMV, the corrected ratio was set to an arbitrary upper cut-off value of 200; (II) If the amount of IFN-γ secreted in response to control moDCs was higher than the one secreted in response to moDCs treated with infectious HCMV, the corrected ratio was set to an arbitrary lower cut-off value of 0.1. The dashed line indicates an A/I ratio of 1.

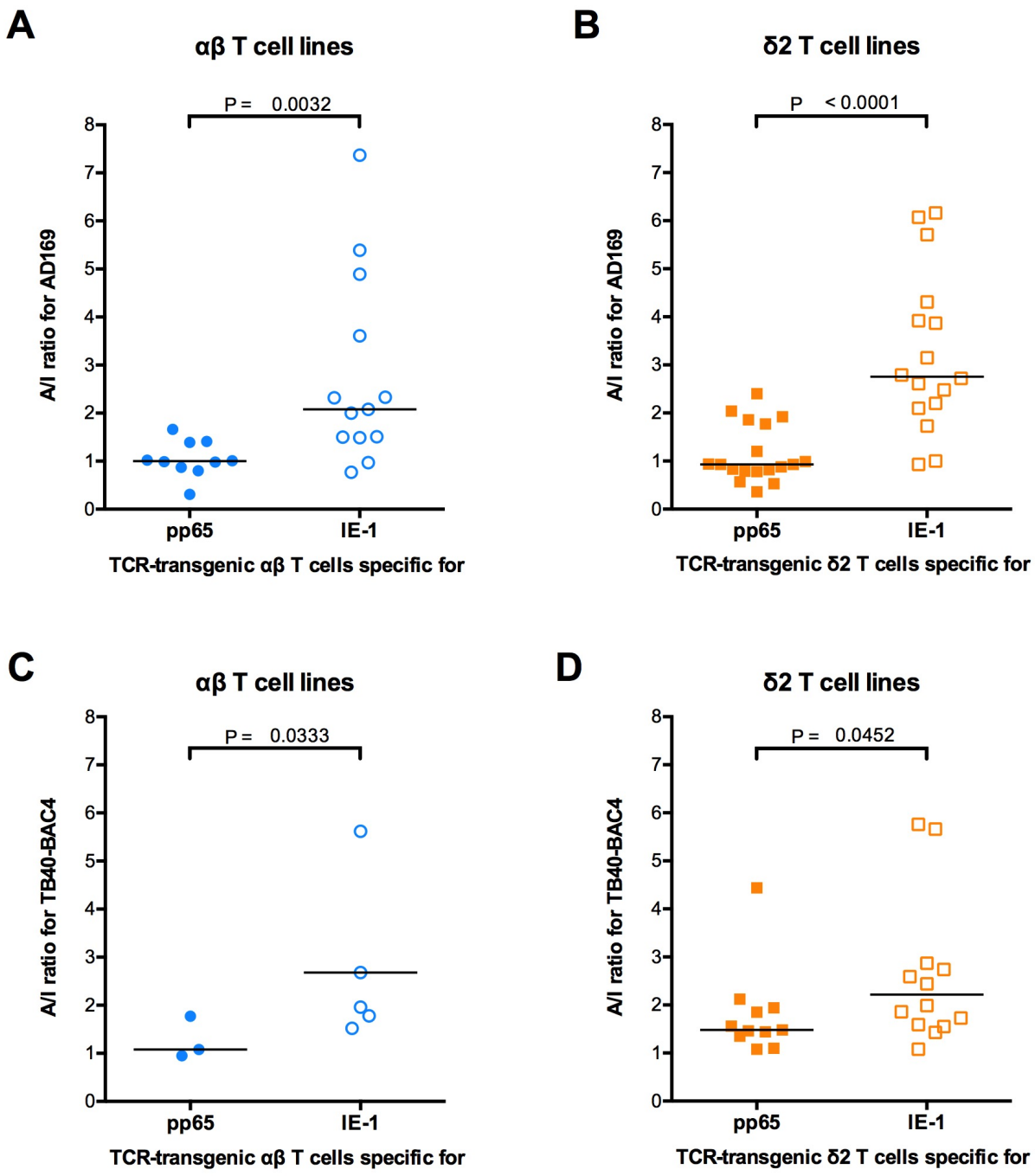

Figure 4.34. Combined active/inactive virus recognition (A/I) ratios of TCR-transgenic αβ T cell lines (blue) **and δ2 T cell lines** (orange) **for AD169** (graphs A & B) **and TB40-BAC4** (graphs C & D)**.** Every dot marks the ratio of IFN-γ secreted by a TCR-transgenic T cell line in response to moDCs treated with infectious HCMV to IFN-γ secreted by identical effector cells in response to moDCs treated with UV-inactivated virus in the same experiment ("A/I ratio"). The A/I ratios for pp65-specific TCRs (filled symbols) and IE-1-specific TCRs (empty symbols) were compared using a Mann-Whitney test for significance, showing the respective P value within each graph.

and IE-1 was highly significant (P<0.004, fig. 4.34 A & B).
For TB40-BAC4 the situation was similar, but due to the smaller number of experiments performed with TCR-transgenic αβ T cells and TB40-BAC4-infected target cells the analysis had reduced statistical strength in that situation (fig. 4.34 C). The median of the A/I ratios of TCR-transgenic αβ T cells for TB40-BAC4 was 1.08 or 2.68 for pp65 or IE-1, respectively (P=0.033). TCR-transgenic δ2 T cells had a median A/I ratio of 1.48 or 2.22 if they expressed a pp65-specific or an IE-1-specific, HLA class II-restricted TCR, respectively (P=0.045, fig. 4.34 D). While three of four median A/I values for pp65 were close to 1.0 (fig. 4.34 A, B & C), the median pp65 A/I ratio was close to 1.5 for the combination of the TB40-BAC4 strain and δ2 T cells (fig. 4.34 D). Thus, in this particular situation endogenous production of pp65 after infection appeared to play a role in provision of antigen for T cell recognition. A possible explanation is that TB40-BAC4 virions carry less pp65 protein than AD169-derived virions (Büscher et al., 2015) and thus UV-inactivated TB40-BAC4 virions introduce less pp65 proteins into an infected target cell than UV-inactivated AD169 at the same MOI. Consequently, DCs that were productively infected with TB40-BAC4 will produce quantities of intracellularly derived pp65 peptides that can detectably contribute to T cell recognition. A comparable increase in A/I ratios was not observed with αβ T cells, which may be due to the low number of experiments that assessed the recognition of TB40-BAC4 infection by pp65-specific, TCR-transgenic αβ T cells (fig. 4.34 C). In the case of HCMV strain AD169, newly synthesised pp65 may play a minor role in recognition of infection by pp65 specific T cells, since these virions already introduce a large amount of pp65 protein into infected cells (Varnum et al., 2004) providing an excess of antigen available for presentation via HLA molecules. This may explain why most of αβ and δ2 T cell lines showed an A/I ratio of approximately 1 in response to pp65 from AD169 (fig. 4.34 A & B).

In summary, figure 4.34 shows that pp65-specific, HLA class II-restricted TCRs most often mediate a comparable IFN-γ response to active and inactive HCMV presented by moDCs. In contrast, IE-1-specific, HLA class II-restricted TCRs conveyed an about 2- to 3-fold increased sensitivity for active infection over inactive HCMV. Thus, a pp65-specific, HLA class II-restricted T cell may be activated by an HLA class II-expressing target cell, such as DCs or monocytes, that presents pp65-derived peptides as a result of infection with HCMV or endocytosis of cellular debris or virions, which contained pp65 protein. An IE-1-specific, HLA class II-restricted T cell, however, is likely activated by an HLA class II-expressing target cell predominantly if the latter was infected with HCMV causing IE-1 production in the infected cell.
The amount of secreted IFN-γ by TCR-transgenic T cells specific for IE-1 was often markedly lower than the amount of secreted IFN-γ by TCR-transgenic T cells specific for pp65 (fig. 4.30 vs. 4.29). This does not necessarily imply that IE-1-specific TCRs are less efficient than pp65-specific TCRs in mediating effective antiviral action against HCMV-infected target cells by TCR-transgenic T cells, but may indicate that protein levels of IE-1 are more limiting that those of the particularly abundant protein pp65 in infected moDCs, which ultimately affects the abundance of corresponding viral peptides presented on HLA class II molecules.
Taken together, HCMV-specific, HLA class II-restricted TCRs effectively mediated recognition of moDCs that presented endogenously processed HCMV antigens. Unspecific reactivity was occasionally observed when using αβ T cells for TCR transduction and partially mismatched

moDCs as targets. This was most likely due to HLA alloreactivity, an expected and relevant aspect that must be optimised for therapeutic application of TCR-transduced αβ T cells. Very interestingly, when δ2 T cells were equipped with HCMV-specific, HLA class II-restricted TCRs, such alloreactivity was absent. Thus, this combination of T cells and HCMV-specific TCRs by TCR gene transfer is very attractive for therapy of HCMV in an allogeneic environment, such as HSCT. HLA class II-restricted TCR-transgenic T cells may be used to complement HLA class I-restricted T cell therapy of HCMV infection in immunocompromised patients.

5 Discussion

HCMV-specific T cells can be produced by transferring the genes of an HCMV-specific TCR into primary T cells (Schub et al., 2009). This approach is a path to therapy of HCMV infection by adoptive transfer of HCMV-specific T cells for immunocompromised patients that lack HCMV-specific T cells even when the HSC donor is HCMV-negative ($HCMV^-$) and therefore has no HCMV-specific memory T cells. To put this idea into practice, HCMV-specific TCRs must be identified, sequenced, and characterised. Ultimately, it must be evaluated whether the TCR, after transfer into suitable effector cells, functionally, sensitively, and specifically recognises HCMV-infected target cells. These tasks were addressed in the present study focussing on TCRs that are restricted by different HLA class I and class II molecules in order to enable their therapeutic use.

5.1 Epitopes, HLA restrictions and TCR sequences of HCMV-specific TCRs

Within this study, the TCR sequences of 9 HLA class I-restricted TCRs and 13 HLA class II-restricted TCRs specific for one of the HCMV antigens pp65, IE-1 or UL28 were identified (tab. 4.2). pp65 is a structural protein that participates in the assembly of new virions and is the most abundant protein of virions from WT strain AD169 (Reyda et al., 2014; Varnum et al., 2004). Due to its high abundance in virions, it is not surprising that pp65 is an immunodominant antigen against which most HCMV-positive ($HCMV^+$) persons develop T cell responses (Sylwester et al., 2005). In contrast, IE-1 was not identified as a component of virions (Büscher et al., 2015; Varnum et al., 2004) or was contained at very low levels only (Reyda et al., 2014). IE-1 functions as a transcriptional regulator and is essential for the expression of viral early proteins, which drive viral DNA replication (Greaves and Mocarski, 1998; Marchini et al., 2001). While $CD8^+$ T cell responses against the antigen IE-1 were also found in most $HCMV^+$ persons, IE-1-specific $CD4^+$ T cell responses were only observed in a third of $HCMV^+$ persons (Sylwester et al., 2005).

Recently, T cell clones specific for IE-$1_{309\text{-}317}$ (CRV) and restricted by HLA-C*07:02 were found to resist the immunomodulatory effect of HCMV's immunoevasins (Ameres et al., 2014, 2013), which are known to down-regulate the expression of HLA class I molecules on infected cells (Jones et al., 1995) and thereby impair T cell recognition. Since HLA-C*07:02 was less affected by the four immunoevasins US2, US3, US6 and US11 in comparison to other HLA class I molecules (Ameres et al., 2014, 2013), TCRs restricted by this HLA may be especially efficacious in controlling HCMV infection in individuals that possess this HLA allele. Thus, four CRV-specific TCRs and five TCRs specific for the epitope UL$28_{327\text{-}335}$ (FRC), which were also restricted by HLA-C*07:02, were analysed and tested in this study. UL28/UL29 codes for an IE protein that enhances IE gene expression and can activate major immediate-early promoter (Mitchell et al., 2009).
To date, no complete TCRs specific for the epitopes CRV or FRC have been described. Recently, 266 and 435 unique TCRβ sequences of HLA class I-restricted TCRs specific for FRC or CRV, respectively, were published (Huth et al., 2019). These TCRβ sequences were identified by deep sequencing of polyclonal, epitope-specific T cell lines that were selectively expanded from $HCMV^+$ donors. This publication confirmed the striking immunodominance of these specificit-

ies in the $CD8^+$ T cell repertoire, but did not address the TCRα repertoire. In contrast, in the present work TCRα and TCRβ sequences of well characterised CRV- and FRC-specific $CD8^+$ T cell clones were identified. All TCRβ sequences (V–CDR3–J) of FRC- or CRV-specific TCRs listed in table 4.2 were already published by Huth et al. (2019) except for the TCRβ sequence of CRV2. Huth et al. (2019), however, did not assess the function of the identified TCRs which has been done for four FRC-specific and four CRV-specific TCRs in the present study.

Among HLA class II-restricted TCRs, six TCRs were specific for pp65 and five TCRs were specific for IE-1. The pp65-specific TCRs were derived from $CD4^+$ T cell clones that had been established and incompletely characterised by Wiesner (2005) and Schub (2010). The epitopes and HLA restrictions of a panel of IE-1-specific $CD4^+$ T cell clones have been analysed in great detail (Ameres et al., 2015). IE-1-specific $CD4^+$ T cell clones were chosen for TCR isolation if their HLA restriction had earlier been confirmed by HLA transfection (see Ameres et al., 2015). Some sequences of HLA class II-restricted TCRs specific for gB, pp65or IE-1 were published, but none of the sequences described in the present study (tab. 4.2) were known to code for an HCMV-specific TCR before:
Abana et al. (2017) studied HLA-DRB1*07:01-restricted T cells specific for $gB_{217\text{-}227}$ (DYSN) or $pp65_{177\text{-}191}$ (EPDV) by peptide/HLA class II multimer stainings *ex vivo*. The TCR EPDV21 which is described in this work is restricted by a different HLA class II complex (HLA-DQB1*03:01, tab. 4.2). Abana et al. (2017) identified several TCRβ chains of DYSN-specific TCRs, but only one TCRα-TCRβ pair, which was subsequently expressed in leukemic Jurkat cells and found to mediate recognition of peptide-loaded target cells.
Crompton et al. (2008) also analysed HLA-DRB1*07:01-restricted, DSYN-specific T cell clones from which they identified seven complete αβ TCRs and five more TCRβ sequences. These TCRs were not recombinantly expressed in other T cells, but the parental T cell clones were shown to recognise endogenously processed antigen presented by LCLs or HCMV-infected target cells. However, HLA-DRB1*07:01-restricted, DYSN-specific $CD4^+$ T cells were shown to cross-react with the alloantigen HLA-DR4, which may be a possible mechanism for the clinical association between HCMV and GvHD after allo-HSCT (Elkington and Khanna, 2005). Thus, DYSN-specific TCRs may not be safe to use in adoptive T cell therapy of HCMV disease.
Simon et al. (2014) described three complete αβ TCRs that are also restricted by HLA-DRB1*07:01 and specific for the epitopes $pp65_{117\text{-}139}$ (PLKM) or $pp65_{337\text{-}359}$ (VELR), but information about the CDR3 of these TCRs was not shown. The TCR sequences were obtained from single $CD4^+$ T cells that had been isolated from pp65-specific T cell lines by IFN-γ capture and magnetic separation. The TCRs were recombinantly expressed in T cells by RNA transfection and mediated recognition of peptide-loaded, HLA-transgenic K562 cells.
Schwele et al. (2012) published nine TCRβ sequences in total from T cell cultures that had been stimulated with either $pp65_{245\text{-}263}$ (TLGS) or $IE\text{-}1_{269\text{-}288}$ (LTHI), but they did not verify HCMV-specificity of these TCRs. Thus, some of the TCRβ sequences may not be specific for HCMV.
van Leeuwen et al. (2006) reported about 80 TCRβ sequences derived from $CD4^+$ T cells that had been isolated from PBMCs of SOT patients by stimulation with HCMV antigens and subsequent IFN-γ capture and magnetic separation. Again, the antigen specificity of the TCRs was not verified independently and thus the list of sequences may include non-HCMV-specific TCRβ

chains.

The epitopes and HLA restrictions of six out of seven pp65-specific $CD4^+$ T cell clones could be determined (see fig. 4.7 & 4.8, as well as tab. 4.1). Each of these epitopes has already been described, but some were allocated to HLA restrictions that were different from the HLA restrictions identified here. This was the case for $pp65_{509\text{-}523}$ (KYQE) described to be restricted through DR1 or DR3 (Khattab et al., 1997; Li Pira et al., 2004; Slezak et al., 2007) and $pp65_{177\text{-}191}$ (EPDV) described to be restricted through DR7 (Li Pira et al., 2004). Misidentification of their HLA restriction in previous studies seems possible, since the possibility of HLA-DQ restriction was neither taken into account nor tested in those studies.
The epitope $pp65_{109\text{-}123}$ (MSIY) that was reported earlier by this group (Wiesner et al., 2005) for the same T cell clone used in the present study (F46P #40) could not be verified. Either the putative HLA-DRB1*15:01 restriction or the epitope may have been wrongly identified because neither MSIY/HLA-DRB1*15:01 tetramers could stain TCR-transgenic T cells, nor did these cells recognise HLA-transgenic peptide-pulsed target cells (data not shown).
The epitopes and HLA restrictions of a large set of IE-1-specific $CD4^+$ T cell clones, from which the TCRs that are used in this study were derived, were analysed in detail by Ameres et al. (2015). The only correction that is made necessary by the results presented here is the HLA restriction of the SVMK-specific $CD4^+$ T cell clones 1021M #6 and #90, and 1826M #27: HLA-DPB1*03:01, but not HLA-DPB1*02:01 and DPB1*04:01, presented SVMK peptide to TCR-transgenic T cells, which suggests that HLA-DPB1*14:01 and DPB1*20:01 may also present the epitope due to their high homology to DPB1*03:01 (99.6% each Marsh et al., 2000). However, this finding is based on a single experiment only and thus needs further confirmation. It was previously described that HLA-DP allotypes are organised in groups that show related behaviour in transplantation, and this correlates with their amino acid similarity (Fleischhauer et al., 2012; Zino et al., 2003). Therefore, it is not unexpected that HLA-DP molecules from the same group can present epitopes to the same T cells.

The expression of 9 HLA class I-restricted TCRs specific for $UL28_{327\text{-}335}$ (FRC) or $IE\text{-}1_{309\text{-}317}$ (CRV) and 12 HLA class II-restricted TCRs specific for various epitopes of either pp65 or IE-1 was analysed. All of those TCRs except for FRC86 could be transferred into primary T cells from $HCMV^-$ healthy donors and were expressed on the cell surface (fig. 4.11-4.18 and 4.20-4.24). The TCR KYQE16 was never transferred because no HLA-DQB1*05:01$^+$ PBMC donor was available from which moDCs could be derived for subsequent testing of TCR-transgenic effector T cells. 8 out of 9 HLA class I-restricted TCRs and 11 out of 12 HLA class II-restricted TCRs recognised endogenously processed antigens that were presented by mini-LCLs, HCMV-infected fibroblasts or HCMV-infected moDCs. Thus, a panel of functional HCMV-specific TCRs was established that covers a variety of HLAs and could be further developed for clinical application (see tab. 4.2).
Based on the HLA allotypes that are covered by it, this panel allows to target HCMV by at least one TCR in large proportions of different ethnicities (see tab. 5.1). Focussing on Caucasians, the CRV- or FRC-specific TCRs that are restricted by HLA-C*07:02 could be applied in adoptive T cell therapy in a quarter of patients, and the YSE-specific TCR, which is HLA-A*01:01-restricted,

Table 5.1. HLA alleles and their phenotype frequency. Allele frequencies (AF) of HLA alleles that are relevant to the TCRs tested in the present study among Caucasians (CAU), African Americans (AFA) and Asian or Pacific Islanders (API) were gathered from Gragert et al. (2013, HLA-A, -C, -DQB & -DRB) or Hollenbach et al. (2012, HLA-DPB). Percentages of phenotype coverage were calculated by assuming distribution according to the Hardy-Weinberg equilibrium: Population coverage (i.e. probability that a specific allele is present in both or one of the two sets of chromosomes of a person) = $(1 - (1 - AF) \cdot (1 - AF))$. Population coverages are highlighted according to low (blue), medium (white) or high (red) frequency.

HLA	allele	estimated population coverage [%]		
		AFA	API	CAU
A	*01:01	9.1	13.8	29.2
C	*07:02	13.9	26.6	25.4
DPB	1*03:01			19.2
	1*14:01			2.3
	1*20:01			1.1
DQB	1*03:01	33.6	33.6	35.4
	1*05:01	27.5	15.5	21.9
DRB	1*13:01	10.5	6.3	12.0
	1*13:02	14.1	7.1	9.1
	1*15:01	5.4	15.8	24.2
	3*01:01	24.9	9.2	26.8
	3*02:02	46.8	37.9	36.2
	3*03:01	18.4	21.7	9.2
	5*01:01	26.9	22.1	24.3

to approximately 30% of patients. According to the HLA restrictions as listed in table 4.2, the HCMV-specific, HLA class II-restricted TCRs could be applied to a proportion of Caucasian patients ranging from 9% to 36% (Gragert et al., 2013, see tab. 5.1) depending on the individual restricting element. Due to restriction by different HLA classes (I and II) as well as different HLA class II isoforms (DP, DQ and DR), more than one HCMV-specific TCR may be applied to patients that harbour the respective HLA alleles: The second and third most frequent HLA haplotype in Caucasians include HLA-C*07:02 and DRB5*01:01 (Gragert et al., 2013) and thus allow for the application of CRV- and FRC-specific TCRs, as well as EFFT22. For these frequent haplotypes, a combined application of HLA class I and class II-restricted TCRs will therefore be possible. The ninth most common Caucasian HLA haplotype includes HLA-DRB1*13:02 and DRB3*03:01 (Gragert et al., 2013) which enables the usage of the TCRs QPFM17, YRIQ18, YRIQ36 and AGIL19. Moreover, HLA heterozygosity may allow for the application of several HCMV-specific TCRs at the same time.

5.2 δ2 T cells as recipients of HCMV-specific αβ TCRs

αβ TCR-transgenic γδ T cells have already been generated by others (van der Veken et al., 2009, 2006) in order to avoid the complication of mixed TCR dimers in TCR-transgenic αβ T cells (Bendle et al., 2010; van Loenen et al., 2010). These γδ T cells, however, had been extensively

purified by FACS and MACS of isolated PBMCs before the TCR genes were transferred (van der Veken et al., 2009, 2006). In contrast, the protocol presented here involves only a single step of immunomagnetic separation in order to deplete αβ TCR^+ cells (αβ T cells) from the PBMC preparation and leaves γδ T cells "untouched". This avoids any interference with the functional state of the γδ T cells due to activation of the TCR (e.g. by antibody-mediated cross-linking) or inactivation of the TCR (e.g. by inhibiting contacts of TCR and target through antibody-mediated blocking). When αβ T cells had not been removed prior to pamidronate (PAM) stimulation, some of them apparently became susceptible to retroviral transduction and thereafter expressed the transgenic TCR (fig. 4.9 & 4.15). Since no foreign antigens were present in the T cell culture except for residual trace amounts of FCS that cannot be entirely excluded, antigen-specific activation of αβ T cells in T cell cultures stimulated with PAM is unlikely. However, it is a long standing observation that a proportion of T cells will be activated *ex vivo* in the presence of cytokines even if no specific antigen is present (Chu et al., 2013; Grimm et al., 1982). This bystander activation cannot be totally avoided in standard T cell cultures and may have activated some αβ T cells, which enabled their retroviral transduction.
In effector assays that evaluated the recognition of HCMV-infected target cells by PAM-stimulated, TCR-transgenic T cell lines, it was not possible to distinguish the contribution of these simultaneously transduced αβ T cells from the effect of the desired TCR-transgenic γδ T cells. Thus, αβ T cells had to be removed from the culture prior to PAM stimulation in order to assess the capability of TCR-transgenic γδ T cells alone. In contrast to van der Veken et al. (2009, 2006), who purified total γδ T cells that encompassed all the different natural subsets (δ1, δ2, and others), PAM stimulation of αβ TCR-depleted PBMCs specifically expands $V\delta2^+$ T cells (Gober et al., 2003; Morita et al., 2007), which are the dominant γδ T cell population in peripheral blood. Out of a range of 1 to 45 µM, 10 µM of PAM showed optimal proliferation of δ2 T cells after three to seven days (fig. 4.9 A). Since susceptibility of δ2 T cells to retroviral transduction was best after three days of PAM stimulation and then gradually decreased up to seven days of PAM stimulation (fig. 4.9 D), the duration of PAM stimulation prior to retroviral transduction was limited to three days. Shorter or longer periods of PAM stimulation were not tested.

