Role of the Nlrp3 inflammasome in regulation of the tolerogenic function of $CD103^{+}$ dendritic cells in $CD4^{+}CD45Rb^{High}$ T cell transfer colitis and in steady state

Rachel Mak'Anyengo

2016

Aus der Abteilung für Klinische Pharmakologie

Leiter: Prof. Dr. med. Stefan Endres

Medizinische Klinik und Poliklinik IV

Klinikum der Universität

Ludwig-Maximilians-Universität München

Direktor: Prof. Dr. med. Martin Reincke

Role of the Nlrp3 inflammasome in regulation of the tolerogenic function of $CD103^{+}$ dendritic cells in $CD4^{+}CD45Rb^{High}$ T cell transfer colitis and in steady state

Dissertation

zum Erwerb des Doktorgrades der Humanbiologie

an der Medizinischen Fakultät der

Ludwig-Maximilians-Universität zu München

vorgelegt von

Rachel Mak'Anyengo

aus

Nairobi, Kenia

2017

Mit Genehmigung der Medizinischen Fakultät

der Universität München

Berichterstatter:	PD Dr. med. Christian Bauer
Mitberichterstatter:	Prof. Dr. med. Martin Storr
	PD Dr. med. Florian Beigel
	PD Dr. med Fabian Schnitzler
Mitbetreuung durch den	
Arbeitsgruppenleiter:	Prof. Dr. med. Max Schnurr
promovierten Mitarbeiter:	Dr. rer. biol. hum. Peter Düwell
Dekan:	Prof. Dr. med. dent. Reinhard Hickel

Tag der mündlichen Prüfung: 04.08.2017

_____ _____

Ort, Datum Unterschrift, Prof. Stefan Endres

Contents

1 Abstract

Inflammatory bowel disease (IBD) is a group of relapsing inflammatory conditions resulting from dysregulation of the mucosal immune system in the colon and small intestine. Although the pathophysiology is not yet fully understood, possible mechanisms include genetic disposition, damage of the mucosal barrier with increased epithelial permeability, endoluminal bacterial triggers resulting in the activation of lymphocytes and macrophages, and imbalance in the production of proinflammatory and antiinflammatory cytokines. Increased levels of interleukin (IL)-1β and IL-18 have been detected in the mucosa of intestines of patients suffering from IBD as well as in IBD animal models. The immature forms (pro-IL-1β and pro-IL-18) of these two inflammatory cytokines are mainly activated via a caspase-1 activating multiprotein complex, the Nlrp3 inflammasome. Dextran sodium sulphate (DSS)-induced colitis is an important model for the study of mucosal damage and innate immunity in IBD. Previous work of our group reported the ability of macrophages to take up DSS, leading to activation of the Nlrp3 inflammasome. Nlrp3-deficient mice were protected from deleterious effects of DSS administration. However, this model is generally believed to be less appropriate for studying the role of the adaptive immune system in IBD. Therefore, further studies with alternative models that are capable of clarifying the immunological mechanisms underlying the regulation of intestinal inflammation are urgently needed.

The main aims of this project were: a) to investigate the role of Nlrp3-dependent cytokines IL-1β and IL-18 in induction of colitis in a T cell transfer model of colitis, b) to characterise intestinal dendritic cells (DCs) as the cellular platform of Nlrp3 effects resulting in the regulation of T cell plasticity, c) to investigate the role of IL-1R and IL-18R signalling in adoptively transferred T cells, and d) to rule out biasing effects of differences in microbiota compositions of Nlrp3-sufficient and Nlrp3-deficient mice.

This study showed that Nlrp3 inflammasome plays a critical role in inducing T cell-mediated inflammation. The balance of Nlrp3-dependent cytokines IL-1β and IL-18 regulated the T cell-induced inflammation with IL-1β proving to be the main inducer of T cell-mediated colon inflammation. Nlrp3-deficient mice that were adoptively transferred with $CD4^{+}CD45Rb^{High}$ T cells had less colonic inflammation. Reduced colonic inflammation correlated with less pronounced T cell infiltration. In Nlrp3-sufficient mice, lamina propria (LP)-infiltrating T helper cells demonstrated an inflammatory Th17/Th1 phenotype, resulting in increased levels of T cell-dependent inflammatory cytokines such as IL-17, IL-22, and IP-10.

Abstract

$CD4^+$ T cells primed with Nlrp3-sufficient DCs demonstrated an inflammatory phenotype, pinpointing DCs as the cellular platform of Nlrp3 effects, resulting in the regulation of T cell plasticity. Nlrp3-deficient DCs had increased expression of CD103, while reduced expression of CD103 on LP-DCs was observed in Nlrp3-sufficient mice after induction of colitis. Increased expression of CD103 on Nlrp3-deficient DCs correlated with increased expression of FLT3L and decreased expression of GM-CSF. Coculture of Nlrp3-deficient DCs with T cells resulted in an increase of FLT3L production by T cells. *Vice versa*, coculture of Nlrp3-sufficient DCs with T cells resulted in an increase of GM-CSF production by T cells.