The phenotype of δ2 T cells from three donors *ex vivo* was composed of roughly 20% naïve (N), 25% central memory (CM), 40% effector memory (EM) and 15% EM re-expressing CD45RA (EMRA) T cells (fig. 4.10 B). After PAM stimulation, the large majority of δ2 T cells from the same three donors predominantly possessed either a CM or a EM phenotype, which is favourable and likely increases the efficacy of a T cell product (Busch et al., 2016). For αβ T cells, transfer of less-differentiated T cell subsets (N, CM) was highly correlated with clinical responses because these T cell subsets were associated with superior T cell engraftment, persistence, and antitumour immunity compared to T_{EM} and T_{EMRA} cells (Klebanoff et al., 2012). For δ2 T cells, it remains unknown which phenotypic subpopulation likely confers best efficacy in adoptive T cell therapy. If less-differentiated δ2 T cells are preferable, the established protocol represents an effective and fast procedure to transfer αβ TCRs into δ2 T cells with a promising potential in adoptive T cell therapy because the proportion of terminally-differentiated δ2 T cells was below 5% after three, five and seven days of PAM stimulation (fig. 4.10).

Both HLA class I- and class II-restricted, HCMV-specific TCRs were expressed on a relatively large proportion of δ2 T cells four to seven days after retroviral transduction (sections 4.3.1 & 4.4.2). TCRs specific for one of the epitopes CRV, FRC, YSE, AGIL, EFFT and YRIQ could be stained by peptide-specific HLA class I or class II multimers, which is discussed in detail in the paragraphs below. In parallel, expression of the majority of transgenic TCRs could be assessed by antibodies that are specific for a particular Vβ domain (8 out of 9 HLA class I-restricted TCRs and 7 out of 12 HLA class II-restricted TCRs). For some Vβ domains, like Vβ6, no specific antibodies can be purchased at the moment, possibly because the epitopes of such Vβ-specific antibodies are too similar to other Vβ domains. In this study, nine out of ten different Vβ-specific antibodies (see tab. 2.14) provided good quality of staining. When the quality of staining with a Vβ-specific antibody was poor (e.g. Vβ5.2) or the particular Vβ-specific antibody was not available (e.g. Vβ6), the expression of the TCR chains was assessed by staining with an antibody that binds to any αβ TCR (antibodies are listed in tab. 2.14). However, the latter staining method was only applicable to δ2 T cells because αβ T cells endogenously express another αβ TCR already.

αβ TCR-transgenic δ2 T cells offer the advantage that they can be stimulated by aminobisphosphonates in the absence of stimulation via the transgenic αβ TCR. However, this is only possible when they express the endogenous γδ TCR and the transgenic αβ TCR simultaneously, as was the case with most TCR-transgenic δ2 T cell lines after transduction with retroviruses coding for an HLA class II-restricted TCR (fig. 4.20-4.24). If HCMV-specific, TCR-transgenic δ2 T cells were applied to an $HCMV^-$ patient (prophylactic therapy) or an $HCMV^+$ patient (preemptive therapy) in the absence of HCMV infection or reactivation, these T cells could be maintained or even expanded by periodical stimulation with clinically approved aminobisphosphonates, like PAM.
Transduction with retroviruses coding for QPFM17 or EFFT22 resulted in an increased fraction of δ2 T cells that expressed the transgenic αβ TCR, but not the endogenous (Vγ9)Vδ2 TCR (fig. 4.20, 4.23 & 4.24), possibly because these two TCRs competed exceptionally well for binding to CD3. Thus, the ability to maintain and expand αβ TCR-transgenic δ2 T cell populations by PAM stimulation in the absence of HCMV infection *in vivo* may negatively correlate with the competitiveness of the particular TCR.

In αβ T cells, hazardous αβ TCR mis-pairing was reported after transfer of additional αβ TCR genes into these cells: virus-specific T cell lines that had been transduced with an αβ TCR showed neo-reactivity directed against allo-, but also auto-HLA which was sometimes as robust as reactivity via the introduced or endogenous TCR against peptide pulsed target cells (van Loenen et al., 2010). Introduction of only TCRα or TCRβ chains also resulted in neo-reactivity. This was also observed in a mouse model, where it was found that self-reactive TCRs were formed by introduced and endogenous TCR chains in transgenic T cells causing lethal autoimmunity (Bendle et al., 2010). In contrast to αβ T cells, potentially harmful αβ TCR mis-pairing is not expected to occur in γδ T cells because the αβ TCR cannot exchange chains with a γδ TCR (Koning et al., 1987; Saito et al., 1988). This makes γδ T cells a safer alternative to αβ T cells. Employing δ2 T cells in particular may even be safer than using total γδ T cells because the endogenous specificity of δ2 T cells is directed towards IPP, HMB-PP and related antigens (re-

viewed in Morita et al., 2007) and δ2 T cells have never been described to recognise a totally different antigen. Also, the repertoire of Vγ9Vδ2 T cells is semi-invariant due to conserved TCR usage (reviewed in Willcox et al., 2018). Thus, δ2 T cells may be considered a mono-specific population, which is further supported by the observation that combining Vγ9 and Vδ2 chains from different TCRs with each other did not alter their specificity, but even increased reactivity of TCR-transgenic T cells (Gründer et al., 2012). In contrast, δ1 and δ3 T cells contain T cell populations of different specificities and may thus be associated with unwanted off-target effects once they are activated by the transgenic TCR.

Almost no unspecific reactivity and in particular no alloreactivity of TCR-transgenic, HLA class II-restricted δ2 T cells from three different donors was observed in this study after testing them against allogeneic target cells from five different donors in 34 different combinations of the three factors TCR, T cell donor and moDC donor (fig. 4.29 & 4.30). In comparison, most TCR-transgenic, HLA class II-restricted αβ T cells showed increased unspecific reactivity in response to allogeneic control target cells. Occasionally, HLA class II-restricted, TCR-transgenic αβ T cells even showed strong reactivity to target cells in comparison to equivalent δ2 T cells in the same allogeneic setting (fig. 4.28). Strong reactivity of TCR-transgenic αβ T cells to allogeneic control moDCs was probably due to alloreactivity and could not have been observed if autologous target cells were used. Alloreactive T cell clones were shown to recognise a single peptide/allo-HLA complex that was unique for each clone (Amir et al., 2011), so alloreactivity in the present study was likely due to a minority of the TCR-transgenic αβ T cells that recognised a "foreign" HLA loaded with a "self" peptide. This is highly relevant in an allogeneic transplant situation because the recipient may develop GvHD (HSCT) or graft rejection (SOT) if alloreactive T cells are transferred. Since δ2 T cells are not considered to be alloreactive, they harbour a safety advantage in an allogeneic setting, which probably is why others use them as well (reviewed in Fisher and Anderson, 2018; Pauza et al., 2018). δ2 T cells also have important functions of relevance in virus control, such as direct killing of target cells and production of antiviral cytokines (reviewed in Fisher and Anderson, 2018; Pauza et al., 2018; Zheng et al., 2013). However, they may not have a functional repertoire comparable to αβ T cells and may not be able to produce all T_H cytokines.

5.3 CRV- and FRC-specific TCRs restricted by HLA-C*07:02

The expression of CRV- and FRC-specific TCRs after transfer of their genes into αβ or δ2 T cells was assessed by HLA-C*07:02 streptamers. All four CRV-specific TCRs and 4 out of 5 FRC-specific TCRs were well expressed on αβ T cells (fig. 4.16 A & 4.18 A). The TCR CRV2 was also well expressed on δ2 T cells in the absence of CD8 (fig. 4.14). While *ex vivo* staining of CRV-specific $CD8^+$ T cells by CRV/HLA-C*07:02 streptamers has already been reported (Ameres et al., 2013; Schlott et al., 2018), the expression of FRC-specific TCRs has not been analysed so far by peptide/HLA multimer analysis in the published literature. However, analyses of this group showed that FRC multimer staining works well and reliably (X. Liang and A. Huth, unpublished results).

HLA-C*07:02 is a promising HLA restriction to employ for T cell therapy of HCMV infection

because CD8$^+$ T cell clones that are restricted by this HLA allotype were able to strongly recognise HCMV-infected fibroblasts despite the presence of immune evasive proteins (Ameres et al., 2014, 2013). HCMV-specific CD8$^+$ T cell clones that were restricted by various HLA-A or -B allotypes were not able to elicit a comparable response in the presence of the four immunoevasins US2, US3, US6 and US11. Even one of the immunoevasins US2 and US11 was potent enough to strongly down-regulate HLA-A*02:01 and other HLA-A molecules on the surface of fibroblasts and thereby evade T cell recognition (Ameres et al., 2014). The mechanism behind the down-regulation of HLA surface expression is the dislocation of HLA class I α chains from the ER to the cytosol, which is driven by US2 and US11, and their subsequent proteasomal degradation (Jones and Sun, 1997; Wiertz et al., 1996). US2 and US11 were shown to directly interact with the α2, α3 and cytoplasmic domains of HLA class I α chains (Barel et al., 2003a,b; Besold and Plachter, 2008). These observations and the fact that these domains of HLA class I α chains are polymorphic explain the allele specificity of HLA class I down-regulation by US2 and US11 (Barel et al., 2003a,b; Schust et al., 1999, 1998). HLA-C was predicted (Barel et al., 2003a,b) and shown to be uniquely resistant to the effects of both US11 and US2 (Schust et al., 1998). The functional relevance of HLA-C for T cell recognition of HCMV infection was shown by Ameres et al. (2014) who confirmed the resistance of HLA-C*07:02 to US2 and US11. However, it remains unclear whether other HLA-C alleles are similarly protected from US2 and US11.
Recognition of AD169-infected fibroblasts by CRV-specific, HLA-C*07:02-restricted CD8$^+$ T cells was reproduced using the two T cell clones 1010M #7 and F46M #36 (fig. 4.16 B II) thereby confirming previous reports from this group (Ameres et al., 2014, 2013). In the present study, both T cell clones were also shown to strongly recognise fibroblasts that had been infected with HCMV WT strain Merlin (fig. 4.16 B II).

Each of the four CRV-specific and the four FRC-specific TCRs that were analysed in the present study mediated strong recognition of peptide-loaded and CMV-Δall-infected fibroblasts, despite low proportions of TCR-transgenic T cells among some of the T cell lines (fig. 4.16 & 4.18). However, neither TCR-transgenic T cells that expressed a CRV-specific TCR, nor those that expressed a FRC-specific TCR recognised fibroblasts infected with AD169 or Merlin. In contrast, fibroblasts infected with CMV-Δall, which is derived from AD169, but lacks the four immunoevasins US2, US3, US6 and US11 (Besold et al., 2009), were strongly recognised by both TCR-transgenic T cells and CRV-specific CD8$^+$ T cell clones. Even T cell lines that were engineered to express the TCR isolated from the CRV-specific T cell clone F46M #36 (i.e. CRV3), which is highly sensitive to infection with WT HCMV, or 1013M #169 (i.e. CRV4) did not recognise fibroblasts infected with AD169 or Merlin.
This insensitivity of HCMV-specific, HLA-C*07:02-restricted TCRs towards WT strains of HCMV was unexpected. Thus, several properties of TCR-transgenic T cells and T cell clones were analysed in order to identify the reason for this deficiency. The intensity of TCR expression on CRV-specific T cell clones as well as their proportion of CRV streptamer$^+$ T cells was comparable to that of TCR-transgenic T cells expressing CRV2 or its murinised variant CRV2mu, as assessed by FACS analysis (fig. 4.17). Thus, TCR expression intensity and the proportion of TCR-expressing T cells were considered unlikely to be the causes. Separation of CD8$^+$ TCR-transgenic T cells in order to eliminate possible TCR-transgenic CD4$^+$ T_{reg} cells and their

subsequent co-cultivation with AD169-infected fibroblasts did not enhance recognition of HCMV infection (data not shown). Moreover, the levels of the anti-inflammatory cytokine IL-10 in the supernatant of these co-cultures were generally low and not elevated due to AD169 infection. The phenotype of TCR-transgenic T cells (≈75% T_{EM} and ≈18% T_{CM}) resembled that of the CRV-specific T cell clones 1010M #7 and F46M #36 (>97% T_{EM}), so an aberrant differentiation of TCR-transgenic T cells was also unlikely to cause the insensitivity of TCR-transgenic T cells towards target cells infected with WT HCMV.

The hypothesis remained that HCMV-specific, HLA-C*07:02-restricted T cell clones may be selected into the memory repertoire *in vivo* depending on an unknown property that confers enhanced sensitivity for HCMV-infected cells. CRV-specific $CD8^+$ T cell clones, and the *in vivo* T cells from which they were derived, may express activating receptors that enable the T cells to overcome the effects of the immunoevasins, but that are not present on most other peripheral T cells. Alternatively, the functional deficiency of TCR-transgenic T cells might be caused by expression of inhibitory receptors that are triggered by AD169-infected fibroblasts, but are not present on CRV-specific $CD8^+$ T cell clones. However, it is not obvious why AD169, but not AD169-derived CMV-Δall, would be able to engage this mechanism.
In order to test both hypotheses, the expression of the activating receptor NKG2D and the inhibitory receptors NKG2A and KIR2DL2/3 on TCR-transgenic T cells and CRV-specific $CD8^+$ T cell clones was assessed. Both types of T cells neither expressed NKG2D (MFI ≤1,000 AU), nor NKG2A (<0.3%). KIR2DL2/3 (CD158b) was not expressed by TCR-transgenic T cell lines and T cell clone 1010M #7 CRV (≤0.3%), but 2.8% of the cells of T cell clone F46M #36 CRV expressed KIR2DL2/3. However, this small fraction of $CD158b^+$ cells did not prevent strong recognition of AD169-infected fibroblasts by the T cell clone F46M #36 CRV (fig. 4.16 B `II`).
Since these analyses only covered three receptors out of many that could modulate T cell functionality (reviewed in Hilton and Parham, 2017; Parham, 2005; Parham and Moffett, 2013), the expression patterns of other inhibitory or activating receptors, such as LILRB1 and several KIRs, remain to be evaluated in order to elucidate the deficiency of TCR-transgenic T cells or the advantage of CRV-specific $CD8^+$ T cell clones. If any one receptor or other molecule could be identified as the root cause, this would greatly help assessing whether the generation of TCR-engineered T cells for adoptive T cell therapy can be optimised in a way to take advantage of these important HLA-C-restricted TCR specificities.

A lack of natural regulation of transgenic TCRs may also impair sensitivity towards WT strains of HCMV. TCR internalisation, resulting in transiently reduced TCR surface expression, usually limits the intensity and duration of signalling as a result of natural TCR engagement (Itoh et al., 1999; Valitutti et al., 1995). The retroviral vector MP71 that was used for delivery of TCR genes in the present study yields stable, high-level transgene expression in T lymphocytes (Engels et al., 2003) and may thus prevent effective limitation of TCR signalling. Although it may seem counter-intuitive, down-regulation of TCR expression may be necessary for an optimal T cell response, as indicated by a study of Eyquem et al. (2017), who showed that targeting gene transfer of a chimeric antigen receptor (CAR) to the *TRAC* locus enhances tumour rejection in comparison to "random" retroviral gene insertion. Therefore, placing the transgenic HCMV-specific TCR

under the regulation of a natural TCR promoter may rescue sensitivity of TCR-transgenic T cells towards target cells infected with WT strains of HCMV. However, this remains speculative and requires further investigation.

5.4 Expression of HCMV-specific HLA class II-restricted TCRs on primary T cells

The intensity of TCR expression and the proportion of TCR-expressing T cells were assessed by staining with TCR-specific antibodies and, if available, with peptide/HLA-specific multimers. The TCRs AGIL19, QPFM17, EPDV21, EFFT22, EFFT50, RRKM18, SVMK6 and SVMK27 could be well expressed on δ2 T cells and most of them also on αβ T cells (fig. 4.20, 4.23 & 4.24 and summary provided in tab. 4.3). In contrast, EIMA236, YRIQ18 and YRIQ36 were expressed on a comparatively small population of δ2 T cells (fig. 4.21 & 4.22).
No Vβ6 (TRBV7)-specific antibody was commercially available for staining the variable domain of the TCRβ chains of YRIQ18, YRIQ36, RRKM18 and EFFT50. Instead, specific peptide/HLA class II tetramers and an antibody that binds to any αβ TCR were used. However, three out of six peptide/HLA tetramers either failed to refold in presence of peptide (KRKM/HLA-DRB3*02:02) or refolded only sub-optimally (QPFM/HLA-DRB1*13:02 and MSIY/HLA-DRB1*15:01) during production by the manufacturer Immunaware (Copenhagen, Denmark), as described in sections 2.8 and 4.4.2. HLA-DRB3*02:02 monomers were deliberately refolded in the presence of KRKM and not RRKM peptide, because the TCR RRKM18 and its parental T cell clone F65M #18 showed superior sensitivity for KRKM (fig. 4.31 A). The reason for the difficulties with refolding HLA monomers is not entirely clear, but may be attributed to a wrong allocation of epitope and/or HLA restriction in at least one case (MSIY/HLA-DRB1*15:01), since neither could be precisely determined for the TCR MSIY40. As a result of the erroneous HLA monomer refolding, KRKM/HLA-DRB3*02:02 was not delivered, QPFM/HLA-DRB1*13:02 failed to stain $CD4^+$ T cell clone F46P #17 QPFM and MSIY/HLA-DRB1*15:01 failed to stain MSIY40-transgenic T cells (data not shown).
Of the three HLA class II tetramers whose monomers had refolded well, EFFT/HLA-DRB5*01:01 and AGIL/HLA-DRB3*03:01 provided stainings of high quality (fig. 4.24). Similar stainings of respective *ex vivo* T cell populations were reported by Braendstrup et al. (2013, 2014) for the epitope IE-$1_{211\text{-}225}$ restricted by HLA-DRB5*01:01. Their IE-$1_{211\text{-}225}$/HLA-DRB5*01:01 tetramers likely stained T cells that are specific for IE-$1_{213\text{-}225}$ (EFFT) as presented here because the latter was identified as the minimal epitope (Ameres et al., 2015). $CD4^+$ T cells specific for $pp65_{489\text{-}503}$ (AGIL) have also been stained *ex vivo* with tetramers previously (Pachnio et al., 2016), but these employed HLA-DRB3*02:02 instead of DRB3*03:01 as used here. In accordance with experiments that assessed the HLA restriction of the T cell clone F46P #45 (fig. 4.7 C), this supports the hypothesis that AGIL may be presented by HLA-DRB3*02:02 as well as DRB3*03:01 and may be recognised on both HLA allotypes by AGIL-specific T cell clones including F46P #19 and #45. These previously reported multimer stainings (Braendstrup et al., 2013, 2014; Pachnio et al., 2016) demonstrated the presence of particular HCMV-specific T cell populations *ex vivo*, but in the work presented here the functional expression of such TCRs after gene transfer into primary T cells is shown for the first time.
In contrast to the high quality stainings with EFFT- or AGIL-specific HLA tetramers, few cells

of a YRIQ18-transgenic αβ or δ2 T cell line stained positive for YRIQ/HLA-DRB1*13:02 tetramer (fig. 4.22). However, due to the lack of a suitable negative control, the quality of this tetramer staining and the proportion of stained cells cannot be properly judged and require further investigation. Staining with an αβ TCR-specific antibody confirmed the small proportions of δ2 T cells that expressed either YRIQ18 or YRIQ36. Thus, expression of those two TCRs was poor in comparison to most other HLA class II-restricted TCRs, possibly because YRIQ18 and YRIQ36 poorly compete with the endogenous γδ TCR for binding of CD3, which is an important prerequisite for strong surface expression of a transgenic TCR (Ahmadi et al., 2011).
In summary, multiple HCMV-specific TCRs derived from $CD4^+$ T cell clones could be transgenically expressed in primary T cells for the first time. HLA class II multimer stainings were successfully used for a subset of epitopes only, but this is expected because HLA class II multimers remain a reagent that is difficult to produce and use (Braendstrup et al., 2013, 2014).

5.5 Recognition of HCMV-infected target cells through HLA class II-restricted TCRs

DCs represent an important target for HCMV-specific $CD4^+$ T cells in order to control HCMV reactivation (Jackson et al., 2017), since the latency reservoir of HCMV is formed by $CD34^+$ hematopoietic progenitor cells (HPCs) and cells of the myeloid lineage (Kondo et al., 1994; Sinclair and Sissons, 2006; Sindre et al., 1996). DCs are professional antigen-presenting cells that possess the unique ability to prime naïve T cells, but are also able to reactivate memory T cells. They are a natural interaction partner of HLA class II-restricted T cells because of their central role in initiating immune responses and their constitutive expression of HLA class II complexes. Since monocyte-derived dendritic cells (moDCs) can be differentiated from peripheral blood monocytes by stimulation with IL4 and GM-CSF and they can be productively infected with HCMV (Moutaftsi et al., 2002; Riegler et al., 2000; Sénéchal et al., 2004), moDCs were chosen as target cells to evaluate the recognition of HCMV infection through HLA class II-restricted TCRs. *In vitro* differentiated moDCs exhibit an interstitial/dermal DC phenotype (Duperrier et al., 2000; Grassi et al., 1998) and thus closely mimic an *in vivo* DC population. More recent protocols of *in vitro* moDC differentiation include the additional use of synthetic TLR agonists (Lichtenegger et al., 2012; Spranger et al., 2010) that mimic natural conditions, in which danger signals are delivered by infection (Matzinger, 1994). But since HCMV infection probably delivers its own danger signals to the infected cells, such as viral DNA that may activate TLR9, this was not considered necessary here.
Fibroblasts that were used as target cells to test HLA class I-restricted TCRs are no adequate target cells for testing HLA class II-restricted TCRs, because fibroblasts usually do not express HLA class II, although stimulation with IFN-γ can promote HLA class II expression to some extent (Pober et al., 1983; Stevanovic et al., 2013).

Contradictory data was reported on the susceptibility of moDCs to HCMV infection owing to different maturation status of the DCs and differently propagated HCMV virus strains. However, Sénéchal et al. (2004) were able to draw a conclusive summary: immature moDCs were most susceptible to HCMV propagated on endothelial cells (35-70% infected DCs at MOI = 1),

while infection by HCMV propagated on fibroblasts was very inefficient. In contrast, mature DCs were efficiently infected by both types of HCMV preparations, but at a lower efficiency (15-30% infected DCs at MOI = 1) than immature moDCs. Some of these observations were confirmed by others as well: TB40/E propagated on human foreskin fibroblasts better infected immature than mature moDCs (Moutaftsi et al., 2002); moDCs were fully permissive for HCMV infection when endothelial cell-adapted HCMV strains were applied, irrespectively of their stage of maturation, but viral replication was abrogated at the level of IE and/or E gene expression when fibroblast-adapted strains were used (Beck et al., 2003); the infection rate of immature moDCs was negligible with fibroblast-adapted HCMV strains, but 80-90% were susceptible to HCMV strains that had been propagated in endothelial cell culture (Riegler et al., 2000).
Since the HCMV virus preparations that were used in the present study had exclusively been propagated on fibroblasts, moDCs were matured prior to infection with HCMV at an MOI of 5 to achieve a sufficient population of HCMV-infected moDCs.

HCMV infection decreases the expression of co-stimulatory molecules, like CD40, CD80 and CD86, and of HLA class I and class II complexes on DCs (Grigoleit et al., 2002; Hertel et al., 2003; Moutaftsi et al., 2002). CD86 and HLA class II molecules, however, may be less affected than CD40, CD80 and HLA class I molecules (Beck et al., 2003; Moutaftsi et al., 2002). Sénéchal et al. (2004) observed a decreased expression of CD80, CD86, HLA class I and class II on immature moDCs after HCMV infection, too, but the latter had comparatively little effect on mature moDCs. HCMV was also shown to decrease the expression of various adhesion molecules, such as CD11c, CD33, CD54, CD58 (Beck et al., 2003) and ICAM-1 (Hertel et al., 2003), on infected DCs.
Probably as a result of the down-regulation of co-stimulatory, adhesion, and HLA molecules, the stimulatory activity of DCs to autologous (Moutaftsi et al., 2002; Raftery et al., 2001) and allogeneic T cells (Grigoleit et al., 2002; Hertel et al., 2003; Sénéchal et al., 2004) was impaired by HCMV infection. CD83, which is an important marker molecule for mature DCs and provides co-stimulation to T cells (Aerts-Toegaert et al., 2007), may play a special role in inhibition of the immunostimulatory activity of HCMV-infected DCs: the expression of CD83 was strongly decreased on HCMV-infected, mature moDCs, while cell-free supernatants from these cells contained a significantly increased amount of soluble CD83 in comparison to uninfected, mature moDCs (Sénéchal et al., 2004). Decreased expression of CD83 on HCMV-infected, mature DCs was also reported by Hertel et al. (2003). The inhibitory effect of soluble CD83 on DC stimulatory activity could be abrogated by immunodepletion of CD83 from the supernatant of HCMV-infected moDCs (Sénéchal et al., 2004).
In accordance with observations of reduced immunostimulatory capacity of infected DC cultures, the immunoevasins US2 and US3 of HCMV were reported to target the HLA class II molecules for degradation (Hegde et al., 2002; Tomazin et al., 1999) in a similar way than HLA class I molecules are affected (Hegde and Johnson, 2003). These studies were conducted using the astrocytoma/glioblastoma cell line U373-MG that was induced to express HLA class II molecules either by IFN-γ or by transfection with the class II transactivator gene (CIITA). However, the previously reported effect of US2 on HLA class II DRα could not be confirmed in moDCs that naturally express HLA class II molecules (Rehm et al., 2002). These contradictory results may

be caused by discrepancies in delivering the viral immunoevasins and enforcing their expression, as well as the difference in cell types. However, it remains somewhat surprising that US2 was reported to cause degradation of both HLA-DRα and DMα (Tomazin et al., 1999), which share only 27% and 24% amino acid homology with HLA-A, respectively, while HLA-C and HLA-G that share about 80% of their sequences with HLA-A (Tomazin et al., 1999) were resistant to the effects of both US2 and US11 (Schust et al., 1998).

In order to assess the effect of the immunoevasins US2, US3, US6 and US11 on peptide presentation via HLA class II, the AD169-derived virus strain CMV-Δall, which is devoid of these four immunoevasins (Besold et al., 2009), was used to infect moDCs. HCMV-specific, HLA class II-restricted T cell lines mainly produced a comparable amount of IFN-γ in response to moDCs that were infected with AD169 or CMV-Δall. YRIQ-, QPFM- and AGIL-specific TCRs mediated a slightly stronger recognition of moDCs infected with CMV-Δall than moDCs infected with AD169 (1.4-, 1.5- and 1.4-fold increased IFN-γ levels, fig. 4.29). However, neither RRKM- nor SVMK-specific TCRs elicited higher IFN-γ levels in response to infection with CMV-Δall when compared with AD169 (fig. 4.30). The sensitivity of EFFT-specific TCRs was not tested against CMV-Δall. This difference in sensitivity of pp65- and IE-1-specific T cell lines for infection with CMV-Δall may not be solely explained by the lack of the four immunoevasins, but may also be caused by a high content of pp65 in virus preparations (Varnum et al., 2004). If virus preparations of the strain CMV-Δall carried more non-infectious enveloped particles (NIEPs) and dense bodies than preparations of AD169, this would increase the sensitivity of pp65-specific, but not IE-1-specific, T cells towards CMV-Δall-infected moDCs. Dense bodies are virus-like structures lacking viral DNA that are readily taken up by antigen-presenting cells, followed by processing and presentation of their antigenic material (Pepperl et al., 2000; Pepperl-Klindworth et al., 2003).
In summary, moDCs infected with CMV-Δall were not recognised more strongly than moDCs that were infected with AD169 by HCMV-specific, TCR-transgenic T cell lines. Thus, the immunoevasins US2, US3, US6 and US11 may have a minor or no effect on peptide presentation via HLA class II in mature moDCs.

The functionality of the HCMV-specific, HLA class II-restricted TCRs AGIL19, EPDV21, EFFT22 and RRKM18 was independent of CD4 co-expression, since HCMV-infected moDCs were recognised similarly well by TCR-transgenic δ2 T cells irrespective of CD4 co-transduction (fig. 4.32). The dependency of SVMK-specific TCRs on CD4 co-expression was not evaluated in the same way by CD4 co-transduction, but δ2 T cells that were only transduced with these TCRs mediated strong recognition of HCMV-infected moDCs (fig. 4.28). This indicates that the two SVMK-specific TCRs work well in the absence of CD4 co-receptor. All YRIQ-specific TCR-transgenic T cell lines, whose function was assessed in this study, had been co-transduced with CD4, so the dependency of YRIQ18 and YRIQ36 on co-expression of CD4 could not be evaluated. Regarding the TCR QPFM17, in two of three experiments only T cells co-transduced with TCR and CD4 were tested, resulting in good recognition of infected cells, but similar and even stronger recognition was achieved in a third experiment with αβ T cells and δ2 T cells, respectively, transduced with TCR but not CD4 (fig. 4.29). Thus, CD4 co-expression appeared

not necessary for this TCR either.
In summary, there was no evidence that recognition of HCMV-infected moDCs by HLA class II-restricted TCRs depended on expression of CD4. This finding is in accordance with a study by van der Veken et al. (2006) who assessed the effector functions of γδ T cells that expressed an HLA class II-restricted TCR specific for the minor histocompatibility antigen HA-2 in the presence or absence of CD4 co-expression. They found that CD4 co-transduction did not increase IFN-γ secretion by TCR-transgenic γδ T cells, but antigen-specific lysis was approximately doubled when CD4 was co-expressed. Whether the HCMV-specific, HLA class II-restricted TCRs presented here rely on CD4 co-expression for enhancement of their cytotoxic efficacy remains to be elucidated.
Murine αβ T cells were reported to be able to detect even a single peptide/MHC class II complex on the surface of an antigen-presenting cell (Irvine et al., 2002). This sensitivity highly depends on the co-receptor CD4 (Irvine et al., 2002; Krogsgaard et al., 2005), which enhances the responsiveness of $CD4^+$ T_H cells by recruiting the tyrosine kinase Lck (Li et al., 2004). However, the recognition of professional APCs, like DCs, may be independent of CD4 expression if sufficient co-stimulation is provided through other molecules, like CD28, which may compensate for a lack of CD4. In δ2 T cells, the activating receptor NKG2D may also help overcoming the activation threshold in recognition of HCMV-infected DCs. At least in $CD8^+$ T cells, NKG2D can have an important co-stimulatory function (Rajasekaran et al., 2010).

10 out of 13 HLA class II-restricted TCRs specific for pp65 or IE-1 that were tested within the present study conveyed sensitivity for HCMV-infected moDCs to TCR-transgenic T cells (fig. 4.29 & 4.30). The recognition of HCMV-infected moDCs mediated by the remaining three TCRs was not assessed due to a lack of HLA-matched moDCs (KYQE16), a lack of mini-LCL recognition (EIMA236) or unresolved HLA restriction (MSIY40).
pp65-specific TCRs that are restricted by HLA class II generally evoked strong IFN-γ secretion by TCR-transgenic T cells regardless of the T cell type that received the TCR genes (αβ vs. δ2, fig. 4.29). In contrast, the amount of secreted IFN-γ by TCR-transgenic T cells specific for IE-1 was often more modest (fig. 4.30). This does not necessarily imply that IE-1-specific TCRs were less efficient than pp65-specific TCRs. pp65 is contained in large quantities in HCMV virions but very little, if any, IE-1 protein (Büscher et al., 2015; Reyda et al., 2014). Moreover, *in vitro* propagation of AD169 generates NIEPs in amounts nearly equivalent to virions (Gibson and Irmiere, 1984) and dense bodies, which are mainly composed of pp65 (>60%, Varnum et al., 2004). Thus, HCMV stock preparations deliver a large amount of pp65 but little IE-1 into infected cells. IE-1 protein is among the first to be expressed after infection and its production peaks at three days p.i. in moDCs (Fish et al., 1995). Nevertheless, at two days p.i., when the recognition of HCMV-infected moDCs by HCMV-specific T cell lines was tested, much higher levels of pp65 protein than IE-1 protein were likely available to HCMV-treated moDCs for processing and presentation, which would explain the difference in IFN-γ secretion between pp65- and IE-1-specific T cells.

Proteins of intracellular and extracellular origin both have a role to play in control of infection. In most infections, the task of DCs is to take up exogenous pathogenic material in order

to prime naïve T cells and activate memory T cells. In the special situation of HCMV, DCs can be infected themselves and are thus part of the viral replication cycle (Gerna et al., 2005; Jahn et al., 1999; Sénéchal et al., 2004). Therefore, it becomes instrumental to distinguish two situations: recognition of DCs that have taken up exogenous viral material, and recognition of DCs that have themselves become infected and intracellularly produce viral antigen.
This distinction is difficult to perform, but different tools are available. Those may include biochemical inhibitors of transcription and translation, such as actinomycin D or cycloheximide. Recently, both compounds were applied in a sequential manner, which allows expression of viral genes whose transcription is independent of *de novo* synthesis of viral proteins, in order to study T cell recognition of early viral products (Hesse et al., 2013). But these compounds have severe disadvantages as they strongly interfere with many biochemical processes of antigen-presenting cells. In the experimental HCMV system, it is hard or impossible to achieve endogenous viral gene expression after infection without co-transfer of protein during infection. Even if dense bodies would be separated from functional virus by density centrifugation (Irmiere and Gibson, 1983; Talbot and Almeida, 1977), a large amount of viral protein would enter the cell as a component of infectious virions. Therefore, the method chosen here was a simple but effective one: to inactivate HCMV particles by UV irradiation, which introduces damage to viral DNA abolishing gene expression from the viral genome. This method has often been used to distinguish the effects of intact virions or UV-inactivated virions on target cells and subsequent T cell recognition (Frascaroli et al., 2018; Jackson et al., 2017; Moutaftsi et al., 2002; Sénéchal et al., 2004).
Although peptides that are presented on HLA class II complexes are generally believed to originate from exogenous sources, peptides derived from endogenous proteins can also be presented efficiently on HLA class II complexes (reviewed in Crotzer and Blum, 2010). Thus, viral proteins that are translated in an infected cell and viral proteins from exogenous sources both contribute to the abundance of viral peptides for presentation by HLA class II complexes.

The active/inactive virus recognition ratio (abbreviated as A/I ratio, see section 4.4.5.6) was calculated to provide an overview whether HCMV-specific, TCR-transgenic T cells preferentially responded to HCMV-infected moDCs or to scavenging moDCs, which had acquired viral proteins through ingestion of extracellular material, but were not functionally infected with HCMV. These calculations showed that pp65-specific, HLA class II-restricted TCRs had similar sensitivity for HCMV-infected moDCs and for moDCs that were treated with UV-inactivated virus (A/I ratio ≈ 1, fig. 4.34). In contrast, IE-1-specific, HLA class II-restricted TCRs reacted more strongly in the active than the inactive situation (A/I ratio ≥ 2). This observation was highly significant for infection with AD169 ($P<0.004$, fig. 4.34 A & B) and also significant for infection with TB40-BAC4 ($P<0.05$, fig. 4.34 C & D) – in both cases regardless of the type of TCR-transgenic T cells ($\alpha\beta$ vs. $\delta 2$). Thus, pp65-specific, HLA class II-restricted TCRs mediate recognition of antigen-expressing target cells irrespective of the antigen source, whereas IE-1-specific, HLA class II-restricted TCRs predominantly mediate recognition of *bona fide* infected target cells.
The benefit of IE-1-specific T cells that selectively recognise true infection is that such cells will only mobilise a response against cells that carry the virus and may be capable of producing new virions. Potentially, T cells that recognise cells which have taken up ubiquitous extracellular antigen may be able to create damage by too broad and indiscriminate activation – especially in

a therapeutic situation where HCMV infection is already widespread in an organ or in the body. In case of massive viral disease, adoptive T cell therapy can be toxic due to overreaction, as an example from EBV shows (Khanna et al., 1999). Therefore, it is useful that HCMV-specific, HLA class II-restricted TCRs with different patterns of reactivity are now available to develop adoptive immunotherapy in different clinical situations.
However, it is not clear yet which T cell specificities are protective against HCMV in humans. Studies by Bunde et al. (2005) and Gratama et al. (2008) suggest that IE-1-specific but not pp65-specific $CD8^+$ T cells may protect from HCMV disease. IE-1-specific T cells have the advantage that they may be able to recognise viral reactivation from latency at an early stage. In contrast, pp65-specific T cells may be able to recognise freshly infected cells more potently because pp65 that is contained in the virion is transferred into the infected cell and may be immediately available for T cell recognition (see Hesse et al., 2013; Riddell et al., 1993, for $CD8^+$ T cells). pp65-specific T cells may also be less limited in their function as their antigen is produced at higher amounts and they may therefore mobilise a stronger response supported by antigen presentation by bystander cells. On the other hand, this will mainly happen late in the infectious cycle. In the end, these different temporal and quantitative patterns of T cell recognition may be complementary, and an optimally functional T cell response against HCMV may require a combination of T cells specific for antigens of different classes, such as IE-1 and pp65. This question remains to be answered by future investigations.

In order to correct the A/I ratios for background due to alloreactivity, such individual backgrounds were subtracted from the A and I values (see section 4.4.5.6, equation 3). As expected, the A/I ratios of TCR-transgenic δ2 T cell lines were little affected by the correction (fig. 4.33) because their background reactivity was very low (fig. 4.29 & 4.30). In contrast, TCR-transgenic αβ T cell lines were more strongly affected by the correction since their background reactivity was slightly increased in general and occasionally even strongly increased. The A/I ratios of IE-1-specific αβ T cell lines were more strongly affected by the correction than the ratios of pp65-specific αβ T cell lines (fig. 4.33) usually because the amounts of cytokine secreted in response to pp65 were higher than those in response to IE-1, as discussed above.

5.6 Development of HCMV-specific TCRs for adoptive T cell therapy

At present, the majority of clinical trials in adoptive T cell therapy focusses on generating HCMV-specific T cells from HCMV-positive donors, who provide an established repertoire of virus-specific memory T cells (https://clinicaltrials.gov, identifier: NCT02227641, NCT01325636 and NCT02985775, accessed on 07.05.19). However, about half of the population of industrialised countries is $HCMV^-$ (reviewed in Cannon et al., 2010), so there is a profound need for clinically feasible protocols to generate HCMV-specific T cells from $HCMV^-$ donors. This can be achieved by priming naïve T cells *in vitro* (Hanley et al., 2009, 2015; Jedema et al., 2011), but the protocols are exceedingly time-consuming and complicated and the resulting T cells have unusual properties and unclear efficacy. Alternatively, $HCMV^-$ HSCT donors or SOT recipients may be vaccinated to generate HCMV-specific T cell immunity, but the antigen components that would provide protective immunity remain unknown (reviewed in Diamond et al., 2018).

Moreover, no vaccine has yet been licensed (Schleiss, 2016), although numerous clinical trials are ongoing (reviewed in Diamond et al., 2018).
Thus, the transfer of genes of HCMV-specific TCRs emerges as the most reliable method to obtain HCMV-specific T cells from HCMV$^-$ donors in a timely, qualitatively and quantitatively appropriate fashion. Moreover, TCR gene transfer may be used to generate HCMV-specific T cells of defined specificity from an HCMV$^+$ HSCT donor or SOT recipient if the existing T cells do not suffice to control HCMV infection. At the moment, only one registered clinical trial (NCT02988258, accessed on 07.05.19) employs gene transfer of a pp65-specific, HLA-A*02:01-restricted TCR into T cells from HCMV$^-$ donors. However, HLA-A*02:01 is particularly susceptible to the effects of HCMV's immunoevasins (Ameres et al., 2014, 2013) and pp65-specific CD8$^+$ T cells may not be able to confer protection from HCMV disease (Bunde et al., 2005; Gratama et al., 2008), as described earlier. Thus, TCRs that may confer protection from HCMV infection and reactivation need to be identified and thoroughly characterised in order to enable the generation of efficacious HCMV-specific T cells.

In this study, the expression and function of 8 HLA class I-restricted TCRs and 10 HLA class II-restricted TCRs specific for one of the HCMV antigens pp65, IE-1 or UL28 were successfully established. Among the CRV (IE-$1_{309\text{-}317}$)-specific TCRs, CRV2 was outstanding because its expression was high even in CD8$^-$ cells (fig. 4.16 A) and its affinity for CRV streptamers was comparable to that of a CRV-specific T cell clone (fig. 4.17). Of the FRC ($UL28_{327\text{-}335}$)-specific TCRs, FRC1 showed the largest fraction of TCR$^+$ CD8$^-$ T cells and FRC32 showed the most intense FRC streptamer staining (fig. 4.18 A). All of the HLA-C*07:02-restricted TCRs either specific for the epitope CRV or FRC conveyed sensitivity for target cells infected with the virus strain CMV-Δall, but not AD169 or Merlin (fig. 4.16 B & 4.18 B). For an efficacious application by transfer of TCR genes in adoptive T cell therapy against HCMV infection, this lack of sensitivity needs to be further investigated and the function of the T cells needs to be improved.

Among the HLA class II-restricted TCRs specific for either pp65 or IE-1, some TCRs showed extraordinary properties that may be useful in adoptive T cell therapy of HCMV infection. δ2 T cells that were transduced with retroviruses coding for one of the two TCRs QPFM17 or EFFT22 showed a relatively high proportion of Vδ2$^-$ $\alpha\beta$ TCR$^+$ cells (fig. 4.20 & 4.23) in comparison to all other HLA class II-restricted TCRs tested in this study. This may indicate that QPFM17 and EFFT22 efficiently competed for CD3, which is limiting for the expression of a transgenic TCR and the conveyed antigen-specific T cell function (Ahmadi et al., 2011). In contrast to other HLA class II-restricted TCRs that presumably compete less successfully for CD3 complexes, QPFM17 and EFFT22 are well expressed on transgenic T cells and thus favourable TCRs for application in adoptive T cell therapy.

Of all HLA class II-restricted TCRs specific for either pp65 or IE-1, AGIL19 mediated the strongest IFN-γ secretion by both TCR-transgenic $\alpha\beta$ and δ2 T cells in response to AD169-treated moDCs (fig. 4.29 & 4.30). This may partly be caused by the high amount of pp65 in AD169 propagated on fibroblasts (Gibson and Irmiere, 1984; Varnum et al., 2004). But since other pp65-specific TCRs did not mediate comparable IFN-γ secretion by TCR-transgenic T cells

in response to AD169, AGIL19 may be an especially sensitive TCR possibly due to enhanced affinity. High competitiveness for binding of CD3 unlikely explains the increased sensitivity of AGIL19 because QPFM17 which apparently competed well for binding of CD3 (described in the previous paragraph) did not confer an increased sensitivity for HCMV infection. Alternatively, AGIL peptides may be more abundantly presented by HCMV-infected moDCs than other peptides, which could explain the increased sensitivity of AGIL19. Differences in peptide processing and peptide/HLA stability may influence peptide abundance, but were too difficult to measure at this point.
In addition to its high sensitivity for pp65, the HLA restriction of AGIL19 (DRB3*02:02 and DRB3*03:01) implies an excellent coverage of various ethnicities (36-47% for DRB3*02:02 and 9-18% for DRB3*03:01, see tab. 5.1) making AGIL19 a promising TCRs for broad application in adoptive T cell therapy.

The HLA class II-restricted TCR RRKM18 had a particular pattern of HCMV strain preference. It consistently mediated higher levels of IFN-γ secretion in response to moDCs infected with TB40-BAC4 than moDCs infected with AD169 (fig. 4.30). This was due to a higher affinity of RRKM18-transgenic T cells for the epitope variant KRKM IYMC YRNV EFF (IE-$1_{201\text{-}215}$) as expressed by HCMV strain Davis than for RRKM MYMC YRNI EFF as expressed by HCMV strains AD169, Towne, Merlin and strain W (fig. 4.31). Since RRKM18 also mediated recognition of moDCs infected with TB40-BAC4 (KRKM IYMC YRNV EFF) and moDCs pulsed with the 15mer peptide derived from HCMV strain Davis, it may also mediate recognition of moDCs infected with HCMV strain Toledo (KRKM MYMC YRNV EFF). If this could be confirmed by future analyses, RRKM18 may confer increased sensitivity for HCMV strains TB40/E, Toledo and Davis to TCR-transgenic T cells, which is a unique property among the HLA class II-restricted TCRs studied here. Consequently, particular HCMV strains that are less susceptible to detection by other HLA class II-restricted TCRs tested in this study may be preferentially targeted with the help of RRKM18. Moreover, RRKM18 is restricted by HLA-DRB3*02:02, which is present in more than a third of Caucasian, African American and Asian or Pacific Islanders (tab. 5.1), and may thus be applicable to a broad range of patients.
The impact of differences among various HCMV strains on T cell recognition of infection still remains largely unknown. There are some publications about HCMV-specific T cells that show the impact of such variants on T cell recognition: Smith et al. (2016) showed this for various $CD8^+$ T cell epitopes including IE-$1_{199\text{-}207}$ (EL RRKM MYM vs. EL KRKM IYM) and two related epitopes (IE-$1_{198\text{-}206}$ and IE-$1_{201\text{-}210}$), which interestingly overlap with the $CD4^+$ T cell epitope RRKM (IE-$1_{201\text{-}215}$) that was just discussed. They reported that some of the HCMV-specific T cell responses were strain-specific. In contrast, earlier work of this group has shown that some HCMV-specific $CD8^+$ T cells can recognise HCMV strain variants equally well (CRVL CCYV L vs. CRVL CCYI L, IE-$1_{309\text{-}317}$, see Ameres et al., 2013). Unfortunately, recognition of genetically different HCMV strains by $CD4^+$ T cells is even less clear than recognition by $CD8^+$ T cells, but this topic will hopefully be addressed by future research as genetic information about HCMV strain variation becomes available (Renzette et al., 2011, 2015).

Since HLA-DP was historically difficult to type, it is often not matched in allo-HSCT (Fleis-

chhauer et al., 2012) and thus a potential source of alloreactivity. Although HLA-DP mismatch increases the risk for mortality and severe GvHD after allo-HSCT (Fleischhauer et al., 2012; Zino et al., 2003), it may also be beneficial to kill leukemia cells by alloreactive T cells (Graft-versus-leukemia, GvL) that are restricted by HLA-DP. Therefore, HLA-DP requires special consideration in allo-HSCT.

HLA-DP-restricted T cells have been studied less frequently than DR- and DQ-restricted T cells. This work identifies the first HCMV-specific TCRs that are restricted by HLA-DP. In contrast to HLA-DR and -DQ, DP is less polymorphic and the repertoires of its α and β chains are dominated by one allele each: HLA-DPA1*01:03 and DPB1*04:01 with frequencies of 81.9% and 43.9%, respectively (Hollenbach et al., 2012, Caucasian population). Both HLA-DP-restricted TCRs that were identified and tested in this study are not restricted by the dominant allele HLA-DPB1*04:01, but by DPB1*03:01 which is the fourth most frequent HLA-DPB1 allele in Caucasians (10.1%, see Hollenbach et al., 2012).

Each of the two SVMK-specific, HLA class II-restricted TCRs conveyed a strong sensitivity for infection with AD169 or TB40-BAC4 to TCR-transgenic T cells (fig. 4.30). Among IE-1-specific TCRs, the IFN-γ levels secreted by TCR-transgenic δ2 T cells were exceptionally high. Evaluation of the function of SVMK6 and SVMK27, however, was only performed with one cell line per TCR and T cell subpopulation each, so more experiments are required to ensure the obtained data were no exception. If the strength of both SVMK-specific TCRs could be confirmed, they may yield a great potential for detecting HCMV-infected target cells that express HLA class II molecules, like professional APCs. Both TCRs are restricted by HLA-DPB1*03:01 that is present in a large proportion of Caucasians (19.2%, see tab. 5.1). Additionally, patients that harbour either DPB1*14:01 or DPB1*20:01, both of which share a high degree of homology with HLA-DPB1*03:01 (99.6% homology each, Marsh et al., 2000), may also benefit from SVMK-specific T cells (2.3% or 1.1% of Caucasians, respectively, see tab. 5.1).

Zino et al. (2003, 2007) redefined HLA-DP matching in a group-specific instead of an allele-specific manner and sorted HLA-DPB1 alleles into three groups associated with high, intermediate or low immunogenicity, as presumed on the basis of a shared alloreactive T cell epitope (TCE3, see Crocchiolo et al., 2009). The purpose of this grouping was to identify HLA-DPB1 mismatches that might be tolerated ("permissive mismatch") and those that would increase risks ("non-permissive mismatch") after allo-HSCT (Fleischhauer et al., 2012), and indeed non-permissive mismatches were associated with a significantly higher risk of adverse outcome than permissive mismatches (Crocchiolo et al., 2009; Zino et al., 2003). HLA-DPB1*03:01, DPB1*14:01 and DPB1*45:01 were allocated to the TCE3 group no. 2 with total frequencies of 14.3%, 7.5% and 6.0% in the "white", Asian and African population, respectively (Zino et al., 2003). Regardless of the apparent sequence similarity to HLA-DPB1*03:01, Zino et al. (2003) allocated DPB1*20:01 to TCE3 group no. 3, which also contained the dominant allele HLA-DPB1*04:01.

In general, HCMV-specific TCRs can be transferred into various subpopulations of T lymphocytes. As shown for such TCRs described in the present study, αβ T cells and δ2 T cells recognise HCMV infection if equipped with an HLA class I- or class II-restricted TCR. Depending on the phenotype of the receiving T cell population, the transfer of TCR genes could direct cytotoxicity of CTLs to target cells or promote T cell help to establish and maintain HCMV immunity.

As described earlier, pp65-specific, HLA class II-restricted TCRs mediated the recognition of both HCMV-infected DCs and DCs that were not infected but presented viral peptides derived from exogenous material equally well (fig. 4.34). If a pp65-specific, HLA class II-restricted TCR is transferred into $CD8^+$ CTLs, these cells will kill HCMV-infected APCs and could thus prevent viral dissemination by hematopoietic and other HLA class II-expressing cells. On the other hand, they would also kill APCs that were not infected but have taken up viral antigens from exogenous sources thereby curtailing HCMV-specific immune responses that depend on the professional presentation of viral antigens to T and B cells. Thus, pp65-specific TCRs may be best put to use by transferring them into $CD4^+$ T_H cells that will subsequently provide help to other HCMV-specific immune cells after encountering HLA class II-expressing APCs.
In contrast, IE-1-specific, HLA class II-restricted TCRs preferentially mediated recognition of HCMV-infected DCs, but not DCs that presented viral peptides derived from exogenous sources (fig. 4.34). By sparing APCs that have not been infected, but present peptides derived from HCMV antigens nonetheless, these APCs will remain available for initiating and promoting immune responses against HCMV infection and will thus strengthen HCMV immunity. However, the genes of IE-1-specific, HLA class II-restricted TCRs may also be transferred into $CD8^+$ CTLs to selectively kill HCMV-infected, HLA class II-expressing APCs, which is important to limit viral dissemination and prevent shedding of virus. Since the latency reservoir is formed by $CD34^+$ HPCs and cells of the myeloid lineage (Kondo et al., 1994; Sinclair and Sissons, 2006; Sindre et al., 1996) all of which express HLA class II molecules, reactivation of HCMV in those cells may be detected early by IE-1-specific, HLA class II-restricted T cells, which could prevent release of virus progeny.
Synergistic effects may even be obtained by combining $CD8^+$ T cells engineered to express an HCMV-specific, HLA class I-restricted TCR with $CD4^+$ T cells engineered to express an HCMV-specific, HLA class II-restricted TCR. This hypothesis is supported by a synergistic anti-tumour effect observed with $CD4^+$ and $CD8^+$ T cells that were engineered to express the same CAR in a murine leukemia model (Sommermeyer et al., 2016) and the efficient treatment of human leukemia patients with CAR-engineered T cells of a defined CD4:CD8 ratio (Turtle et al., 2016). It will be important to determine what may be an optimal composition of HCMV-specific T cells that will respond to HLA class I- and class II-expressing target cells and whether there is a combination of certain TCRs that is particularly protective.

In order to successfully control HCMV infection and reactivation in immunocompromised patients after adoptive T cell transfer, specificities that correlate with protection from HCMV disease remain to be determined. Moreover, the efficacy and toxicity profiles of αβ T cells and δ2 T cells have to be compared to decide which T cell subset is better suited for application in adoptive T cell therapy in the long run. Both questions must ultimately be answered in clinical trials.
Within the next decades, HCMV will remain a serious threat to immunocompromised individuals. A new and promising virostatic compound has been discovered (Letermovir, see Marty et al., 2017), but HCMV may develop resistances against it. On the other hand, big clinical studies have been initiated ("CORDIS": EU-funded, multi-national trial, targeting CMV, EBV and Adenovirus) to establish adoptive T cell transfer as treatment for refractory viral infection post-

HSCT. However, as previously mentioned, such trials only include HCMV$^+$ HSCT donors to date, but a solution for HCMV$^-$ HSCT donors, whose HCMV$^+$ recipients are at higher risk of viral reactivation (Ganepola et al., 2007; Ljungman et al., 2014), is urgently needed. Recombinant antigen receptor techniques have now been established in clinical application due to many studies on CD19-specific CARs and their excellent success (Chow et al., 2018; Kochenderfer et al., 2015; Schuster et al., 2017). Thus, adoptive immunotherapy using TCR-transgenic T cells will be easier to perform in the future because the production of genetically engineered T cells will become much more widespread and commonplace. Therefore, HCMV-specific TCR therapy stands a realistic chance of becoming clinical routine in the future, potentially as part of a platform of multiple virus- and tumour-specific TCRs that will be available as libraries to choose from according to the needs of a particular patient.

6 References

Abana, C.O., Pilkinton, M.A., Gaudieri, S., Chopra, A., McDonnell, W.J., Wanjalla, C., Barnett, L., Gangula, R., Hager, C., Jung, D.K., et al. (2017). Cytomegalovirus (CMV) Epitope-Specific CD4+ T Cells Are Inflated in HIV+ CMV+ Subjects. *Journal of Immunology*, 199(9): 3187–3201. DOI: 10.4049/jimmunol.1700851.

Aerts-Toegaert, C., Heirman, C., Tuyaerts, S., Corthals, J., Aerts, J.L., Bonehill, A., Thielemans, K., and Breckpot, K. (2007). CD83 expression on dendritic cells and T cells: Correlation with effective immune responses. *European Journal of Immunology*, 37(3): 686–695. DOI: 10.1002/eji.200636535.

Ahmadi, M., King, J.W., Xue, S.A., Voisine, C., Holler, A., Wright, G.P., Waxman, J., Morris, E., and Stauss, H.J. (2011). CD3 limits the efficacy of TCR gene therapy in vivo. *Blood*, 118(13): 3528–3537. DOI: 10.1182/blood-2011-04-346338.

Ahn, K., Gruhler, A., Galocha, B., Jones, T.R., Wiertz, E.J., Ploegh, H.L., Peterson, P.A., Yang, Y., and Früh, K. (1997). The ER-luminal domain of the HCMV glycoprotein US6 inhibits peptide translocation by TAP. *Immunity*, 6(5): 613–21. DOI: 10.1016/S1074-7613(00)80349-0.

Alp, N.J., Allport, T.D., Van Zanten, J., Rodgers, B., Sissons, J.G., and Borysiewicz, L.K. (1991). Fine specificity of cellular immune responses in humans to human cytomegalovirus immediate-early 1 protein. *Journal of Virology*, 65(9): 4812–20.

Altman, J.D., Moss, P.A., Goulder, P.J., Barouch, D.H., McHeyzer-Williams, M.G., Bell, J.I., McMichael, A.J., and Davis, M.M. (1996). Phenotypic analysis of antigen-specific T lymphocytes. *Science*, 274(5284): 94–6. DOI: 10.1126/science.274.5284.94.

Ameres, S. (2012). *The T cell repertoire specific for the IE-1 protein of human cytomegalovirus: diversity, function and evasion*. Ph.D. thesis, Ludwig-Maximilians-Universität München. URL: https://edoc.ub.uni-muenchen.de/16717/.

Ameres, S., Besold, K., Plachter, B., and Moosmann, A. (2014). CD8 T Cell-Evasive Functions of Human Cytomegalovirus Display Pervasive MHC Allele Specificity, Complementarity, and Cooperativity. *Journal of Immunology*, 192(12): 5894–5905. DOI: 10.4049/jimmunol.1302281.

Ameres, S., Liang, X., Wiesner, M., Mautner, J., and Moosmann, A. (2015). A diverse repertoire of CD4 T cells targets the immediate-early 1 protein of human cytomegalovirus. *Frontiers in Immunology*, 6(NOV): 1–12. DOI: 10.3389/fimmu.2015.00598.

Ameres, S., Mautner, J., Schlott, F., Neuenhahn, M., Busch, D.H., Plachter, B., and Moosmann, A. (2013). Presentation of an Immunodominant Immediate-Early CD8+ T Cell Epitope Resists Human Cytomegalovirus Immunoevasion. *PLoS Pathogens*, 9(5): e1003,383. DOI: 10.1371/journal.ppat.1003383.

Amir, A.L., Orsogna, L.J.a.D., Roelen, D.L., Loenen, M.M.V., Renate, S., Boer, R.D., Hoorn, M.a.W.G.V.D., Kester, M.G.D., Doxiadis, I.I.N., Falkenburg, J.H.F., et al. (2011). Allo-HLA reactivity of virus-specific memory T cells is common Allo-HLA reactivity of virus-specific memory T cells is common. *Library*, 115(15): 3146–3157. DOI: 10.1182/blood-2009-07-234906.

Arber, C., Abhyankar, H., Heslop, H.E., Brenner, M.K., Liu, H., Dotti, G., and Savoldo, B. (2013). The immunogenicity of virus-derived 2A sequences in immunocompetent individuals. *Gene Therapy*, 20(9): 958–62. DOI: 10.1038/gt.2013.25.

Archbold, J.K., Macdonald, W.A., Gras, S., Ely, L.K., Miles, J.J., Bell, M.J., Brennan, R.M., Beddoe, T., Wilce, M.C., Clements, C.S., et al. (2009). Natural micropolymorphism in human leukocyte antigens provides a basis for genetic control of antigen recognition. *The Journal of Experimental Medicine*, 206(1): 209–219. DOI: 10.1084/jem.20082136.

Arden, B., Clark, S.P., Kabelitz, D., and Mak, T.W. (1995). Human T-cell receptor variable gene segment families. *Immunogenetics*, 42(6): 455–500.

Attaf, M., Huseby, E., and Sewell, A.K. (2015). $\alpha\beta$ T cell receptors as predictors of health and disease. *Cellular & Molecular Immunology*, 12(4): 391–9. DOI: 10.1038/cmi.2014.134.

Baldanti, F., Paolucci, S., Campanini, G., Sarasini, A., Percivalle, E., Revello, M.G., and Gerna, G. (2006). Human cytomegalovirus UL131A, UL130 and UL128 genes are highly conserved among field isolates. *Archives of Virology*, 151(6): 1225–1233. DOI: 10.1007/s00705-005-0696-5.

Barel, M.T., Pizzato, N., Le Bouteiller, P., Wiertz, E.J.H.J., and Lenfant, F. (2006). Subtle sequence variation among MHC class I locus products greatly influences sensitivity to HCMV US2- and US11-mediated degradation. *International Immunology*, 18(1): 173–182. DOI: 10.1093/intimm/dxh362.

Barel, M.T., Pizzato, N., van Leeuwen, D., Bouteiller, P.L., Wiertz, E.J.H.J., and Lenfant, F. (2003a). Amino acid composition of $\alpha 1/\alpha 2$ domains and cytoplasmic tail of MHC class I molecules determine their susceptibility to human cytomegalovirus US11-mediated down-regulation. *European Journal of Immunology*, 33(6): 1707–1716. DOI: 10.1002/eji.200323912.

Barel, M.T., Ressing, M., Pizzato, N., van Leeuwen, D., Le Bouteiller, P., Lenfant, F., and Wiertz, E.J.H.J. (2003b). Human Cytomegalovirus-Encoded US2 Differentially Affects Surface Expression of MHC Class I Locus Products and Targets Membrane-Bound, but Not Soluble HLA-G1 for Degradation. *Journal of Immunology*, 171(12): 6757–6765. DOI: 10.4049/jimmunol.171.12.6757.

Barnes, P.D. and Grundy, J.E. (1992). Down-regulation of the class I HLA heterodimer and $\beta 2$-microglobulin on the surface of cells infected with cytomegalovirus. *Journal of General Virology*, 73(9): 2395–2403. DOI: 10.1099/0022-1317-73-9-2395.

Beck, K., Meyer-König, U., Weidmann, M., Nern, C., and Hufert, F. (2003). Human cytomegalovirus impairs dendritic cell function: a novel mechanism of human cytomegalovirus immune escape. *European Journal of Immunology*, 33(6): 1528–1538. DOI: 10.1002/eji.200323612.

Bendle, G.M., Linnemann, C., Hooijkaas, A.I., Bies, L., de Witte, M.A., Jorritsma, A., Kaiser, A.D.M., Pouw, N., Debets, R., Kieback, E., et al. (2010). Lethal graft-versus-host disease in mouse models of T cell receptor gene therapy. *Nature Medicine*, 16(5): 565–70. DOI: 10.1038/nm.2128.

Benson, D.A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., and Sayers, E.W. (2012). GenBank. *Nucleic Acids Research*, 41(D1): D36–D42. DOI: 10.1093/nar/gks1195.

Besold, K. and Plachter, B. (2008). Recombinant viruses as tools to study human cytomegalovirus immune modulation. *Medical Microbiology and Immunology*, 197(2): 215–222. DOI: 10.1007/s00430-008-0083-4.

Besold, K., Wills, M., and Plachter, B. (2009). Immune evasion proteins gpUS2 and gpUS11 of human cytomegalovirus incompletely protect infected cells from CD8 T cell recognition. *Virology*, 391(1): 5–19. DOI: 10.1016/j.virol.2009.06.004.

Biron, C.A., Byron, K.S., and Sullivan, J.L. (1989). Severe herpesvirus infections in an adolescent without natural killer cells. *The New England Journal of Medicine*, 320(26): 1731–5. DOI: 10.1056/NEJM198906293202605.

Boeckh, M., Leisenring, W., Riddell, S.R., Bowden, R.A., Huang, M.L., Myerson, D., Stevens-Ayers, T., Flowers, M.E.D., Cunningham, T., and Corey, L. (2003). Late cytomegalovirus disease and mortality in recipients of allogeneic hematopoietic stem cell transplants: importance of viral load and T-cell immunity. *Blood*, 101(2): 407–414. DOI: 10.1182/blood-2002-03-0993.

Boeckh, M. and Nichols, W.G. (2004). The impact of cytomegalovirus serostatus of donor and recipient before hematopoietic stem cell transplantation in the era of antiviral prophylaxis and preemptive therapy. *Blood*, 103(6): 2003–2008. DOI: 10.1182/blood-2003-10-3616.

Bondinas, G.P., Moustakas, A.K., and Papadopoulos, G.K. (2007). The spectrum of HLA-DQ and HLA-DR alleles, 2006: A listing correlating sequence and structure with function. *Immunogenetics*, 59(7): 539–553. DOI: 10.1007/s00251-007-0224-8.

Borrego, F., Ulbrecht, M., Weiss, E.H., Coligan, J.E., and Brooks, A.G. (1998). Recognition of human histocompatibility leukocyte antigen (HLA)-E complexed with HLA class I signal sequence-derived peptides by CD94/NKG2 confers protection from natural killer cell-mediated lysis. *The Journal of Experimental Medicine*, 187(5): 813–8. DOI: 10.1084/jem.187.5.813.

Borysiewicz, L.K., Graham, S., Hickling, J.K., Mason, P.D., and Sissons, J.G. (1988). Human cytomegalovirus-specific cytotoxic T cells: their precursor frequency and stage specificity. *European Journal of Immunology*, 18(2): 269–75. DOI: 10.1002/eji.1830180214.

Bradley, A.J., Lurain, N.S., Ghazal, P., Trivedi, U., Cunningham, C., Baluchova, K., Gatherer, D., Wilkinson, G.W.G., Dargan, D.J., and Davison, A.J. (2009). High-throughput sequence analysis of variants of human cytomegalovirus strains Towne and AD169. *Journal of General Virology*, 90(10): 2375–2380. DOI: 10.1099/vir.0.013250-0.

Braendstrup, P., Justesen, S., Osterbye, T., Nielsen, L.L.B., Mallone, R., Vindeløv, L., Stryhn, A., and Buus, S. (2013). MHC class II tetramers made from isolated recombinant α and β chains refolded with affinity-tagged peptides. *PLoS ONE*, 8(9): e73,648. DOI: 10.1371/journal.pone.0073648.

Braendstrup, P., Mortensen, B.K., Justesen, S., Østerby, T., Rasmussen, M., Hansen, A.M., Christiansen, C.B., Hansen, M.B., Nielsen, M., Vindeløv, L., et al. (2014). Identification and HLA-tetramer-validation of human CD4+and CD8+T cell responses against HCMV proteins IE1 and IE2. *PLoS ONE*, 9(4). DOI: 10.1371/journal.pone.0094892.

Bresnahan, W.A. and Shenk, T. (2000). A subset of viral transcripts packaged within human cytomegalovirus particles. *Science*, 288(5475): 2373–6. DOI: 10.1126/science.288.5475.2373.

Brochet, X., Lefranc, M.P., and Giudicelli, V. (2008). IMGT/V-QUEST: the highly customized and integrated system for IG and TR standardized V-J and V-D-J sequence analysis. *Nucleic Acids Research*, 36(Web Server): W503–W508. DOI: 10.1093/nar/gkn316.

Broers, A.E., van Der Holt, R., van Esser, J.W., Gratama, J.W., Henzen-Logmans, S., Kuenen-Boumeester, V., Löwenberg, B., and Cornelissen, J.J. (2000). Increased transplant-related morbidity and mortality in CMV-seropositive patients despite highly effective prevention of CMV disease after allogeneic T-cell-depleted stem cell transplantation. *Blood*, 95(7): 2240–5.

Bukowski, J.F., Morita, C.T., Tanaka, Y., Bloom, B.R., Brenner, M.B., and Band, H. (1995). Vgamma2Vdelta2 TCR-dependent recognition of non-peptide antigens and Daudi cells analyzed by TCR gene transfer. *Journal of Immunology*, 154(3): 998–1006.

Bunde, T., Kirchner, A., Hoffmeister, B., Habedank, D., Hetzer, R., Cherepnev, G., Proesch, S., Reinke, P., Volk, H.D., Lehmkuhl, H., et al. (2005). Protection from cytomegalovirus after transplantation is correlated with immediate early 1-specific CD8 T cells. *The Journal of Experimental Medicine*, 201(7): 1031–6. DOI: 10.1084/jem.20042384.

Burgdorf, S. and Kurts, C. (2008). Endocytosis mechanisms and the cell biology of antigen presentation. *Current Opinion in Immunology*, 20(1): 89–95. DOI: 10.1016/j.coi.2007.12.002.

Busch, D.H., Fräßle, S.P., Sommermeyer, D., Buchholz, V.R., and Riddell, S.R. (2016). Role of memory T cell subsets for adoptive immunotherapy. *Seminars in Immunology*, 28(1): 28–34. DOI: 10.1016/j.smim.2016.02.001.

Büscher, N., Paulus, C., Nevels, M., Tenzer, S., and Plachter, B. (2015). The proteome of human cytomegalovirus virions and dense bodies is conserved across different strains. *Medical Microbiology and Immunology*, 204(3): 285–293. DOI: 10.1007/s00430-015-0397-y.

Cannon, M.J., Schmid, D.S., and Hyde, T.B. (2010). Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. *Reviews in Medical Virology*, 20(4): 202–213. DOI: 10.1002/rmv.655.

Carding, S.R. and Egan, P.J. (2002). $\gamma\delta$ T cells: functional plasticity and heterogeneity. *Nature Reviews Immunology*, 2(5): 336–345. DOI: 10.1038/nri797.

Chalupny, N.J., Rein-Weston, A., Dosch, S., and Cosman, D. (2006). Down-regulation of the NKG2D ligand MICA by the human cytomegalovirus glycoprotein UL142. *Biochemical and Biophysical Research Communications*, 346(1): 175–81. DOI: 10.1016/j.bbrc.2006.05.092.

Chapman, T.L., Heikeman, A.P., and Bjorkman, P.J. (1999). The inhibitory receptor LIR-1 uses a common binding interaction to recognize class I MHC molecules and the viral homolog UL18. *Immunity*, 11(5): 603–13. DOI: 10.1016/S1074-7613(00)80135-1.

Chen, D.H., Jiang, H., Lee, M., Liu, F., and Zhou, Z.H. (1999). Three-dimensional visualization of tegument/capsid interactions in the intact human cytomegalovirus. *Virology*, 260(1): 10–6. DOI: 10.1006/viro.1999.9791.

Chow, V.A., Shadman, M., and Gopal, A.K. (2018). Translating anti-CD19 CAR T-Cell therapy into clinical practice for relapsed/refractory diffuse large B-Cell lymphoma. *Blood*, 132(8): 777–781. DOI: 10.1182/blood-2018-04-839217.

Chu, T., Tyznik, A.J., Roepke, S., Berkley, A.M., Woodward-Davis, A., Pattacini, L., Bevan, M.J., Zehn, D., and Prlic, M. (2013). Bystander-Activated Memory CD8 T Cells Control Early Pathogen Load in an Innate-like, NKG2D-Dependent Manner. *Cell Reports*, 3(3): 701–708. DOI: 10.1016/j.celrep.2013.02.020.

Cobbold, M., Khan, N., Pourgheysari, B., Tauro, S., McDonald, D., Osman, H., Assenmacher, M., Billingham, L., Steward, C., Crawley, C., et al. (2005). Adoptive transfer of cytomegalovirus-specific CTL to stem cell transplant patients after selection by HLA-peptide tetramers. *The Journal of Experimental Medicine*, 202(3): 379–386. DOI: 10.1084/jem.20040613.

Collins, T., Korman, A.J., Wake, C.T., Boss, J.M., Kappes, D.J., Fiers, W., Ault, K.A., Gimbrone, M.A., Strominger, J.L., and Pober, J.S. (1984). Immune interferon activates multiple class II major histocompatibility complex genes and the associated invariant chain gene in human endothelial cells and dermal fibroblasts. *Proceedings of the National Academy of Sciences of the United States of America*, 81(15): 4917–21. DOI: 10.1073/PNAS.81.15.4917.

Compton, T., Kurt-Jones, E.A., Boehme, K.W., Belko, J., Latz, E., Golenbock, D.T., and Finberg, R.W. (2003). Human cytomegalovirus activates inflammatory cytokine responses via CD14 and Toll-like receptor 2. *Journal of Virology*, 77(8): 4588–96. DOI: 10.1128/JVI.77.8.4588-4596.2003.

Compton, T., Nepomuceno, R.R., and Nowlin, D.M. (1992). Human cytomegalovirus penetrates host cells by pH-independent fusion at the cell surface. *Virology*, 191(1): 387–95. DOI: 10.1016/0042-6822(92)90200-9.

Constant, P., Davodeau, F., Peyrat, M.A., Poquet, Y., Puzo, G., Bonneville, M., and Fournié, J.J. (1994). Stimulation of human gamma delta T cells by nonpeptidic mycobacterial ligands. *Science*, 264(5156): 267–70. DOI: 10.1126/science.8146660.

Crocchiolo, R., Zino, E., Vago, L., Oneto, R., Bruno, B., Pollichieni, S., Sacchi, N., Sormani, M.P., Marcon, J., Lamparelli, T., et al. (2009). Nonpermissive HLA-DPB1 disparity is a significant independent risk factor for mortality after unrelated hematopoietic stem cell transplantation. *Blood*, 114(7): 1437–1444. DOI: 10.1182/blood-2009-01-200378.

Crompton, L., Khan, N., Khanna, R., Nayak, L., and Moss, P.A.H. (2008). CD4+ T cells specific for glycoprotein B from cytomegalovirus exhibit extreme conservation of T-cell receptor usage between different individuals. *Blood*, 111(4): 2053–61. DOI: 10.1182/blood-2007-04-079863.

Crotzer, V.L. and Blum, J.S. (2010). Autophagy and adaptive immunity. *Immunology*, 131(1): 9–17. DOI: 10.1111/j.1365-2567.2010.03321.x.

Das, H., Wang, L., Kamath, A., and Bukowski, J.F. (2001). Vγ2Vδ2 T-cell receptor-mediated recognition of aminobisphosphonates. *Blood*, 98(5): 1616–8. DOI: 10.1182/blood.V98.5.1616.

Davies, T.F., Martin, A., Concepcion, E.S., Graves, P., Cohen, L., and Ben-Nun, A. (1991). Evidence of limited variability of antigen receptors on intrathyroidal T cells in autoimmune thyroid disease. *The New England Journal of Medicine*, 325(4): 238–44. DOI: 10.1056/NEJM199107253250404.

Davignon, J.L., Castanié, P., Yorke, J.A., Gautier, N., Clément, D., and Davrinche, C. (1996). Anti-human cytomegalovirus activity of cytokines produced by CD4+ T-cell clones specifically activated by IE1 peptides in vitro. *Journal of Virology*, 70(4): 2162–9.

Davison, A.J., Dolan, A., Akter, P., Addison, C., Dargan, D.J., Alcendor, D.J., McGeoch, D.J., and Hayward, G.S. (2003). The human cytomegalovirus genome revisited: comparison with the chimpanzee cytomegalovirus genome. *Journal of General Virology*, 84(1): 17–28. DOI: 10.1099/vir.0.18606-0.

Davodeau, F., Peyrat, M.A., Hallet, M.M., Gaschet, J., Houde, I., Vivien, R., Vie, H., and Bonneville, M. (1993). Close correlation between Daudi and mycobacterial antigen recognition by human gamma delta T cells and expression of V9JPC1 gamma/V2DJC delta-encoded T cell receptors. *Journal of Immunology*, 151(3): 1214–23.

Déchanet, J., Merville, P., Lim, A., Retière, C., Pitard, V., Lafarge, X., Michelson, S., Méric, C., Hallet, M.M., Kourilsky, P., et al. (1999). Implication of $\gamma\delta$ T cells in the human immune response to cytomegalovirus. *Journal of Clinical Investigation*, 103(10): 1437–1449. DOI: 10.1172/JCI5409.

Diamond, D.J., La Rosa, C., Chiuppesi, F., Contreras, H., Dadwal, S., Wussow, F., Bautista, S., Nakamura, R., and Zaia, J.A. (2018). A fifty-year odyssey: prospects for a cytomegalovirus vaccine in transplant and congenital infection. *Expert Review of Vaccines*, 17(10): 889–911. DOI: 10.1080/14760584.2018.1526085.

Dieli, F., Poccia, F., Lipp, M., Sireci, G., Caccamo, N., Sano, C.D., Salerno, A., Di Sano, C., and Salerno, A. (2003). Differentiation of Effector/Memory Vδ2 T Cells and Migratory Routes in Lymph Nodes or Inflammatory Sites. *The Journal of Experimental Medicine*, 198(3): 391–397. DOI: 10.1084/jem.20030235.

Donnelly, M.L.L., Luke, G., Mehrotra, A., Li, X., Hughes, L.E., Gani, D., and Ryan, M.D. (2001). Analysis of the aphthovirus 2A/2B polyprotein 'cleavage' mechanism indicates not a proteolytic reaction, but a novel translational effect: a putative ribosomal 'skip'. *Journal of General Virology*, 82(5): 1013–1025. DOI: 10.1099/0022-1317-82-5-1013.

Dunn, C., Chalupny, N.J., Sutherland, C.L., Dosch, S., Sivakumar, P., Johnson, D.C., and Cosman, D. (2003). Human Cytomegalovirus Glycoprotein UL16 Causes Intracellular Sequestration of NKG2D Ligands, Protecting Against Natural Killer Cell Cytotoxicity. *The Journal of Experimental Medicine*, 197(11): 1427–1439. DOI: 10.1084/jem.20022059.

Duperrier, K., Eljaafari, A., Dezutter-Dambuyant, C., Bardin, C., Jacquet, C., Yoneda, K., Schmitt, D., Gebuhrer, L., and Rigal, D. (2000). Distinct subsets of dendritic cells resembling dermal DCs can be generated in vitro from monocytes, in the presence of different serum supplements. *Journal of Immunological Methods*, 238(1-2): 119–31. DOI: 10.1016/S0022-1759(00)00147-2.

Einsele, H., Roosnek, E., Rufer, N., Sinzger, C., Riegler, S., Löffler, J., Grigoleit, U., Moris, A., Rammensee, H.G., Kanz, L., et al. (2002). Infusion of cytomegalovirus (CMV)-specific T cells for the treatment of CMV infection not responding to antiviral chemotherapy. *Blood*, 99(11): 3916–3922. DOI: 10.1182/blood.V99.11.3916.

Elkington, R. and Khanna, R. (2005). Cross-recognition of human alloantigen by cytomegalovirus glycoprotein-specific CD4+ cytotoxic T lymphocytes: implications for graft-versus-host disease. *Blood*, 105(3): 1362–1364. DOI: 10.1182/blood-2004-07-2602.

Elkington, R., Shoukry, N.H., Walker, S., Crough, T., Fazou, C., Kaur, A., Walker, C.M., and Khanna, R. (2004). Cross-reactive recognition of human and primate cytomegalovirus sequences by human CD4 cytotoxic T lymphocytes specific for glycoprotein B and H. *European Journal of Immunology*, 34(11): 3216–3226. DOI: 10.1002/eji.200425203.

Elkington, R., Walker, S., Crough, T., Menzies, M., Tellam, J., Bharadwaj, M., and Khanna, R. (2003). Ex vivo profiling of CD8+ T-cell responses to human cytomegalovirus reveals broad and multispecific reactivities in healthy virus carriers. *Journal of Virology*, 77(9): 5226–40. DOI: 10.1128/JVI.77.9.5226-5240.2003.

van Endert, P.M. (1999). Role of Nucleotides and Peptide Substrate for Stability and Functional State of the Human ABC Family Transporters Associated with Antigen Processing. *Journal of Biological Chemistry*, 274(21): 14,632–14,638. DOI: 10.1074/jbc.274.21.14632.

Engels, B., Cam, H., Schüler, T., Indraccolo, S., Gladow, M., Baum, C., Blankenstein, T., and Uckert, W. (2003). Retroviral vectors for high-level transgene expression in T lymphocytes. *Human Gene Therapy*, 14(12): 1155–68. DOI: 10.1089/104303403322167993.

Evans, P.C., Soin, A., Wreghitt, T.G., Taylor, C.J., Wight, D.G., and Alexander, G.J. (2000). An association between cytomegalovirus infection and chronic rejection after liver transplantation. *Transplantation*, 69(1): 30–5.

Eyquem, J., Mansilla-Soto, J., Giavridis, T., van der Stegen, S.J.C., Hamieh, M., Cunanan, K.M., Odak, A., Gönen, M., and Sadelain, M. (2017). Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. *Nature*, 543(7643): 113–117. DOI: 10.1038/nature21405.

Falagas, M.E., Snydman, D.R., Ruthazer, R., Griffith, J., Werner, B.G., Freeman, R., and Rohrer, R. (1997). Cytomegalovirus immune globulin (CMVIG) prophylaxis is associated with increased survival after orthotopic liver transplantation. The Boston Center for Liver Transplantation CMVIG Study Group. *Clinical Transplantation*, 11(5 Pt 1): 432–7.

Fish, K.N., Depto, A.S., Moses, A.V., Britt, W., and Nelson, J.A. (1995). Growth kinetics of human cytomegalovirus are altered in monocyte-derived macrophages. *Journal of Virology*, 69(6): 3737–43.

Fisher, J. and Anderson, J. (2018). Engineering Approaches in Human Gamma Delta T Cells for Cancer Immunotherapy. *Frontiers in Immunology*, 9: 1409. DOI: 10.3389/fimmu.2018.01409.

Fleischhauer, K., Shaw, B.E., Gooley, T., Malkki, M., Bardy, P., Bignon, J.D., Dubois, V., Horowitz, M.M., Madrigal, J.A., Morishima, Y., et al. (2012). Effect of T-cell-epitope matching at HLA-DPB1 in recipients of unrelated-donor haemopoietic-cell transplantation: a retrospective study. *The Lancet Oncology*, 13(4): 366–374. DOI: 10.1016/S1470-2045(12)70004-9.

Folch, G. and Lefranc, M.P. (2000). The Human T cell Receptor Beta Variable (TRBV) Genes. *Experimental and Clinical Immunogenetics*, 17(1): 42–54. DOI: 10.1159/000019123.

Foolad, F., Aitken, S.L., and Chemaly, R.F. (2018). Letermovir for the prevention of cytomegalovirus infection in adult cytomegalovirus-seropositive hematopoietic stem cell transplant recipients. *Expert Review of Clinical Pharmacology*, 11(10): 931–941. DOI: 10.1080/17512433.2018.1500897.

Fowler, K.B., Stagno, S., Pass, R.F., Britt, W.J., Boll, T.J., and Alford, C.A. (1992). The Outcome of Congenital Cytomegalovirus Infection in Relation to Maternal Antibody Status. *New England Journal of Medicine*, 326(10): 663–667. DOI: 10.1056/NEJM199203053261003.

Frascaroli, G., Lecher, C., Varani, S., Setz, C., van der Merwe, J., Brune, W., and Mertens, T. (2018). Human macrophages escape inhibition of major histocompatibility complex-dependent antigen presentation by cytomegalovirus and drive proliferation and activation of memory CD4+ and CD8+ T cells. *Frontiers in Immunology*, 9(1129). DOI: 10.3389/fimmu.2018.01129.

Freed, D.C., Tang, Q., Tang, A., Li, F., He, X., Huang, Z., Meng, W., Xia, L., Finnefrock, A.C., Durr, E., et al. (2013). Pentameric complex of viral glycoprotein H is the primary target for potent neutralization by a human cytomegalovirus vaccine. *Proceedings of the National Academy of Sciences of the United States of America*, 110(51): E4997–5005. DOI: 10.1073/pnas.1316517110.

Gabanti, E., Bruno, F., Lilleri, D., Fornara, C., Zelini, P., Cane, I., Migotto, C., Sarchi, E., Furione, M., and Gerna, G. (2014). Human Cytomegalovirus (HCMV)-specific CD4+ and CD8+ T cells are both required for prevention of HCMV disease in seropositive solid-organ transplant recipients. *PLoS ONE*, 9(8). DOI: 10.1371/journal.pone.0106044.

Gallot, G., Vivien, R., Ibisch, C., Lulé, J., Davrinche, C., Gaschet, J., and Vié, H. (2001). Purification of Ag-Specific T Lymphocytes After Direct Peripheral Blood Mononuclear Cell Stimulation Followed by CD25 Selection. I. Application to CD4+ or CD8+ Cytomegalovirus Phosphoprotein pp65 Epitope Determination. *The Journal of Immunology*, 167: 4196–4206. DOI: 10.4049/jimmunol.167.8.4196.

Gamadia, L.E., Remmerswaal, E.B.M., Weel, J.F., Bemelman, F., van Lier, R.A.W., and Ten Berge, I.J.M. (2003). Primary immune responses to human CMV: a critical role for IFN-γ-producing CD4+ T cells in protection against CMV disease. *Blood*, 101(7): 2686–2692. DOI: 10.1182/blood-2002-08-2502.

Gandhi, M.K. and Khanna, R. (2004). Human cytomegalovirus: clinical aspects, immune regulation, and emerging treatments. *The Lancet Infectious diseases*, 4(12): 725–38. DOI: 10.1016/S1473-3099(04)01202-2.

Ganepola, S., Gentilini, C., Hilbers, U., Lange, T., Rieger, K., Hofmann, J., Maier, M., Liebert, U.G., Niederwieser, D., Engelmann, E., et al. (2007). Patients at high risk for CMV infection and disease show delayed CD8+ T-cell immune recovery after allogeneic stem cell transplantation. *Bone Marrow Transplantation*, 39(5): 293–9. DOI: 10.1038/sj.bmt.1705585.

Gary, R., Aigner, M., Moi, S., Schaffer, S., Gottmann, A., Maas, S., Zimmermann, R., Zingsem, J., Strobel, J., Mackensen, A., et al. (2018). Clinical-grade generation of peptide-stimulated CMV/EBV-specific T cells from G-CSF mobilized stem cell grafts. *Journal of Translational Medicine*, 16(1): 124. DOI: 10.1186/s12967-018-1498-3.

Gerna, G. and Lilleri, D. (2019). Human cytomegalovirus (HCMV) infection/re-infection: development of a protective HCMV vaccine. *The New Microbiologica*, 42(1): 1–68.

Gerna, G., Percivalle, E., Lilleri, D., Lozza, L., Fornara, C., Hahn, G., Baldanti, F., and Revello, M.G. (2005). Dendritic-cell infection by human cytomegalovirus is restricted to strains carrying functional UL131-128 genes and mediates efficient viral antigen presentation to CD8+ T cells. *The Journal of General Virology*, 86(Pt 2): 275–84. DOI: 10.1099/vir.0.80474-0.

Gibson, W. and Irmiere, A. (1984). Selection of particles and proteins for use as human cytomegalovirus subunit vaccines. *Birth Defects Original Article Series*, 20(1): 305–24.

Giudicelli, V., Brochet, X., and Lefranc, M.P. (2011). IMGT/V-QUEST: IMGT Standardized Analysis of the Immunoglobulin (IG) and T Cell Receptor (TR) Nucleotide Sequences. *Cold Spring Harbor Protocols*, 2011(6): 695–715. DOI: 10.1101/pdb.prot5633.

Gober, H.J., Kistowska, M., Angman, L., Jenö, P., Mori, L., and De Libero, G. (2003). Human T Cell Receptor $\gamma\delta$ Cells Recognize Endogenous Mevalonate Metabolites in Tumor Cells. *The Journal of Experimental Medicine*, 197(2): 163–168. DOI: 10.1084/jem.20021500.

Gragert, L., Madbouly, A., Freeman, J., and Maiers, M. (2013). Six-locus high resolution HLA haplotype frequencies derived from mixed-resolution DNA typing for the entire US donor registry. *Human Immunology*, 74(10): 1313–1320. DOI: 10.1016/j.humimm.2013.06.025.

Grassi, F., Dezutter-Dambuyant, C., McIlroy, D., Jacquet, C., Yoneda, K., Imamura, S., Boumsell, L., Schmitt, D., Autran, B., Debre, P., et al. (1998). Monocyte-derived dendritic cells have a phenotype comparable to that of dermal dendritic cells and display ultrastructural granules distinct from Birbeck granules. *Journal of Leukocyte Biology*, 64(4): 484–493. DOI: 10.1002/jlb.64.4.484.

Gratama, J.W., Brooimans, R.A., Van Der Holt, B., Sintnicolaas, K., Van Doornum, G., Niesters, H.G., Löwenberg, B., and Cornelissen, J.J. (2008). Monitoring cytomegalovirus IE-1 and pp65-specific CD4+ and CD8+ T-cell responses after allogeneic stem cell transplantation may identify patients at risk for recurrent CMV reactivations. *Cytometry Part B*, 74(4): 211–220. DOI: 10.1002/cyto.b.20420.

Grazia Revello, M., Baldanti, F., Percivalle, E., Sarasini, A., De-Giuli, L., Genini, E., Lilleri, D., Labò, N., and Gerna, G. (2001). In vitro selection of human cytomegalovirus variants unable to transfer virus and virus products from infected cells to polymorphonuclear leukocytes and to grow in endothelial cells. *Journal of General Virology*, 82(6): 1429–1438. DOI: 10.1099/0022-1317-82-6-1429.

Greaves, R.F. and Mocarski, E.S. (1998). Defective growth correlates with reduced accumulation of a viral DNA replication protein after low-multiplicity infection by a human cytomegalovirus ie1 mutant. *Journal of Virology*, 72(1): 366–79.

Grigoleit, U., Riegler, S., Einsele, H., Laib Sampaio, K., Jahn, G., Hebart, H., Brossart, P., Frank, F., and Sinzger, C. (2002). Human cytomegalovirus induces a direct inhibitory effect on antigen presentation by monocyte-derived immature dendritic cells. *British Journal of Haematology*, 119(1): 189–98. DOI: 10.1046/j.1365-2141.2002.03798.x.

Grimm, E.A., Mazumder, A., Zhang, H.Z., and Rosenberg, S.A. (1982). Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. *The Journal of Experimental Medicine*, 155(6): 1823–41. DOI: 10.1084/jem.155.6.1823.

Groh, V., Porcelli, S., Fabbi, M., Lanier, L.L., Picker, L.J., Anderson, T., Warnke, R.A., Bhan, A.K., Strominger, J.L., and Brenner, M.B. (1989). Human lymphocytes bearing T cell receptor gamma/delta are phenotypically diverse and evenly distributed throughout the lymphoid system. *The Journal of Experimental Medicine*, 169(4): 1277–94. DOI: 10.1084/jem.169.4.1277.

Gründer, C., van Dorp, S., Hol, S., Drent, E., Straetemans, T., Heijhuurs, S., Scholten, K., Scheper, W., Sebestyen, Z., Martens, A., et al. (2012). γ9 and δ2CDR3 domains regulate functional avidity of T cells harboring γ9δ2TCRs. *Blood*, 120(26): 5153–62. DOI: 10.1182/blood-2012-05-432427.

Hahn, G., Revello, M.G., Patrone, M., Percivalle, E., Campanini, G., Sarasini, A., Wagner, M., Gallina, A., Milanesi, G., Koszinowski, U., et al. (2004). Human Cytomegalovirus UL131-128 Genes Are Indispensable for Virus Growth in Endothelial Cells and Virus Transfer to Leukocytes. *Journal of Virology*, 78(18): 10,023–10,033. DOI: 10.1128/JVI.78.18.10023-10033.2004.

Halary, F., Pitard, V., Dlubek, D., Krzysiek, R., de la Salle, H., Merville, P., Dromer, C., Emilie, D., Moreau, J.F., and Déchanet-Merville, J. (2005). Shared reactivity of Vδ2neg $\gamma\delta$ T cells against cytomegalovirus-infected cells and tumor intestinal epithelial cells. *The Journal of Experimental Medicine*, 201(10): 1567–1578. DOI: 10.1084/jem.20041851.

Hanley, P.J., Cruz, C.R.Y., Savoldo, B., Leen, A.M., Stanojevic, M., Khalil, M., Decker, W., Molldrem, J.J., Liu, H., Gee, A.P., et al. (2009). Functionally active virus-specific T cells that target CMV, adenovirus, and EBV can be expanded from naive T-cell populations in cord blood and will target a range of viral epitopes. *Blood*, 114(9): 1958–1967. DOI: 10.1182/blood-2009-03-213256.

Hanley, P.J., Melenhorst, J.J., Nikiforow, S., Scheinberg, P., Blaney, J.W., Demmler-Harrison, G., Cruz, C.R., Lam, S., Krance, R.A., Leung, K.S., et al. (2015). CMV-specific T cells generated from naïve T cells recognize atypical epitopes and may be protective in vivo. *Science Translational Medicine*, 7(285): 285ra63. DOI: 10.1126/scitranslmed.aaa2546.

Harly, C., Guillaume, Y., Nedellec, S., Peigne, C.M., Monkkonen, H., Monkkonen, J., Li, J., Kuball, J., Adams, E.J., Netzer, S., et al. (2012). Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human $\gamma\delta$ T-cell subset. *Blood*, 120(11): 2269–2279. DOI: 10.1182/blood-2012-05-430470.

Hegde, N.R. and Johnson, D.C. (2003). Human cytomegalovirus US2 causes similar effects on both major histocompatibility complex class I and II proteins in epithelial and glial cells. *Journal of Virology*, 77(17): 9287–94. DOI: 10.1128/JVI.77.17.9287-9294.2003.

Hegde, N.R., Tomazin, R.A., Wisner, T.W., Dunn, C., Boname, J.M., Lewinsohn, D.M., and Johnson, D.C. (2002). Inhibition of HLA-DR assembly, transport, and loading by human cytomegalovirus glycoprotein US3: a novel mechanism for evading major histocompatibility complex class II antigen presentation. *Journal of Virology*, 76(21): 10,929–41. DOI: 10.1128/jvi.76.21.10929-10941.2002.

Hennecke, J. and Wiley, D.C. (2002). Structure of a Complex of the Human α/β T Cell Receptor (TCR) HA1.7, Influenza Hemagglutinin Peptide, and Major Histocompatibility Complex Class II Molecule, HLA-DR4 (DRA*0101 and DRB1*0401). *The Journal of Experimental Medicine*, 195(5): 571–581. DOI: 10.1084/jem.20011194.

Hertel, L., Lacaille, V.G., Strobl, H., Mellins, E.D., and Mocarski, E.S. (2003). Susceptibility of immature and mature Langerhans cell-type dendritic cells to infection and immunomodulation by human cytomegalovirus. *Journal of Virology*, 77(13): 7563–74. DOI: 10.1128/jvi.77.13.7563-7574.2003.

Hesse, J., Ameres, S., Besold, K., Krauter, S., Moosmann, A., and Plachter, B. (2013). Suppression of CD8+ T-cell recognition in the immediate-early phase of human cytomegalovirus infection. *Journal of General Virology*, 94(Pt_2): 376–386. DOI: 10.1099/vir.0.045682-0.

Hiller, K., Grote, A., Scheer, M., Munch, R., and Jahn, D. (2004). PrediSi: prediction of signal peptides and their cleavage positions. *Nucleic Acids Research*, 32(Web Server): W375–W379. DOI: 10.1093/nar/gkh378.

Hilton, H.G. and Parham, P. (2017). Missing or altered self: human NK cell receptors that recognize HLA-C. *Immunogenetics*, 69(8-9): 567–579. DOI: 10.1007/s00251-017-1001-y.

Hintz, M., Reichenberg, A., Altincicek, B., Bahr, U., Gschwind, R.M., Kollas, A.K., Beck, E., Wiesner, J., Eberl, M., and Jomaa, H. (2001). Identification of (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate as a major activator for human gammadelta T cells in Escherichia coli. *FEBS letters*, 509(2): 317–22. DOI: 10.1016/s0014-5793(01)03191-x.

Hoeres, T., Smetak, M., Pretscher, D., and Wilhelm, M. (2018). Improving the Efficiency of Vγ9Vδ2 T-Cell Immunotherapy in Cancer. *Frontiers in Immunology*, 9: 800. DOI: 10.3389/fimmu.2018.00800.

Hollenbach, J.A., Madbouly, A., Gragert, L., Vierra-Green, C., Flesch, S., Spellman, S., Begovich, A., Noreen, H., Trachtenberg, E., Williams, T., et al. (2012). A combined DPA1˜DPB1 amino acid epitope is the primary unit of selection on the HLA-DP heterodimer. *Immunogenetics*, 64(8): 559–69. DOI: 10.1007/s00251-012-0615-3.

Holst, J., Vignali, K.M., Burton, A.R., and Vignali, D.A.A. (2006). Rapid analysis of T-cell selection in vivo using T cell-receptor retrogenic mice. *Nature Methods*, 3(3): 191–7. DOI: 10.1038/nmeth858.

Hook, L., Hancock, M., Landais, I., Grabski, R., Britt, W., and Nelson, J.A. (2014). Cytomegalovirus microRNAs. *Current Opinion in Virology*, 7(1): 40–46. DOI: 10.1016/j.coviro.2014.03.015.

Hopkins, J., Fiander, A., Evans, A., Delchambre, M., Gheysen, D., and Borysiewicz, L. (1996). Cytotoxic T cell immunity to human cytomegalovirus glycoprotein B. *Journal of Medical Virology*, 49(2): 124–131. DOI: 10.1002/(SICI)1096-9071(199606)49:2<124::AID-JMV9>3.0.CO;2-7.

Huth, A., Liang, X., Krebs, S., Blum, H., and Moosmann, A. (2019). Antigen-Specific TCR Signatures of Cytomegalovirus Infection. *Journal of Immunology*, 202(3): 979–990. DOI: 10.4049/jimmunol.1801401.

Irmiere, A. and Gibson, W. (1983). Isolation and characterization of a noninfectious virion-like particle released from cells infected with human strains of cytomegalovirus. *Virology*, 130(1): 118–33. DOI: 10.1016/0042-6822(83)90122-8.

Irvine, D.J., Purbhoo, M.A., Krogsgaard, M., and Davis, M.M. (2002). Direct observation of ligand recognition by T cells. *Nature*, 419(6909): 845–849. DOI: 10.1038/nature01076.

Itoh, Y., Hemmer, B., Martin, R., and Germain, R.N. (1999). Serial TCR engagement and down-modulation by peptide:MHC molecule ligands: relationship to the quality of individual TCR signaling events. *Journal of Immunology*, 162(4): 2073–80.

Jackson, S.E., Mason, G.M., and Wills, M.R. (2011). Human cytomegalovirus immunity and immune evasion. *Virus Research*, 157(2): 151–160. DOI: 10.1016/j.virusres.2010.10.031.

Jackson, S.E., Sedikides, G.X., Mason, G.M., Okecha, G., and Wills, M.R. (2017). Human Cytomegalovirus (HCMV)-Specific CD4+ T Cells Are Polyfunctional and Can Respond to HCMV-Infected Dendritic Cells In Vitro. *Journal of Virology*, 91(6). DOI: 10.1128/JVI.02128-16.

Jacobs, J.P., Jones, C.M., and Baille, J.P. (1970). Characteristics of a human diploid cell designated MRC-5. *Nature*, 227(5254): 168–70. DOI: 10.1038/227168a0.

Jahn, G., Stenglein, S., Riegler, S., Einsele, H., and Sinzger, C. (1999). Human Cytomegalovirus Infection of Immature Dendritic Cells and Macrophages. *Intervirology*, 42(5-6): 365–372. DOI: 10.1159/000053973.

Janeway, C.A. (1992). The T cell receptor as a multicomponent signalling machine: CD4/CD8 coreceptors and CD45 in T cell activation. *Annual Review of Immunology*, 10(1): 645–74. DOI: 10.1146/annurev.iy.10.040192.003241.

Jean Beltran, P.M. and Cristea, I.M. (2014). The life cycle and pathogenesis of human cytomegalovirus infection: lessons from proteomics. *Expert Review of Proteomics*, 11(6): 697–711. DOI: 10.1586/14789450.2014.971116.

Jedema, I., van de Meent, M., Pots, J., Kester, M.G.D., van der Beek, M.T., and Falkenburg, J.H.F. (2011). Successful generation of primary virus-specific and anti-tumor T-cell responses from the naive donor T-cell repertoire is determined by the balance between antigen-specific precursor T cells and regulatory T cells. *Haematologica*, 96(8): 1204–1212. DOI: 10.3324/haematol.2010.039099.

Jensen, K.D., Su, X., Shin, S., Li, L., Youssef, S., Yamasaki, S., Steinman, L., Saito, T., Locksley, R.M., Davis, M.M., et al. (2008). Thymic Selection Determines $\gamma\delta$ T Cell Effector Fate: Antigen-Naive Cells Make Interleukin-17 and Antigen-Experienced Cells Make Interferon γ. *Immunity*, 29(1): 90–100. DOI: 10.1016/j.immuni.2008.04.022.

Jones, T.R., Hanson, L.K., Sun, L., Slater, J.S., Stenberg, R.M., and Campbell, A.E. (1995). Multiple independent loci within the human cytomegalovirus unique short region down-regulate expression of major histocompatibility complex class I heavy chains. *Journal of Virology*, 69(8): 4830–41.

Jones, T.R. and Sun, L. (1997). Human cytomegalovirus US2 destabilizes major histocompatibility complex class I heavy chains. *Journal of Virology*, 71(4): 2970–9.

Jones, T.R., Wiertz, E.J., Sun, L., Fish, K.N., Nelson, J.A., and Ploegh, H.L. (1996). Human cytomegalovirus US3 impairs transport and maturation of major histocompatibility complex class I heavy chains. *Proceedings of the National Academy of Sciences*, 93(21): 11,327–11,333. DOI: 10.1073/pnas.93.21.11327.

Jonjić, S., Pavić, I., Lucin, P., Rukavina, D., and Koszinowski, U.H. (1990). Efficacious control of cytomegalovirus infection after long-term depletion of CD8+ T lymphocytes. *Journal of Virology*, 64(11): 5457–64.

Jonjić, S., Pavić, I., Polić, B., Crnković, I., Lucin, P., and Koszinowski, U.H. (1994). Antibodies are not essential for the resolution of primary cytomegalovirus infection but limit dissemination of recurrent virus. *The Journal of Experimental Medicine*, 179(5): 1713–7. DOI: 10.1084/jem.179.5.1713.

Jonuleit, H., Giesecke, A., Kandemir, A., Paragnik, L., Knop, J., and Enk, A.H. (2000). Induction of tumor peptide-specific cytotoxic T cells under serum-free conditions by mature human dendritic cells. *Archives of Dermatological Research*, 292(7): 325–32.

Jonuleit, H., Kühn, U., Müller, G., Steinbrink, K., Paragnik, L., Schmitt, E., Knop, J., and Enk, A.H. (1997). Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf serum-free conditions. *European Journal of Immunology*, 27(12): 3135–42. DOI: 10.1002/eji.1830271209.

Kalejta, R.F. (2008). Functions of human cytomegalovirus tegument proteins prior to immediate early gene expression. *Current Topics in Microbiology and Immunology*, 325: 101–15.

Kärber, G. (1931). Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. *Naunyn-Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie*, 162(4): 480–483. DOI: 10.1007/BF01863914.

Kempkes, B., Pich, D., Zeidler, R., Sugden, B., and Hammerschmidt, W. (1995). Immortalization of human B lymphocytes by a plasmid containing 71 kilobase pairs of Epstein-Barr virus DNA. *Journal of Virology*, 69(1): 231–8.

Kern, F., Bunde, T., Faulhaber, N., Kiecker, F., Khatamzas, E., Rudawski, I.M., Pruss, A., Gratama, J.W., Volkmer-Engert, R., Ewert, R., et al. (2002). Cytomegalovirus (CMV) phosphoprotein 65 makes a large contribution to shaping the T cell repertoire in CMV-exposed individuals. *The Journal of Infectious Diseases*, 185(12): 1709–16. DOI: 10.1086/340637.

Kern, F., Surel, I.P., Faulhaber, N., Frömmel, C., Schneider-Mergener, J., Schönemann, C., Reinke, P., and Volk, H.D. (1999). Target structures of the CD8(+)-T-cell response to human cytomegalovirus: the 72-kilodalton major immediate-early protein revisited. *Journal of Virology*, 73(10): 8179–84.

Khairallah, C., Déchanet-Merville, J., and Capone, M. (2017). $\gamma\delta$ T Cell-Mediated Immunity to Cytomegalovirus Infection. *Frontiers in Immunology*, 8: 105. DOI: 10.3389/fimmu.2017.00105.

Khan, M.W.A., Curbishley, S.M., Chen, H.C.C., Thomas, A.D., Pircher, H., Mavilio, D., Steven, N.M., Eberl, M., and Moser, B. (2014). Expanded Human Blood-Derived $\gamma\delta$T Cells Display Potent Antigen-Presentation Functions. *Frontiers in Immunology*, 5(JUL): 1–13. DOI: 10.3389/fimmu.2014.00344.

Khan, N., Best, D., Rickinson, A.B., Bruton, R., Nayak, L., and Moss, P.A.H. (2007). T Cell Recognition Patterns of Immunodominant Cytomegalovirus Antigens in Primary and Persistent Infection. *Journal of Immunology*, 178(7): 4455–4465. DOI: 10.4049/jimmunol.178.7.4455.

Khan, N., Bruton, R., Taylor, G.S., Cobbold, M., Jones, T.R., Rickinson, A.B., and Moss, P.A.H. (2005). Identification of Cytomegalovirus-Specific Cytotoxic T Lymphocytes In Vitro Is Greatly Enhanced by the Use of Recombinant Virus Lacking the US2 to US11 Region or Modified Vaccinia Virus Ankara Expressing Individual Viral Genes. *Journal of Virology*, 79(5): 2869–2879. DOI: 10.1128/JVI.79.5.2869-2879.2005.

Khanna, R., Bell, S., Sherritt, M., Galbraith, A., Burrows, S.R., Rafter, L., Clarke, B., Slaughter, R., Falk, M.C., Douglass, J., et al. (1999). Activation and adoptive transfer of Epstein-Barr virus-specific cytotoxic T cells in solid organ transplant patients with posttransplant lymphoproliferative disease. *Proceedings of the National Academy of Sciences of the United States of America*, 96(18): 10,391–6. DOI: 10.1073/pnas.96.18.10391.

Khattab, B.A.M., Lindenmaier, W., Frank, R., and Link, H. (1997). Three T-cell epitopes within the C-terminal 265 amino acids of the matrix protein pp65 of human cytomegalovirus recognized by human lymphocytes. *Journal of Medical Virology*, 52(1): 68–76. DOI: 10.1002/(SICI)1096-9071(199705)52:1<68::AID-JMV11>3.0.CO;2-X.

Kim, J.H., Lee, S.R., Li, L.H., Park, H.J., Park, J.H., Lee, K.Y., Kim, M.K., Shin, B.A., and Choi, S.Y. (2011a). High cleavage efficiency of a 2A peptide derived from porcine teschovirus-1 in human cell lines, zebrafish and mice. *PLoS ONE*, 6(4): 1–8. DOI: 10.1371/journal.pone.0018556.

Kim, S., Lee, S., Shin, J., Kim, Y., Evnouchidou, I., Kim, D., Kim, Y.K., Kim, Y.E., Ahn, J.H., Riddell, S.R., et al. (2011b). Human cytomegalovirus microRNA miR-US4-1 inhibits CD8+ T cell responses by targeting the aminopeptidase ERAP1. *Nature Immunology*, 12(10): 984–991. DOI: 10.1038/ni.2097.

Kinzler, E.R. and Compton, T. (2005). Characterization of human cytomegalovirus glycoprotein-induced cell-cell fusion. *Journal of Virology*, 79(12): 7827–37. DOI: 10.1128/JVI.79.12.7827-7837.2005.

Klebanoff, C.A., Gattinoni, L., and Restifo, N.P. (2012). Sorting Through Subsets: which T-cell populations mediate highly effective adoptive immunotherapy? *Journal of Immunotherapy*, 35(9): 651–660. DOI: 10.1097/CJI.0b013e31827806e6.

Kline, J.N., Hunninghake, G.M., He, B., Monick, M.M., and Hunninghake, G.W. (1998). Synergistic activation of the human cytomegalovirus major immediate early promoter by prostaglandin E2 and cytokines. *Experimental Lung Research*, 24(1): 3–14.

Klitz, W., Maiers, M., Spellman, S., Baxter-Lowe, L.A., Schmeckpeper, B., Williams, T.M., and Fernandez-Viña, M. (2003). New HLA haplotype frequency reference standards : High-resolution and large sample typing of HLA DR-DQ haplotypes in a sample of European Americans. *Tissue Antigens*, 62(4): 296–307. DOI: 10.1034/j.1399-0039.2003.00103.x.

Knabel, M., Franz, T.J., Schiemann, M., Wulf, A., Villmow, B., Schmidt, B., Bernhard, H., Wagner, H., and Busch, D.H. (2002). Reversible MHC multimer staining for functional isolation of T-cell populations and effective adoptive transfer. *Nature Medicine*, 8(6): 631–7. DOI: 10.1038/nm0602-631.

Knight, A., Madrigal, A.J., Grace, S., Sivakumaran, J., Kottaridis, P., Mackinnon, S., Travers, P.J., and Lowdell, M.W. (2010). The role of Vδ2-negative $\gamma\delta$ T cells during cytomegalovirus reactivation in recipients of allogeneic stem cell transplantation. *Blood*, 116(12): 2164–2172. DOI: 10.1182/blood-2010-01-255166.

Knipper, A.J., Hakenberg, P., Enczmann, J., Kuhröber, A., Kiesel, U., Kögler, G., and Wernet, P. (2000). HLA-DRB 1, 3, 4, 5 and -DQB1 allele frequencies and HLA-DR/DQ linkage disequilibrium of 231 German Caucasoid patients and their corresponding 821 potential unrelated stem cell transplants. *Human Immunology*, 61(6): 605–614. DOI: 10.1016/S0198-8859(00)00114-2.

Kochenderfer, J.N., Dudley, M.E., Kassim, S.H., Somerville, R.P., Carpenter, R.O., Stetler-Stevenson, M., Yang, J.C., Phan, G.Q., Hughes, M.S., Sherry, R.M., et al. (2015). Chemotherapy-Refractory Diffuse Large B-Cell Lymphoma and Indolent B-Cell Malignancies Can Be Effectively Treated With Autologous T Cells Expressing an Anti-CD19 Chimeric Antigen Receptor. *Journal of Clinical Oncology*, 33(6): 540–549. DOI: 10.1200/JCO.2014.56.2025.

Komanduri, K.V., Viswanathan, M.N., Wieder, E.D., Schmidt, D.K., Bredt, B.M., Jacobson, M.A., and McCune, J.M. (1998). Restoration of cytomegalovirus-specific CD4+ T-lymphocyte responses after ganciclovir and highly active antiretroviral therapy in individuals infected with HIV-1. *Nature Medicine*, 4(8): 953–6. DOI: 10.1038/nm0898-953.

Kondo, K., Kaneshima, H., and Mocarski, E.S. (1994). Human cytomegalovirus latent infection of granulocyte-macrophage progenitors. *Proceedings of the National Academy of Sciences of the United States of America*, 91(25): 11,879–83. DOI: 10.1073/pnas.91.25.11879.

Koning, F., Maloy, W.L., Cohen, D., and Coligan, J.E. (1987). Independent association of T cell receptor beta and gamma chains with CD3 in the same cell. *The Journal of Experimental Medicine*, 166(2): 595–600. DOI: 10.1084/jem.166.2.595.

Koop, B.F., Rowen, L., Wang, K., Kuo, C.L., Seto, D., Lenstra, J.A., Howard, S., Shan, W., Deshpande, P., and Hood, L. (1994). The Human T-Cell Receptor TCRAC/TCRDC (C alpha/C delta) Region: Organization, Sequence, and Evolution of 97.6 kb of DNA. *Genomics*, 19(3): 478–493. DOI: 10.1006/geno.1994.1097.

Krause, H., Hebart, H., Jahn, G., Müller, C., and Einsele, H. (1997). Screening for CMV-specific T cell proliferation to identify patients at risk of developing late onset CMV disease. *Bone Marrow Transplantation*, 19(11): 1111–1116. DOI: 10.1038/sj.bmt.1700801.

Krogsgaard, M., Li, Q.j., Sumen, C., Huppa, J.B., Huse, M., and Davis, M.M. (2005). Agonist/endogenous peptide-MHC heterodimers drive T cell activation and sensitivity. *Nature*, 434(7030): 238–243. DOI: 10.1038/nature03391.

Kropshofer, H., Hämmerling, G.J., and Vogt, A.B. (1999). The impact of the non-classical MHC proteins HLA-DM and HLA-DO on loading of MHC class II molecules. *Immunological Reviews*, 172: 267–78. DOI: 10.1111/j.1600-065X.1999.tb01371.x.

Kutza, A.S., Muhl, E., Hackstein, H., Kirchner, H., and Bein, G. (1998). High incidence of active cytomegalovirus infection among septic patients. *Clinical Infectious Diseases*, 26(5): 1076–82. DOI: 10.1086/520307.

Le Roy, E., Baron, M., Faigle, W., Clément, D., Lewinsohn, D.M., Streblow, D.N., Nelson, J.A., Amigorena, S., Davignon, J.L.J.L., Clement, D., et al. (2002). Infection of APC by Human Cytomegalovirus Controlled Through Recognition of Endogenous Nuclear Immediate Early Protein 1 by Specific CD4+ T Lymphocytes. *Journal of Immunology*, 169(3): 1293–1301. DOI: 10.4049/jimmunol.169.3.1293.

van Leeuwen, E.M.M., Remmerswaal, E.B.M., Heemskerk, M.H.M., ten Berge, I.J.M., and van Lier, R.A.W. (2006). Strong selection of virus-specific cytotoxic CD4+ T-cell clones during primary human cytomegalovirus infection. *Blood*, 108(9): 3121–3127. DOI: 10.1182/blood-2006-03-006809.

Lefranc, M.P., Giudicelli, V., Ginestoux, C., Bodmer, J., Müller, W., Bontrop, R., Lemaitre, M., Malik, A., Barbié, V., and Chaume, D. (1999). IMGT, the international ImMunoGeneTics database. *Nucleic Acids Research*, 27(1): 209–12. DOI: 10.1093/nar/27.1.209.

Leisegang, M., Engels, B., Meyerhuber, P., Kieback, E., Sommermeyer, D., Xue, S.A., Reuß, S., Stauss, H., and Uckert, W. (2008). Enhanced functionality of T cell receptor-redirected T cells is defined by the transgene cassette. *Journal of Molecular Medicine*, 86(5): 573–583. DOI: 10.1007/s00109-008-0317-3.

Lemmermann, N.A. and Reddehase, M.J. (2016). Refining human T-cell immunotherapy of cytomegalovirus disease: a mouse model with 'humanized' antigen presentation as a new preclinical study tool. *Medical Microbiology and Immunology*, 205(6): 549–561. DOI: 10.1007/s00430-016-0471-0.

Li, Q.J., Dinner, A.R., Qi, S., Irvine, D.J., Huppa, J.B., Davis, M.M., and Chakraborty, A.K. (2004). CD4 enhances T cell sensitivity to antigen by coordinating Lck accumulation at the immunological synapse. *Nature Immunology*, 5(8): 791–799. DOI: 10.1038/ni1095.

Li Pira, G., Bottone, L., Ivaldi, F., Pelizzoli, R., Del Galdo, F., Lozzi, L., Bracci, L., Loregian, A., Palù, G., De Palma, R., et al. (2004). Identification of new Th peptides from the cytomegalovirus protein pp65 to design a peptide library for generation of CD4 T cell lines for cellular immunoreconstitution. *International Immunology*, 16(5): 635–642. DOI: 10.1093/intimm/dxh065.

Lichtenegger, F.S., Mueller, K., Otte, B., Beck, B., Hiddemann, W., Schendel, D.J., and Subklewe, M. (2012). CD86 and IL-12p70 are key players for T helper 1 polarization and natural killer cell activation by Toll-like receptor-induced dendritic cells. *PloS ONE*, 7(9): e44,266. DOI: 10.1371/journal.pone.0044266.

Lilleri, D., Fornara, C., Chiesa, A., Caldera, D., Alessandrino, E.P., and Gerna, G. (2008). Human cytomegalovirus-specific CD4+ and CD8+ T-cell reconstitution in adult allogeneic hematopoietic stem cell transplant recipients and immune control of viral infection. *Haematologica*, 93(2): 248–256. DOI: 10.3324/haematol.11912.

Ljungman, P. (2007). Risk assessment in haematopoietic stem cell transplantation: Viral status. *Best Practice & Research Clinical Haematology*, 20(2): 209–217. DOI: 10.1016/j.beha.2006.09.003.

Ljungman, P., Aschan, J., Azinge, J.N., Brandt, L., Ehrnst, A., Hammarström, V., Klaesson, S., Linde, A., Lönnqvist, B., and Ringdén, O. (1993). Cytomegalovirus viraemia and specific T-helper cell responses as predictors of disease after allogeneic marrow transplantation. *British Journal of Haematology*, 83(1): 118–24. DOI: 10.1111/j.1365-2141.1993.tb04641.x.

Ljungman, P., Brand, R., Hoek, J., de la Camara, R., Cordonnier, C., Einsele, H., Styczynski, J., Ward, K.N., Cesaro, S., and Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation (2014). Donor Cytomegalovirus Status Influences the Outcome of Allogeneic Stem Cell Transplant: A Study by the European Group for Blood and Marrow Transplantation. *Clinical Infectious Diseases*, 59(4): 473–481. DOI: 10.1093/cid/ciu364.

van Loenen, M.M., de Boer, R., Amir, A.L., Hagedoorn, R.S., Volbeda, G.L., Willemze, R., van Rood, J.J., Falkenburg, J.H.F., and Heemskerk, M.H.M. (2010). Mixed T cell receptor dimers harbor potentially harmful neoreactivity. *Proceedings of the National Academy of Sciences of the United States of America*, 107(24): 10,972–7. DOI: 10.1073/pnas.1005802107.

Loewendorf, A. and Benedict, C.A. (2010). Modulation of host innate and adaptive immune defenses by cytomegalovirus: timing is everything. *Journal of Internal Medicine*, 267(5): 483–501. DOI: 10.1111/j.1365-2796.2010.02220.x.

Lucin, P., Pavić, I., Polić, B., Jonjić, S., and Koszinowski, U.H. (1992). Gamma interferon-dependent clearance of cytomegalovirus infection in salivary glands. *Journal of Virology*, 66(4): 1977–84.

Lurain, N.S. and Chou, S. (2010). Antiviral Drug Resistance of Human Cytomegalovirus. *Clinical Microbiology Reviews*, 23(4): 689–712. DOI: 10.1128/CMR.00009-10.

Macagno, A., Bernasconi, N.L., Vanzetta, F., Dander, E., Sarasini, A., Revello, M.G., Gerna, G., Sallusto, F., and Lanzavecchia, A. (2010). Isolation of human monoclonal antibodies that potently neutralize human cytomegalovirus infection by targeting different epitopes on the gH/gL/UL128-131A complex. *Journal of Virology*, 84(2): 1005–13. DOI: 10.1128/JVI.01809-09.

Macfarlane Burnet, F. (1954). The newer approach to immunity in its bearing on medicine and biology. *British Medical Journal*, 2(4881): 189–93. DOI: 10.1136/bmj.2.4881.189.

Mandelboim, O., Reyburn, H.T., Valés-Gómez, M., Pazmany, L., Colonna, M., Borsellino, G., and Strominger, J.L. (1996). Protection from lysis by natural killer cells of group 1 and 2 specificity is mediated by residue 80 in human histocompatibility leukocyte antigen C alleles and also occurs with empty major histocompatibility complex molecules. *The Journal of Experimental Medicine*, 184(3): 913–22. DOI: 10.1084/jem.184.3.913.

Manicklal, S., Emery, V.C., Lazzarotto, T., Boppana, S.B., and Gupta, R.K. (2013). The "silent" global burden of congenital cytomegalovirus. *Clinical Microbiology Reviews*, 26(1): 86–102. DOI: 10.1128/CMR.00062-12.

Mantegazza, A.R., Magalhaes, J.G., Amigorena, S., and Marks, M.S. (2013). Presentation of phagocytosed antigens by MHC class I and II. *Traffic*, 14(2): 135–52. DOI: 10.1111/tra.12026.

Marchini, A., Liu, H., and Zhu, H. (2001). Human cytomegalovirus with IE-2 (UL122) deleted fails to express early lytic genes. *Journal of Virology*, 75(4): 1870–8. DOI: 10.1128/JVI.75.4.1870-1878.2001.

Marsh, S.G.E., Parham, P., and Barber, L.D. (2000). *The HLA FactsBook*. Academic Press. ISBN 978-0-12-545025-6.

Martinez-Martin, N., Marcandalli, J., Huang, C.S., Arthur, C.P., Perotti, M., Foglierini, M., Ho, H., Dosey, A.M., Shriver, S., Payandeh, J., et al. (2018). An Unbiased Screen for Human Cytomegalovirus Identifies Neuropilin-2 as a Central Viral Receptor. *Cell*, 174(5): 1158–1171.e19. DOI: 10.1016/j.cell.2018.06.028.

Marty, F.M., Ljungman, P., Chemaly, R.F., Maertens, J., Dadwal, S.S., Duarte, R.F., Haider, S., Ullmann, A.J., Katayama, Y., Brown, J., et al. (2017). Letermovir Prophylaxis for Cytomegalovirus in Hematopoietic-Cell Transplantation. *New England Journal of Medicine*, 377(25): 2433–2444. DOI: 10.1056/NEJMoa1706640.

Matzinger, P. (1994). Tolerance, Danger, and the Extended Family. *Annual Review of Immunology*, 12(1): 991–1045. DOI: 10.1146/annurev.iy.12.040194.005015.

Matzinger, P. (2012). The evolution of the danger theory. *Expert Review of Clinical Immunology*, 8(4): 311–7. DOI: 10.1586/eci.12.21.

Messori, A., Rampazzo, R., Scroccaro, G., and Martini, N. (1994). Efficacy of hyperimmune anti-cytomegalovirus immunoglobulins for the prevention of cytomegalovirus infection in recipients of allogeneic bone marrow transplantation: a meta-analysis. *Bone Marrow Transplantation*, 13(2): 163–7.

Mitchell, D.P., Savaryn, J.P., Moorman, N.J., Shenk, T., and Terhune, S.S. (2009). Human Cytomegalovirus UL28 and UL29 Open Reading Frames Encode a Spliced mRNA and Stimulate Accumulation of Immediate-Early RNAs. *Journal of Virology*, 83(19): 10,187–10,197. DOI: 10.1128/JVI.00396-09.

Miyagawa, F., Tanaka, Y., Yamashita, S., and Minato, N. (2001). Essential requirement of antigen presentation by monocyte lineage cells for the activation of primary human $\gamma\delta$ T cells by aminobisphosphonate antigen. *Journal of Immunology*, 166(9): 5508–14. DOI: 10.4049/jimmunol.166.9.5508.

Mizuguchi, H., Xu, Z., Ishii-Watabe, A., Uchida, E., and Hayakawa, T. (2000). IRES-Dependent Second Gene Expression Is Significantly Lower Than Cap-Dependent First Gene Expression in a Bicistronic Vector. *Molecular Therapy*, 1(4): 376–382. DOI: 10.1006/mthe.2000.0050.

Moosmann, A., Bigalke, I., Tischer, J., Schirrmann, L., Kasten, J., Tippmer, S., Leeping, M., Prevalsek, D., Jaeger, G., Ledderose, G., et al. (2010). Effective and long-term control of EBV PTLD after transfer of peptide-selected T cells. *Blood*, 115(14): 2960–2970. DOI: 10.1182/blood-2009-08-236356.

Moosmann, A., Khan, N., Cobbold, M., Zentz, C., Delecluse, H.J., Hollweck, G., Hislop, A.D., Blake, N.W., Croom-Carter, D., Wollenberg, B., et al. (2002). B cells immortalized by a mini-Epstein-Barr virus encoding a foreign antigen efficiently reactivate specific cytotoxic T cells. *Blood*, 100(5): 1755–64.

Morita, C.T., Jin, C., Sarikonda, G., and Wang, H. (2007). Nonpeptide antigens, presentation mechanisms, and immunological memory of human Vγ2Vδ2 T cells: discriminating friend from foe through the recognition of prenyl pyrophosphate antigens. *Immunological Reviews*, 215(1): 59–76. DOI: 10.1111/j.1600-065X.2006.00479.x.

Moss, P. (2010). The emerging role of cytomegalovirus in driving immune senescence: a novel therapeutic opportunity for improving health in the elderly. *Current Opinion in Immunology*, 22(4): 529–534. DOI: 10.1016/j.coi.2010.07.001.

Moutaftsi, M., Mehl, A.M., Borysiewicz, L.K., and Tabi, Z. (2002). Human cytomegalovirus inhibits maturation and impairs function of monocyte-derived dendritic cells. *Blood*, 99(8): 2913–2921. DOI: 10.1182/blood.v99.8.2913.

Muixí, L., Carrascal, M., Alvarez, I., Daura, X., Martí, M., Armengol, M.P., Pinilla, C., Abian, J., Pujol-Borrell, R., and Jaraquemada, D. (2008). Thyroglobulin peptides associate in vivo to HLA-DR in autoimmune thyroid glands. *Journal of Immunology*, 181(1): 795–807. DOI: 10.4049/jimmunol.181.1.795.

Muñoz, I., Gutiérrez, A., Gimeno, C., Farga, A., Alberola, J., Solano, C., Prósper, F., García-Conde, J., and Navarro, D. (2001). Lack of association between the kinetics of human cytomegalovirus (HCMV) glycoprotein B (gB)-specific and neutralizing serum antibodies and development or recovery from HCMV active infection in patients undergoing allogeneic stem cell transplant. *Journal of Medical Virology*, 65(1): 77–84.

Murphy, E. and Shenk, T. (2008). Human cytomegalovirus genome. *Current Topics in Microbiology and Immunology*, 325: 1–19.

Mutimer, D.J., Shaw, J., O'Donnell, K., and Elias, E. (1997). Enhanced (cytomegalovirus) viral replication after transplantation for fulminant hepatic failure. *Liver Transplantation and Surgery*, 3(5): 506–12. DOI: 10.1002/lt.500030505.

Nastke, M.D., Herrgen, L., Walter, S., Wernet, D., Rammensee, H.G., and Stevanović, S. (2005). Major contribution of codominant CD8 and CD4 T cell epitopes to the human cytomegalovirus-specific T cell repertoire. *Cellular and Molecular Life Sciences*, 62(1): 77–86. DOI: 10.1007/s00018-004-4363-x.

Neudorfer, J., Schmidt, B., Huster, K.M., Anderl, F., Schiemann, M., Holzapfel, G., Schmidt, T., Germeroth, L., Wagner, H., Peschel, C., et al. (2007). Reversible HLA multimers (Streptamers) for the isolation of human cytotoxic T lymphocytes functionally active against tumor- and virus-derived antigens. *Journal of Immunological Methods*, 320(1-2): 119–131. DOI: 10.1016/j.jim.2007.01.001.

Neuenhahn, M., Albrecht, J., Odendahl, M., Schlott, F., Dössinger, G., Schiemann, M., Lakshmipathi, S., Martin, K., Bunjes, D., Harsdorf, S., et al. (2017). Transfer of minimally manipulated CMV-specific T cells from stem cell or third-party donors to treat CMV infection after allo-HSCT. *Leukemia*, 31(10): 2161–2171. DOI: 10.1038/leu.2017.16.

Nigro, G., Adler, S.P., La Torre, R., Best, A.M., and Congenital Cytomegalovirus Collaborating Group (2005). Passive immunization during pregnancy for congenital cytomegalovirus infection. *The New England Journal of Medicine*, 353(13): 1350–62. DOI: 10.1056/NEJMoa043337.

Nimmerjahn, F., Milosevic, S., Behrends, U., Jaffee, E., Pardoll, D., Bornkamm, G., and Mautner, J. (2003). Major histocompatibility complex class II-restricted presentation of a cytosolic antigen by autophagy. *European Journal of Immunology*, 33(5): 1250–1259. DOI: 10.1002/eji.200323730.

Odeberg, J. and Söderberg-Nauclér, C. (2001). Reduced expression of HLA class II molecules and interleukin-10- and transforming growth factor beta1-independent suppression of T-cell proliferation in human cytomegalovirus-infected macrophage cultures. *Journal of Virology*, 75(11): 5174–81. DOI: 10.1128/JVI.75.11.5174-5181.2001.

Pachnio, A., Ciaurriz, M., Begum, J., Lal, N., Zuo, J., Beggs, A., and Moss, P. (2016). Cytomegalovirus Infection Leads to Development of High Frequencies of Cytotoxic Virus-Specific CD4 + T Cells Targeted to Vascular Endothelium. *PLoS Pathogens*, 12(9): 1–25. DOI: 10.1371/journal.ppat.1005832.

Pahl-Seibert, M.F., Juelch, M., Podlech, J., Thomas, D., Deegen, P., Reddehase, M.J., and Holtappels, R. (2005). Highly protective in vivo function of cytomegalovirus IE1 epitope-specific memory CD8 T cells purified by T-cell receptor-based cell sorting. *Journal of Virology*, 79(9): 5400–13. DOI: 10.1128/JVI.79.9.5400-5413.2005.

Papadopoulou, A., Gerdemann, U., Katari, U.L., Tzannou, I., Liu, H., Martinez, C., Leung, K., Carrum, G., Gee, A.P., Vera, J.F., et al. (2014). Activity of broad-spectrum T cells as treatment for AdV, EBV, CMV, BKV, and HHV6 infections after HSCT. *Science Translational Medicine*, 6(242): 242ra83. DOI: 10.1126/scitranslmed.3008825.

Parham, P. (2005). MHC class I molecules and kirs in human history, health and survival. *Nature Reviews Immunology*, 5(3): 201–214. DOI: 10.1038/nri1570.

Parham, P. and Moffett, A. (2013). Variable NK cell receptors and their MHC class I ligands in immunity, reproduction and human evolution. *Nature Reviews Immunology*, 13(2): 133–144. DOI: 10.1038/nri3370.

Park, B., Kim, Y., Shin, J., Lee, S., Cho, K., Früh, K., Lee, S., and Ahn, K. (2004). Human cytomegalovirus inhibits tapasin-dependent peptide loading and optimization of the MHC class I peptide cargo for immune evasion. *Immunity*, 20(1): 71–85. DOI: 10.1016/S1074-7613(03)00355-8.

Park, B., Lee, S., Kim, E., Cho, K., Riddell, S.R., Cho, S., and Ahn, K. (2006). Redox Regulation Facilitates Optimal Peptide Selection by MHC Class I during Antigen Processing. *Cell*, 127(2): 369–382. DOI: 10.1016/j.cell.2006.08.041.

Park, B., Spooner, E., Houser, B.L., Strominger, J.L., and Ploegh, H.L. (2010). The HCMV membrane glycoprotein US10 selectively targets HLA-G for degradation. *The Journal of Experimental Medicine*, 207(9): 2033–41. DOI: 10.1084/jem.20091793.

Pass, R.F., Zhang, C., Evans, A., Simpson, T., Andrews, W., Huang, M.L., Corey, L., Hill, J., Davis, E., Flanigan, C., et al. (2009). Vaccine Prevention of Maternal Cytomegalovirus Infection. *New England Journal of Medicine*, 360(12): 1191–1199. DOI: 10.1056/NEJMoa0804749.

Pauza, C.D., Liou, M.L., Lahusen, T., Xiao, L., Lapidus, R.G., Cairo, C., and Li, H. (2018). Gamma Delta T Cell Therapy for Cancer: It Is Good to be Local. *Frontiers in Immunology*, 9: 1305. DOI: 10.3389/fimmu.2018.01305.

Peggs, K., Verfuerth, S., and Mackinnon, S. (2001). Induction of cytomegalovirus (CMV)-specific T-cell responses using dendritic cells pulsed with CMV antigen: a novel culture system free of live CMV virions. *Blood*, 97(4): 994–1000. DOI: 10.1182/blood.v97.4.994.

Peggs, K.S., Verfuerth, S., Pizzey, A., Khan, N., Guiver, M., Moss, P.A., and Mackinnon, S. (2003). Adoptive cellular therapy for early cytomegalovirus infection after allogeneic stem-cell transplantation with virus-specific T-cell lines. *Lancet (London, England)*, 362(9393): 1375–7. DOI: 10.1016/S0140-6736(03)14634-X.

Pepperl, S., Münster, J., Mach, M., Harris, J.R., and Plachter, B. (2000). Dense bodies of human cytomegalovirus induce both humoral and cellular immune responses in the absence of viral gene expression. *Journal of Virology*, 74(13): 6132–46. DOI: 10.1128/jvi.74.13.6132-6146.2000.

Pepperl-Klindworth, S., Frankenberg, N., Riegler, S., and Plachter, B. (2003). Protein delivery by subviral particles of human cytomegalovirus. *Gene Therapy*, 10(3): 278–84. DOI: 10.1038/sj.gt.3301879.

Peters, B., Bulik, S., Tampe, R., Van Endert, P.M., and Holzhütter, H.G. (2003). Identifying MHC class I epitopes by predicting the TAP transport efficiency of epitope precursors. *Journal of Immunology*, 171(4): 1741–9. DOI: 10.4049/jimmunol.171.4.1741.

Pitard, V., Roumanes, D., Lafarge, X., Couzi, L., Garrigue, I., Lafon, M.E., Merville, P., Moreau, J.F., and Dechanet-Merville, J. (2008). Long-term expansion of effector/memory Vδ2- $\gamma\delta$ T cells is a specific blood signature of CMV infection. *Blood*, 112(4): 1317–1324. DOI: 10.1182/blood-2008-01-136713.

Plotkin, S.A. and Boppana, S.B. (2018). Vaccination against the human cytomegalovirus. *Vaccine*. DOI: 10.1016/j.vaccine.2018.02.089.

Pober, J.S., Collins, T., Gimbrone, M.A., Cotran, R.S., Gitlin, J.D., Fiers, W., Clayberger, C., Krensky, A.M., Burakoff, S.J., and Reiss, C.S. (1983). Lymphocytes recognize human vascular endothelial and dermal fibroblast Ia antigens induced by recombinant immune interferon. *Nature*, 305(5936): 726–9. DOI: 10.1038/305726a0.

Prösch, S., Staak, K., Stein, J., Liebenthal, C., Stamminger, T., Volk, H.D., and Krüger, D.H. (1995). Stimulation of the Human Cytomegalovirus IE Enhancer/Promoter in HL-60 Cells by TNFα Is Mediated via Induction of NF-κB. *Virology*, 208(1): 197–206. DOI: 10.1006/viro.1995.1143.

Prösch, S., Wendt, C.E., Reinke, P., Priemer, C., Oppert, M., Krüger, D.H., Volk, H.D., and Döcke, W.D. (2000). A Novel Link between Stress and Human Cytomegalovirus (HCMV) Infection: Sympathetic Hyperactivity Stimulates HCMV Activation. *Virology*, 272(2): 357–365. DOI: 10.1006/viro.2000.0367.

Pulvertaft, J.V. (1964). Cytology of Burkitt's Tumour (African Lymphoma). *Lancet (London, England)*, 1(7327): 238–40. DOI: 10.1016/S0140-6736(64)92345-1.

Qi, Q., Liu, Y., Cheng, Y., Glanville, J., Zhang, D., Lee, J.Y., Olshen, R.A., Weyand, C.M., Boyd, S.D., and Goronzy, J.J. (2014). Diversity and clonal selection in the human T-cell repertoire. *Proceedings of the National Academy of Sciences*, 111(36): 13,139–13,144. DOI: 10.1073/pnas.1409155111.

Quinnan, G.V., Kirmani, N., Rook, A.H., Manischewitz, J.F., Jackson, L., Moreschi, G., Santos, G.W., Saral, R., and Burns, W.H. (1982). Cytotoxic T Cells in Cytomegalovirus Infection. *New England Journal of Medicine*, 307(1): 7–13. DOI: 10.1056/NEJM198207013070102.

Raftery, M.J., Schwab, M., Eibert, S.M., Samstag, Y., Walczak, H., and Schönrich, G. (2001). Targeting the function of mature dendritic cells by human cytomegalovirus: a multilayered viral defense strategy. *Immunity*, 15(6): 997–1009. DOI: 10.1016/S1074-7613(01)00239-4.

Rajasekaran, K., Xiong, V., Fong, L., Gorski, J., and Malarkannan, S. (2010). Functional dichotomy between NKG2D and CD28-mediated co-stimulation in human CD8+ T cells. *PloS ONE*, 5(9): e12,635. DOI: 10.1371/journal.pone.0012635.

Reddehase, M.J. (2002). Antigens and immunoevasins: opponents in cytomegalovirus immune surveillance. *Nature Reviews Immunology*, 2(11): 831–844. DOI: 10.1038/nri932.

Rehm, A., Engelsberg, A., Tortorella, D., Korner, I.J., Lehmann, I., Ploegh, H.L., Hopken, U.E., Körner, I.J., Lehmann, I., Ploegh, H.L., et al. (2002). Human Cytomegalovirus Gene Products US2 and US11 Differ in Their Ability To Attack Major Histocompatibility Class I Heavy Chains in Dendritic Cells. *Journal of Virology*, 76(10): 5043–5050. DOI: 10.1128/jvi.76.10.5043-5050.2002.

Reinhardt, A., Ravens, S., Fleige, H., Haas, J.D., Oberdörfer, L., Łyszkiewicz, M., Förster, R., and Prinz, I. (2014). CCR7-mediated migration in the thymus controls $\gamma\delta$ T-cell development. *European Journal of Immunology*, 44(5): 1320–1329. DOI: 10.1002/eji.201344330.

Reiser, M., Wieland, A., Plachter, B., Mertens, T., Greiner, J., and Schirmbeck, R. (2011). The immunodominant CD8 T cell response to the human cytomegalovirus tegument phosphoprotein pp65(495-503) epitope critically depends on CD4 T cell help in vaccinated HLA-A*0201 transgenic mice. *Journal of Immunology*, 187(5): 2172–80. DOI: 10.4049/jimmunol.1002512.

Rentenaar, R.J., Gamadia, L.E., van DerHoek, N., van Diepen, F.N., Boom, R., Weel, J.F., Wertheim-van Dillen, P.M., van Lier, R.A., and ten Berge, I.J. (2000). Development of virus-specific CD4+ T cells during primary cytomegalovirus infection. *The Journal of Clinical Investigation*, 105(4): 541–8. DOI: 10.1172/JCI8229.

Renzette, N., Bhattacharjee, B., Jensen, J.D., Gibson, L., and Kowalik, T.F. (2011). Extensive genome-wide variability of human cytomegalovirus in congenitally infected infants. *PLoS Pathogens*, 7(5): e1001,344. DOI: 10.1371/journal.ppat.1001344.

Renzette, N., Pokalyuk, C., Gibson, L., Bhattacharjee, B., Schleiss, M.R., Hamprecht, K., Yamamoto, A.Y., Mussi-Pinhata, M.M., Britt, W.J., Jensen, J.D., et al. (2015). Limits and patterns of cytomegalovirus genomic diversity in humans. *Proceedings of the National Academy of Sciences*, 112(30): E4120–E4128. DOI: 10.1073/pnas.1501880112.

Reusser, P., Riddell, S.R., Meyers, J.D., and Greenberg, P.D. (1991). Cytotoxic T-lymphocyte response to cytomegalovirus after human allogeneic bone marrow transplantation: pattern of recovery and correlation with cytomegalovirus infection and disease. *Blood*, 78(5): 1373–80.

Revello, M.G., Lazzarotto, T., Guerra, B., Spinillo, A., Ferrazzi, E., Kustermann, A., Guaschino, S., Vergani, P., Todros, T., Frusca, T., et al. (2014). A Randomized Trial of Hyperimmune Globulin to Prevent Congenital Cytomegalovirus. *New England Journal of Medicine*, 370(14): 1316–1326. DOI: 10.1056/NEJMoa1310214.

Reyda, S., Büscher, N., Tenzer, S., and Plachter, B. (2014). Proteomic analyses of human cytomegalovirus strain AD169 derivatives reveal highly conserved patterns of viral and cellular proteins in infected fibroblasts. *Viruses*, 6(1): 172–188. DOI: 10.3390/v6010172.

Riddell, S.R., Gilbert, M.J., and Greenberg, P.D. (1993). CD8+ cytotoxic T cell therapy of cytomegalovirus and HIV infection. *Current Opinion in Immunology*, 5(4): 484–91. DOI: 10.1016/0952-7915(93)90027-P.

Riddell, S.R. and Greenberg, P.D. (1995). Principles for Adoptive T Cell Therapy of Human Viral Diseases. *Annual Review of Immunology*, 13(1): 545–586. DOI: 10.1146/annurev.iy.13.040195.002553.

Riddell, S.R., Watanabe, K.S., Goodrich, J.M., Li, C.R., Agha, M.E., and Greenberg, P.D. (1992). Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones. *Science*, 257(5067): 238–41. DOI: 10.1126/science.1352912.

Riegler, S., Hebart, H., Einsele, H., Brossart, P., Jahn, G., and Sinzger, C. (2000). Monocyte-derived dendritic cells are permissive to the complete replicative cycle of human cytomegalovirus. *The Journal of General Virology*, 81(Pt 2): 393–9. DOI: 10.1099/0022-1317-81-2-393.

Ringdén, O., Pihlstedt, P., Volin, L., Nikoskelainen, J., Lönnqvist, B., Ruutu, P., Ruutu, T., Toivanen, A., and Wahren, B. (1987). Failure to prevent cytomegalovirus infection by cytomegalovirus hyperimmune plasma: a randomized trial by the Nordic Bone Marrow Transplantation Group. *Bone Marrow Transplantation*, 2(3): 299–305.

Ross, S.A. and Boppana, S.B. (2005). Congenital cytomegalovirus infection: Outcome and diagnosis. *Seminars in Pediatric Infectious Diseases*, 16(1): 44–49. DOI: 10.1053/j.spid.2004.09.011.

Rubin, R.H. (2007). The pathogenesis and clinical management of cytomegalovirus infection in the organ transplant recipient: the end of the 'silo hypothesis'. *Current Opinion in Infectious Diseases*, 20(4): 399–407. DOI: 10.1097/QCO.0b013e328285a358.

Ryckman, B.J., Chase, M.C., and Johnson, D.C. (2010). Human cytomegalovirus TR strain glycoprotein O acts as a chaperone promoting gH/gL incorporation into virions but is not present in virions. *Journal of Virology*, 84(5): 2597–609. DOI: 10.1128/JVI.02256-09.

Sabbaj, S., Pass, R.F., Goepfert, P.A., and Pichon, S. (2011). Glycoprotein B vaccine is capable of boosting both antibody and CD4 T-cell responses to cytomegalovirus in chronically infected women. *The Journal of Infectious Diseases*, 203(11): 1534–41. DOI: 10.1093/infdis/jir138.

Sacre, K., Nguyen, S., Deback, C., Carcelain, G., Vernant, J.P., Leblond, V., Autran, B., and Dhedin, N. (2008). Expansion of Human Cytomegalovirus (HCMV) Immediate-Early 1-Specific CD8+ T Cells and Control of HCMV Replication after Allogeneic Stem Cell Transplantation. *Journal of Virology*, 82(20): 10,143–10,152. DOI: 10.1128/JVI.00688-08.

Saito, T., Hochstenbach, F., Marusic-Galesic, S., Kruisbeek, A.M., Brenner, M., and Germain, R.N. (1988). Surface expression of only gamma delta and/or alpha beta T cell receptor heterodimers by cells with four (alpha, beta, gamma, delta) functional receptor chains. *The Journal of Experimental Medicine*, 168(3): 1003–20. DOI: 10.1084/jem.168.3.1003.

Sallusto, F., Geginat, J., and Lanzavecchia, A. (2004). Central Memory and Effector Memory T Cell Subsets: Function, Generation, and Maintenance. *Annual Review of Immunology*, 22(1): 745–763. DOI: 10.1146/annurev.immunol.22.012703.104702.

Sandstrom, A., Peigné, C.M., Léger, A., Crooks, J.E., Konczak, F., Gesnel, M.C., Breathnach, R., Bonneville, M., Scotet, E., and Adams, E.J. (2014). The intracellular B30.2 domain of butyrophilin 3A1 binds phosphoantigens to mediate activation of human Vγ9Vδ2 T cells. *Immunity*, 40(4): 490–500. DOI: 10.1016/j.immuni.2014.03.003.

Savage, P.A., Boniface, J.J., and Davis, M.M. (1999). A kinetic basis for T cell receptor repertoire selection during an immune response. *Immunity*, 10(4): 485–92. DOI: 10.1016/S1074-7613(00)80048-5.

Saveanu, L., Carroll, O., Lindo, V., Del Val, M., Lopez, D., Lepelletier, Y., Greer, F., Schomburg, L., Fruci, D., Niedermann, G., et al. (2005). Concerted peptide trimming by human ERAP1 and ERAP2 aminopeptidase complexes in the endoplasmic reticulum. *Nature Immunology*, 6(7): 689–697. DOI: 10.1038/ni1208.

Scaviner, D. and Lefranc, M.P. (2000a). The Human T Cell Receptor Alpha Joining (TRAJ) Genes. *Experimental and Clinical Immunogenetics*, 17(2): 97–106. DOI: 10.1159/000019129.

Scaviner, D. and Lefranc, M.P. (2000b). The Human T Cell Receptor Alpha Variable (TRAV) Genes. *Experimental and Clinical Immunogenetics*, 17(2): 83–96. DOI: 10.1159/000019128.

Scheper, W., Sebestyen, Z., and Kuball, J. (2014). Cancer Immunotherapy Using $\gamma\delta$T Cells: Dealing with Diversity. *Frontiers in Immunology*, 5: 601. DOI: 10.3389/fimmu.2014.00601.

Schleiss, M.R. (2016). Cytomegalovirus vaccines under clinical development. *Journal of Virus Eradication*, 2(4): 198–207.

Schlott, F., Steubl, D., Ameres, S., Moosmann, A., Dreher, S., Heemann, U., Hösel, V., Busch, D.H., and Neuenhahn, M. (2018). Characterization and clinical enrichment of HLA-C*07:02-restricted Cytomegalovirus-specific CD8+ T cells. *PLoS ONE*, 13(2): e0193,554. DOI: 10.1371/journal.pone.0193554.

Schmitt, A., Tonn, T., Busch, D.H., Grigoleit, G.U., Einsele, H., Odendahl, M., Germeroth, L., Ringhoffer, M., Ringhoffer, S., Wiesneth, M., et al. (2011). Adoptive transfer and selective reconstitution of streptamer-selected cytomegalovirus-specific CD8+ T cells leads to virus clearance in patients after allogeneic peripheral blood stem cell transplantation. *Transfusion*, 51(3): 591–599. DOI: 10.1111/j.1537-2995.2010.02940.x.

Schub, A. (2010). *Herpesvirusspezifische T-Zellen für die Immuntherapie: Von ihrer Identifikation zum Transfer ihrer Spezifität.* Ph.D. thesis, Ludwig-Maximilians-Universität München. URL: https://edoc.ub.uni-muenchen.de/11331/.

Schub, A., Schuster, I.G., Hammerschmidt, W., and Moosmann, A. (2009). CMV-specific TCR-transgenic T cells for immunotherapy. *Journal of Immunology*, 183(10): 6819–30. DOI: 10.4049/jimmunol.0902233.

Schulze, M.S.E.D. and Wucherpfennig, K.W. (2012). The mechanism of HLA-DM induced peptide exchange in the MHC class II antigen presentation pathway. *Current Opinion in Immunology*, 24(1): 105–11. DOI: 10.1016/j.coi.2011.11.004.

Schust, D.J., Tortorella, D., and Ploegh, H.L. (1999). Viral immunoevasive strategies and trophoblast class I major histocompatibility complex antigens. *Journal of Reproductive Immunology*, 43(2): 243–251. DOI: 10.1016/S0165-0378(99)00024-8.

Schust, D.J., Tortorella, D., Seebach, J., Phan, C., and Ploegh, H.L. (1998). Trophoblast Class I Major Histocompatibility Complex (MHC) Products Are Resistant to Rapid Degradation Imposed by the Human Cytomegalovirus (HCMV) Gene Products US2 and US11. *The Journal of Experimental Medicine*, 188(3): 497–503. DOI: 10.1084/jem.188.3.497.

Schuster, I. (2008). *Identifikation, Klonierung und retroviraler Transfer allorestringierter FMNL1-peptidspezifischer T-Zellrezeptoren für die Entwicklung adoptiver Immuntherapien gegen B-Zell-Non-Hodgkin-Lymphome.* Ph.D. thesis, Ludwig-Maximilians-Universität München. URL: https://edoc.ub.uni-muenchen.de/9269/.

Schuster, S.J., Svoboda, J., Chong, E.A., Nasta, S.D., Mato, A.R., Anak, Ö., Brogdon, J.L., Pruteanu-Malinici, I., Bhoj, V., Landsburg, D., et al. (2017). Chimeric Antigen Receptor T Cells in Refractory B-Cell Lymphomas. *New England Journal of Medicine*, 377(26): 2545–2554. DOI: 10.1056/NEJMoa1708566.

Schwele, S., Fischer, A.M., Brestrich, G., Wlodarski, M.W., Wagner, L., Schmueck, M., Roemhild, A., Thomas, S., Hammer, M.H., Babel, N., et al. (2012). Cytomegalovirus-specific regulatory and effector T cells share TCR clonality–possible relation to repetitive CMV infections. *American Journal of Transplantation*, 12(3): 669–81. DOI: 10.1111/j.1600-6143.2011.03842.x.

Scrivano, L., Sinzger, C., Nitschko, H., Koszinowski, U.H., and Adler, B. (2011). HCMV spread and cell tropism are determined by distinct virus populations. *PLoS Pathogens*, 7(1). DOI: 10.1371/journal.ppat.1001256.

Sebestyen, Z., Scheper, W., Vyborova, A., Gu, S., Rychnavska, Z., Schiffler, M., Cleven, A., Chéneau, C., Van Noorden, M., Peigné, C.M.M., et al. (2016). RhoB Mediates Phosphoantigen Recognition by Vγ9Vδ2 T Cell Receptor. *Cell Reports*, 15(9): 1973–1985. DOI: 10.1016/j.celrep.2016.04.081.

Sellar, R.S. and Peggs, K.S. (2012). Therapeutic strategies for the prevention and treatment of cytomegalovirus infection. *Expert Opinion on Biological Therapy*, 12(9): 1161–1172. DOI: 10.1517/14712598.2012.693471.

Sénéchal, B., Boruchov, A.M., Reagan, J.L., Hart, D.N., and Young, J.W. (2004). Infection of mature monocyte-derived dendritic cells with human cytomegalovirus inhibits stimulation of T-cell proliferation via the release of soluble CD83. *Blood*, 103(11): 4207–4215. DOI: 10.1182/blood-2003-12-4350.

Sester, M., Sester, U., Gärtner, B., Heine, G., Girndt, M., Mueller-Lantzsch, N., Meyerhans, A., and Köhler, H. (2001). Levels of virus-specific CD4 T cells correlate with cytomegalovirus control and predict virus-induced disease after renal transplantation. *Transplantation*, 71(9): 1287–94. DOI: 10.1097/00007890-200105150-00018.

Sharland, M. and Khare, M.D. (2001). Cytomegalovirus treatment options in immunocompromised patients. *Expert Opinion on Pharmacotherapy*, 2(8): 1247–1257. DOI: 10.1517/14656566.2.8.1247.

Shen, Y., Zhou, D., Qiu, L., Lai, X., Simon, M., Shen, L., Kou, Z., Wang, Q., Jiang, L., Estep, J., et al. (2002). Adaptive immune response of Vγ2Vδ2+ T cells during mycobacterial infections. *Science*, 295(5563): 2255–8. DOI: 10.1126/science.1068819.

Sijmons, S., Van Ranst, M., and Maes, P. (2014). Genomic and functional characteristics of human cytomegalovirus revealed by next-generation sequencing. *Viruses*, 6(3): 1049–72. DOI: 10.3390/v6031049.

Simon, P., Omokoko, T.A., Breitkreuz, A., Hebich, L., Kreiter, S., Attig, S., Konur, A., Britten, C.M., Paret, C., Dhaene, K., et al. (2014). Functional TCR retrieval from single antigen-specific human T cells reveals multiple novel epitopes. *Cancer Immunology Research*, 2(12): 1230–44. DOI: 10.1158/2326-6066.CIR-14-0108.

Sinclair, J. and Sissons, P. (2006). Latency and reactivation of human cytomegalovirus. *Journal of General Virology*, 87(7): 1763–1779. DOI: 10.1099/vir.0.81891-0.

Sindre, H., Tjønnfjord, G.E., Rollag, H., Ranneberg-Nilsen, T., Veiby, O.P., Beck, S., Degré, M., and Hestdal, K. (1996). Human cytomegalovirus suppression of and latency in early hematopoietic progenitor cells. *Blood*, 88(12): 4526–33.

Sinzger, C., Digel, M., and Jahn, G. (2008a). Cytomegalovirus cell tropism. *Current Topics in Microbiology and Immunology*, 325: 63–83.

Sinzger, C., Hahn, G., Digel, M., Katona, R., Sampaio, K.L., Messerle, M., Hengel, H., Koszinowski, U., Brune, W., and Adler, B. (2008b). Cloning and sequencing of a highly productive, endotheliotropic virus strain derived from human cytomegalovirus TB40/E. *The Journal of General Virology*, 89(Pt 2): 359–68. DOI: 10.1099/vir.0.83286-0.

Slezak, S.L., Bettinotti, M., Selleri, S., Adams, S., Marincola, F.M., and Stroncek, D.F. (2007). CMV pp65 and IE-1 T cell epitopes recognized by healthy subjects. *Journal of Translational Medicine*, 5: 1–16. DOI: 10.1186/1479-5876-5-17.

Smith, C., Brennan, R.M., Tey, S.K., Smyth, M.J., Burrows, S.R., Miles, J.J., Hill, G.R., and Khanna, R. (2016). Coinfection with Human Cytomegalovirus Genetic Variants in Transplant Recipients and Its Impact on Antiviral T Cell Immune Reconstitution. *Journal of Virology*, 90(16): 7497–7507. DOI: 10.1128/JVI.00297-16.

Snydman, D.R., Werner, B.G., Heinze-Lacey, B., Berardi, V.P., Tilney, N.L., Kirkman, R.L., Milford, E.L., Cho, S.I., Bush, H.L., Levey, A.S., et al. (1987). Use of Cytomegalovirus Immune Globulin to Prevent Cytomegalovirus Disease in Renal-Transplant Recipients. *New England Journal of Medicine*, 317(17): 1049–1054. DOI: 10.1056/NEJM198710223171703.

Sommermeyer, D., Hudecek, M., Kosasih, P.L., Gogishvili, T., Maloney, D.G., Turtle, C.J., and Riddell, S.R. (2016). Chimeric antigen receptor-modified T cells derived from defined CD8+ and CD4+ subsets confer superior antitumor reactivity in vivo. *Leukemia*, 30(2): 492–500. DOI: 10.1038/leu.2015.247.

Spearman, C. (1908). The method of 'right and wrong cases' ('constant stimuli') without Gauss's formulae. *British Journal of Psychology*, 2(3): 227–242. DOI: 10.1111/j.2044-8295.1908.tb00176.x.

Spranger, S., Javorovic, M., Bürdek, M., Wilde, S., Mosetter, B., Tippmer, S., Bigalke, I., Geiger, C., Schendel, D.J., and Frankenberger, B. (2010). Generation of Th1-polarizing dendritic cells using the TLR7/8 agonist CL075. *Journal of Immunology*, 185(1): 738–47. DOI: 10.4049/jimmunol.1000060.

Stein, J., Volk, H.D., Liebenthal, C., Krüger, D.H., and Prösch, S. (1993). Tumour necrosis factor alpha stimulates the activity of the human cytomegalovirus major immediate early enhancer/promoter in immature monocytic cells. *The Journal of General Virology*, 74 (Pt 11(11): 2333–8. DOI: 10.1099/0022-1317-74-11-2333.

Steinle, A., Reinhardt, C., Jantzer, P., and Schendel, D.J. (1995). In vivo expansion of HLA-B35 alloreactive T cells sharing homologous T cell receptors: evidence for maintenance of an oligoclonally dominated allospecificity by persistent stimulation with an autologous MHC/peptide complex. *The Journal of Experimental Medicine*, 181(2): 503–13. DOI: 10.1084/jem.181.2.503.

Stevanovic, S., van Bergen, C.A.M., van Luxemburg-Heijs, S.A.P., van der Zouwen, B., Jordanova, E.S., Kruisselbrink, A.B., van de Meent, M., Harskamp, J.C., Claas, F.H.J., Marijt, E.W.A., et al. (2013). HLA class II upregulation during viral infection leads to HLA-DP-directed graft-versus-host disease after CD4+ donor lymphocyte infusion. *Blood*, 122(11): 1963–1973. DOI: 10.1182/blood-2012-12-470872.

Stinski, M.F. (1978). Sequence of protein synthesis in cells infected by human cytomegalovirus: early and late virus-induced polypeptides. *Journal of Virology*, 26(3): 686–701.

Stitz, J., Buchholz, C., Engelstädter, M., Uckert, W., Bloemer, U., Schmitt, I., and Cichutek, K. (2000). Lentiviral Vectors Pseudotyped with Envelope Glycoproteins Derived from Gibbon Ape Leukemia Virus and Murine Leukemia Virus 10A1. *Virology*, 273(1): 16–20. DOI: 10.1006/viro.2000.0394.

Straetemans, T., Kierkels, G.J.J., Doorn, R., Jansen, K., Heijhuurs, S., Dos Santos, J.M., van Muyden, A.D.D., Vie, H., Clemenceau, B., Raymakers, R., et al. (2018). GMP-Grade Manufacturing of T Cells Engineered to Express a Defined $\gamma\delta$TCR. *Frontiers in Immunology*, 9: 1062. DOI: 10.3389/fimmu.2018.01062.

Sutherland, C.L., Chalupny, N.J., and Cosman, D. (2001). The UL16-binding proteins, a novel family of MHC class I-related ligands for NKG2D, activate natural killer cell functions. *Immunological Reviews*, 181: 185–92. DOI: 10.1034/j.1600-065X.2001.1810115.x.

Sylwester, A.W., Mitchell, B.L., Edgar, J.B., Taormina, C., Pelte, C., Ruchti, F., Sleath, P.R., Grabstein, K.H., Hosken, N.A., Kern, F., et al. (2005). Broadly targeted human cytomegalovirus-specific CD4+ and CD8+ T cells dominate the memory compartments of exposed subjects. *The Journal of Experimental Medicine*, 202(5): 673–685. DOI: 10.1084/jem.20050882.

Szymczak, A.L., Workman, C.J., Wang, Y., Vignali, K.M., Dilioglou, S., Vanin, E.F., and Vignali, D.A.A. (2004). Correction of multi-gene deficiency in vivo using a single 'self-cleaving' 2A peptide-based retroviral vector. *Nature Biotechnology*, 22(5): 589–94. DOI: 10.1038/nbt957.

Talbot, P. and Almeida, J.D. (1977). Human Cytomegalovirus: Purification of Enveloped Virions and Dense Bodies. *Journal of General Virology*, 36(2): 345–349. DOI: 10.1099/0022-1317-36-2-345.

Tanaka, Y., Morita, C.T., Tanaka, Y., Nieves, E., Brenner, M.B., and Bloom, B.R. (1995). Natural and synthetic non-peptide antigens recognized by human $\gamma\delta$ T cells. *Nature*, 375(6527): 155–158. DOI: 10.1038/375155a0.

Thakral, D., Dobbins, J., Devine, L., and Kavathas, P.B. (2008). Differential Expression of the Human CD8 Splice Variants and Regulation of the M-2 Isoform by Ubiquitination. *Journal of Immunology*, 180(11): 7431–7442. DOI: 10.4049/jimmunol.180.11.7431.

The UniProt Consortium (2017). UniProt: the universal protein knowledgebase. *Nucleic Acids Research*, 45(D1): D158–D169. DOI: 10.1093/nar/gkw1099.

Thomas, S., Klobuch, S., Podlech, J., Plachter, B., Hoffmann, P., Renzaho, A., Theobald, M., Reddehase, M.J., Herr, W., and Lemmermann, N.A.W. (2015). Evaluating Human T-Cell Therapy of Cytomegalovirus Organ Disease in HLA-Transgenic Mice. *PLoS Pathogens*, 11(7): e1005,049. DOI: 10.1371/journal.ppat.1005049.

Tomasec, P., Braud, V.M., Rickards, C., Powell, M.B., McSharry, B.P., Gadola, S., Cerundolo, V., Borysiewicz, L.K., McMichael, A.J., and Wilkinson, G.W. (2000). Surface expression of HLA-E, an inhibitor of natural killer cells, enhanced by human cytomegalovirus gpUL40. *Science*, 287(5455): 1031. DOI: 10.1126/science.287.5455.1031.

Tomazin, R., Boname, J., Hedge, N.R., Lewinsohn, D.M., Altschuler, Y., Jones, T.R., Cresswell, P., Nelson, J.A., Riddell, S.R., and Johnson, D.C. (1999). Cytomegalovirus US2 destroys two components of the MHC class II pathway, preventing recognition by CD4+ T cells. *Nature Medicine*, 5(9): 1039–1043. DOI: 10.1038/12478.

Trowsdale, J. (2002). The gentle art of gene arrangement: the meaning of gene clusters. *Genome Biology*, 3(comment2002.1). DOI: 10.1186/gb-2002-3-3-comment2002.

Tu, W., Chen, S., Sharp, M., Dekker, C., Manganello, A.M., Tongson, E.C., Maecker, H.T., Holmes, T.H., Wang, Z., Kemble, G., et al. (2004). Persistent and selective deficiency of CD4+ T cell immunity to cytomegalovirus in immunocompetent young children. *Journal of Immunology*, 172(5): 3260–7. DOI: 10.4049/jimmunol.172.5.3260.

Turtle, C.J., Hanafi, L.A., Berger, C., Gooley, T.A., Cherian, S., Hudecek, M., Sommermeyer, D., Melville, K., Pender, B., Budiarto, T.M., et al. (2016). CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. *Journal of Clinical Investigation*, 126(6): 2123–2138. DOI: 10.1172/JCI85309.

Uckert, W., Becker, C., Gladow, M., Klein, D., Kammertoens, T., Pedersen, L., and Blankenstein, T. (2000). Efficient Gene Transfer into Primary Human CD8+ T Lymphocytes by MuLV-10A1 Retrovirus Pseudotype. *Human Gene Therapy*, 11(7): 1005–1014. DOI: 10.1089/10430340050015310.

Valitutti, S., Müller, S., Cella, M., Padovan, E., and Lanzavecchia, A. (1995). Serial triggering of many T-cell receptors by a few peptide-MHC complexes. *Nature*, 375(6527): 148–151. DOI: 10.1038/375148a0.

Varnum, S.M., Streblow, D.N., Monroe, M.E., Smith, P., Auberry, K.J., Pas, L., Wang, D., Ii, D.G.C., Rodland, K., Wiley, S., et al. (2004). Identification of Proteins in Human Cytomegalovirus (HCMV) Particles: the HCMV Proteome. *Virology*, 78(20): 10,960–10,966. DOI: 10.1128/JVI.78.20.10960-10966.2004.

Vavassori, S., Kumar, A., Wan, G.S., Ramanjaneyulu, G.S., Cavallari, M., El Daker, S., Beddoe, T., Theodossis, A., Williams, N.K., Gostick, E., et al. (2013). Butyrophilin 3A1 binds phosphorylated antigens and stimulates human $\gamma\delta$ T cells. *Nature Immunology*, 14(9): 908–916. DOI: 10.1038/ni.2665.

van der Veken, L.T., Coccoris, M., Swart, E., Falkenburg, J.H.F., Schumacher, T.N., and Heemskerk, M.H.M. (2009). $\alpha\beta$ T cell receptor transfer to $\gamma\delta$ T cells generates functional effector cells without mixed TCR dimers in vivo. *The Journal of Immunology*, 182(1): 164–170. DOI: 10.4049/jimmunol.182.1.164.

van der Veken, L.T., Hagedoorn, R.S., Van Loenen, M.M., Willemze, R., Falkenburg, J.H.F., and Heemskerk, M.H.M. (2006). $\alpha\beta$ T-cell receptor engineered $\gamma\delta$ T cells mediate effective antileukemic reactivity. *Cancer Research*, 66(6): 3331–3337. DOI: 10.1158/0008-5472.CAN-05-4190.

Virgin, H.W., Wherry, E.J., and Ahmed, R. (2009). Redefining Chronic Viral Infection. *Cell*, 138(1): 30–50. DOI: 10.1016/j.cell.2009.06.036.

Walker, C.M., van Burik, J.A.H., De For, T.E., and Weisdorf, D.J. (2007). Cytomegalovirus Infection after Allogeneic Transplantation: Comparison of Cord Blood with Peripheral Blood and Marrow Graft Sources. *Biology of Blood and Marrow Transplantation*, 13(9): 1106–1115. DOI: 10.1016/j.bbmt.2007.06.006.

Walter, E.A., Greenberg, P.D., Gilbert, M.J., Finch, R.J., Watanabe, K.S., Thomas, E.D., and Riddell, S.R. (1995). Reconstitution of Cellular Immunity against Cytomegalovirus in Recipients of Allogeneic Bone Marrow by Transfer of T-Cell Clones from the Donor. *New England Journal of Medicine*, 333(16): 1038–1044. DOI: 10.1056/NEJM199510193331603.

Wang, D. and Shenk, T. (2005). Human Cytomegalovirus UL131 Open Reading Frame Is Required for Epithelial Cell Tropism. *Journal of Virology*, 79(16): 10,330–10,338. DOI: 10.1128/JVI.79.16.10330-10338.2005.

Weekes, M.P., Wills, M.R., Sissons, J.G.P., and Carmichael, A.J. (2004). Long-term stable expanded human CD4+ T cell clones specific for human cytomegalovirus are distributed in both CD45RAhigh and CD45ROhigh populations. *Journal of Immunology*, 173(9): 5843–51. DOI: 10.4049/jimmunol.173.9.5843.

Wiertz, E.J., Jones, T.R., Sun, L., Bogyo, M., Geuze, H.J., and Ploegh, H.L. (1996). The human cytomegalovirus US11 gene product dislocates MHC class I heavy chains from the endoplasmic reticulum to the cytosol. *Cell*, 84(5): 769–79. DOI: 10.1016/s0092-8674(00)81054-5.

Wiesner, M. (2005). *Die Diversität Cytomegalovirus-spezifischer T-Zellen nach Selektion mittels B-Zell-Vektoren.* Ph.D. thesis, Ludwig-Maximilians-Universität München. URL: https://edoc.ub.uni-muenchen.de/4203/.

Wiesner, M., Zentz, C., Hammer, M.H., Cobbold, M., Kern, F., Kolb, H.J., Hammerschmidt, W., Zeidler, R., and Moosmann, A. (2005). Selection of CMV-specific CD8+ and CD4+ T cells by mini-EBV-transformed B cell lines. *European Journal of Immunology*, 35(7): 2110–2121. DOI: 10.1002/eji.200425936.

Wilhelm, M., Kunzmann, V., Eckstein, S., Reimer, P., Weissinger, F., Ruediger, T., and Tony, H.P. (2003). $\gamma\delta$ T cells for immune therapy of patients with lymphoid malignancies. *Blood*, 102(1): 200–6. DOI: 10.1182/blood-2002-12-3665.

Willcox, C.R., Davey, M.S., and Willcox, B.E. (2018). Development and Selection of the Human Vγ9Vδ2+ T-Cell Repertoire. *Frontiers in Immunology*, 9: 1501. DOI: 10.3389/fimmu.2018.01501.

Willcox, C.R., Pitard, V., Netzer, S., Couzi, L., Salim, M., Silberzahn, T., Moreau, J.F., Hayday, A.C., Willcox, B.E., and Déchanet-Merville, J. (2012). Cytomegalovirus and tumor stress surveillance by binding of a human $\gamma\delta$ T cell antigen receptor to endothelial protein C receptor. *Nature Immunology*, 13(9): 872–9. DOI: 10.1038/ni.2394.

Yasui, M., Suenaga, E., Koyama, N., Masutani, C., Hanaoka, F., Gruz, P., Shibutani, S., Nohmi, T., Hayashi, M., and Honma, M. (2008). Miscoding properties of 2'-deoxyinosine, a nitric oxide-derived DNA Adduct, during translesion synthesis catalyzed by human DNA polymerases. *Journal of Molecular Biology*, 377(4): 1015–23. DOI: 10.1016/j.jmb.2008.01.033.

Yunis, J., Farrell, H.E., Bruce, K., Lawler, C., Sidenius, S., Wyer, O., Davis-Poynter, N., and Stevenson, P.G. (2018). Murine cytomegalovirus degrades MHC class II to colonize the salivary glands. *PLoS Pathogens*, 14(2): 1–17. DOI: 10.1371/journal.ppat.1006905.

Zaia, J.A., Gallez-Hawkins, G.M., Tegtmeier, B.R., ter Veer, A., Li, X., Niland, J.C., and Forman, S.J. (1997). Late cytomegalovirus disease in marrow transplantation is predicted by virus load in plasma. *The Journal of Infectious Diseases*, 176(3): 782–5. DOI: 10.1086/517301.

Zhao, Y. and Biegalke, B.J. (2003). Functional analysis of the human cytomegalovirus immune evasion protein, pUS3 (22kDa). *Virology*, 315(2): 353–61. DOI: 10.1016/s0042-6822(03)00545-2.

Zheng, J., Liu, Y., Lau, Y.L., and Tu, W. (2013). $\gamma\delta$-T cells: an unpolished sword in human anti-infection immunity. *Cellular & Molecular Immunology*, 10(1): 50–57. DOI: 10.1038/cmi.2012.43.

Zhou, D., Srivastava, R., Grummel, V., Cepok, S., Hartung, H.P., and Hemmer, B. (2006). High throughput analysis of TCR-beta rearrangement and gene expression in single T cells. *Laboratory Investigation*, 86(3): 314–21. DOI: 10.1038/labinvest.3700381.

Zino, E., Frumento, G., Marktel, S., Sormani, M.P., Ficara, F., Di Terlizzi, S., Parodi, A.M., Sergeant, R., Martinetti, M., Bontadini, A., et al. (2003). A T-cell epitope encoded by a subset of HLA-DPB1 alleles determines nonpermissive mismatches for hematologic stem cell transplantation. *Blood*, 103(4): 1417–1424. DOI: 10.1182/blood-2003-04-1279.

Zino, E., Vago, L., Di Terlizzi, S., Mazzi, B., Zito, L., Sironi, E., Rossini, S., Bonini, C., Ciceri, F., Roncarolo, M.G., et al. (2007). Frequency and targeted detection of HLA-DPB1 T cell epitope disparities relevant in unrelated hematopoietic stem cell transplantation. *Biology of Blood and Marrow Transplantation*, 13(9): 1031–40. DOI: 10.1016/j.bbmt.2007.05.010.

List of Abbreviations

Abbreviation	Full name
A	Adenosine
aa	Amino acid
AF	Allele frequency
Ag	Antigen
A/I ratio	Active/inactive virus recognition ratio
AIDS	Acquired immunodeficiency syndrome
ALP	Alkaline phosphatase
APC	Allophycocyanin
APC	Antigen presenting cell
ATP	Adenosine triphosphate
AU	Arbitrary units
β2-m	β2-microglobulin
BCR	B cell receptor
bp	Base pairs
BTN	Butyrophilin
BV	Brilliant violet
C	Constant
C	Cytidine
C (terminus)	Carboxy (terminus)
CAR	Chimeric antigen receptor
Cat.No.	Catalogue number
CD	Cluster of differentiation (cell surface marker)
cDNA	Complementary desoxyribonucleic acid
CDR	Complementarity determining region
CIITA	(HLA) Class II transactivator
CM	Central memory (T cell subpopulation)
CTL	Cytotoxic T lymphocyte
Ctrl	Control
Cy	Cyanine (dye)
D	Diversity
DC	Dendritic cell
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
DNA	Desoxyribonucleic acid
dNTP	Desoxynucleoside triphosphate
DOI	Digital object idenfifier
DP	Double-positive (for CD4 and CD8 co-receptor)
EBV	Epstein-Barr virus (HHV-4)
EC_{50}	Half maximal effective concentration
EDTA	Ethylenediaminetetraacetic acid
e.g.	Latin: exempli gratia (meaning "for example")
ELISA	Enzyme-linked immunosorbent assay
EM	Effector memory (T cell subpopulation)
EMRA	Effector memory (T cell subpopulation), re-expressing CD45RA
ER	Endoplasmic reticulum
ERAAP	ER aminopeptidase associated with antigen processing
e:t (ratio)	Effector-to-target (ratio)

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Abbreviation	Full name
et al.	Latin: et alii (meaning “and others”)
FACS	Fluorescence-activated cell sorting
FBS	Fetal bovine serum (also called FCS)
FCS	Fetal calf serum (also called FBS)
Fig.	Figure
FITC	Fluoresceinisothiocyanate
FSC	Forward scatter
G	Guanosine
GFP	Green fluorescent protein
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GMP	Good manufacturing practice
gp	Glycoprotein
GTP	Guanosine triphosphate
GTPase	Enzyme that binds and hydrolyses GTP
GvHD	Graft-versus-host disease
GvL	Graft-versus-leukemia
Gy	Gray (unit of energy dose, e.g. for ionising radiation; Gy = J/kg
HCMV	Human cytomegalovirus
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HHV	Human herpes virus
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HMB-PP	(E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate
HPC	Hematopoietic progenitor cell
HS	Human serum
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplantation
IC_{50}	Half maximal inhibitory concentration
ICOS	Inducible co-stimulator
i.e.	Latin: id est (meaning “that is to say”)
IE	Immediate-early
IE-1	Immediate-early 1 protein
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IMGT	International ImMunoGeneTics information system (http://www.imgt.org/, founder and director: Marie-Paule Lefranc, Montpellier, France)
IPP	Isopentenyl pyrophosphate
IRES	Internal ribosome entry site
ITAM	Immunoreceptor tyrosine-based activation motif
J	Joining
kb	Kilobases (or kilo base pairs)
kDa	Kilodalton
KIR	Killer cell immunoglobulin-like receptor
L	Ligand (e.g. CD40L)
LCL	Lymphoblastoid cell line
LILRB1	Leukocyte immunoglobulin-like receptor subfamily B member 1

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Abbreviation	Full name
LPS	Lipopolysaccharide
LTR	Long terminal repeat
MACS	Magnetic-activated cell sorting
M-CSF	Macrophage colony-stimulating factor
MCMV	Murine cytomegalovirus
MEP pathway	2-C-methyl-D-erythritol 4-phosphate pathway
MFI	Mean fluorescence intensity
MHC	Major histocompatibility complex
mini-EBV	Genetically modified Epstein Barr Virus that contains a reduced set of viral genes but all necessary genes and elements for transformation of primary B cells
mini-LCL	Lymphoblastoid cell line created by using mini-EBV
mio	Million (10^6)
miRNA	Micro-ribonucleic acid (non-coding)
moDC	Monocyte-derived dendritic cell
MOI	Multiplicity of infection
mRNA	Messenger ribonucleic acid
mu	Murinised
MVA	Modified vaccinia Ankara
N	Naïve (T cell subpopulation)
N (terminus)	Amino (terminus)
n/a	Not applicable
NCBI	National Center for Biotechnology Information https://www.ncbi.nlm.nih.gov
n.d.	Not determined
NEAA	Non-essential amino acids
NK cell	Natural killer cell
NOD	Nuclear oligomerisation domain
nt	Nucleotide
ORF	Open reading frame
P2A	Picornavirus 'self-cleaving' 2A peptide
PAM	Pamidronate
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
pCMV	Human cytomegalovirus immediate-early enhancer and promoter
PCR	Polymerase chain reaction
PE	Phycoerythrin
PerCp	Peridin-chlorophyll-protein complex
PFA	Paraformaldehyde
pfu	Plaque-forming unit
PGE	Prostaglandine
p.i.	Post infection
PLC	Peptide-loading complex
PMT	Photomultiplier tube
pNPP	Para-Nitrophenylphosphate
pos.	Position
pp65	Phosphoprotein 65

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Abbreviation	Full name
PRR	Pattern recognition receptor
rhu	Recombinant human
RNA	Ribonucleic acid
rpm	Revolutions per minute
RPMI	Roswell Park Memorial Institute (cell culture medium)
SD	Standard deviation
SSC	Side scatter
SOT	Solid organ transplantation
SP	Single-positive (for CD4 and CD8 co-receptor)
T	Thymidine
Tab.	Table
TAE	Tris, acetate & EDTA
TAP	Transporter associated with antigen processing
$TCID_{50}$	50% infectious tissue culture dose
TCR	T cell receptor
T_H cell	Helper T cell or T helper cell
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TPA	Tetradecanoylphorbol acetate
U	Units
UL	Unique long
US	Unique short
UV	Ultraviolet
V	Variable
vs.	Versus
v/v	Volume per volume
w/v	Weight per volume
WT	Wild-type
× g	Unit of gravity (“times gravity”)

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