It was also shown that T cell-mediated inflammation was negatively regulated by IL-18R signalling of adoptively transferred T cells, as lack of IL-18R expression resulted in more severe colonic inflammation, increased expression of proinflammatory cytokines and increased colonic infiltration with immune cells. Biasing effects of differences in microbiota of Nlrp3-sufficient and Nlrp3-deficient mice were ruled out by cohousing of the two mouse strains, as evidenced by PCR-based microbial analysis.

These data suggest a mechanism, through which Nlrp3-dependent IL-1β promotes a Th-17/Th1-dependent intestinal pathology. Additionally, antigen presentation to T cells by Nlrp3-deficient DCs results in a shift in the balance of the growth factors FLT3L and GM-CSF towards FLT3L. This microenvironment could be the deciding factor in the induction of tolerogenic $CD103^+$ DCs as well as T cells with a non-inflammatory phenotype, a finding with potential therapeutic application for the treatment of IBD.

2 Zusammenfassung

Als chronisch entzündliche Darmerkrankungen (CED) bezeichnet man rezidivierende entzündliche Erkrankungen des Dünn- und Dickdarms, die als Folge einer Dysregulation des mukosalen Immunsystems auftreten. Die Pathophysiologie der CED ist unvollständig verstanden; zu den möglichen Ursachen zählen eine genetische Disposition, Schädigung der Schleimhautbarriere mit erhöhter epithelialer Permeabilität, endoluminale bakterielle Auslöser, welche zur Aktivierung von Lymphozyten und Makrophagen führen, sowie ein Ungleichgewicht der Produktion von pro-inflammatorischen und anti-inflammatorischen Zytokinen. Erhöhte Spiegel an Interleukin (IL)-1β und IL-18 konnten in der Schleimhaut des Darms von Patienten mit CED sowie in CED-Tiermodellen nachgewiesen werden. Die unreifen Formen (pro-IL-1β und pro-IL-18) dieser beiden entzündlichen Zytokine werden hauptsächlich über einen Caspase-1-aktivierenden Multiproteinkomplex, das Nlrp3-Inflammasom, aktiviert. Die Dextran Sulfat Sodium (DSS)-induzierte Kolitis ist ein wichtiges Tiermodell, das essentielle Aspekte der mukosalen Schädigung und der angeborenen Immunität bei CED widerspiegelt. Unsere Arbeitsgruppe konnte im Mausmodell zeigen, dass das Nlrp3-Inflammasom bei der Pathogenese der DSS-induzierten Kolitis eine zentrale Rolle spielt. Hierbei erwies sich die Nlrp3-Defizienz in diesem Modell als protektiv. Zusätzlich wurde gezeigt, dass die Aufnahme von DSS durch Makrophagen zu einer Aktivierung des Nlrp3-Inflammasoms führt. Jedoch ist das DSS-Modell nicht geeignet Vorgänge der adaptiven Immunantwort im Rahmen der CED-Pathogenese zu untersuchen. Daher sind weitere Studien mit alternativen Modellen, die die immunologischen Mechanismen der Regulierung der Darmentzündung klären, dringend erforderlich.

Die Hauptziele dieses Projekt waren: a) die Untersuchung der Rolle der Nlrp3-abhängigen Zytokine IL-1β und IL-18 bei der Induktion von Kolitis in einem T-Zelltransfermodell der Kolitis, b) die Charakterisierung der Darm-dendritischen Zellen (DCs) und deren Nlrp3-vermittelter Einfluss auf die T-Zell-Plastizität, c) die Untersuchung der Rolle des IL-1R- und IL-18R-vermittelten Signalweges in adoptiv transferierten T-Zellen und d) die Untersuchung des Einflusses der Mikrobiota von Nlrp3-suffizienten und Nlrp3-defizienten Mäusen.

Diese Arbeit zeigt, dass das Nlrp3-Inflammasom eine entscheidende Rolle bei der Induktion von T-Zell-vermittelten Entzündungen spielt. Das Gleichgewicht der Nlrp3-abhängigen Zytokine IL-1β und IL-18 ist essentiell für die Induktion T-Zell-basierter Entzündungsprozesse im Darm, wobei IL-1β hier eine Schlüsselrolle übernimmt.

Nlrp3-defiziente Mäuse, die $CD4^{+}CD45Rb^{High}$ T-Zellen transferiert bekamen, wiesen ein geringeres Maß an Entzündung und eine reduzierte T-Zell-Infiltration im Kolon auf. In Nlrp3-suffizienten Mäusen zeigten Lamina Propria (LP)-infiltrierende T-Helferzellen einen entzündlichen Th17/Th1-Phänotyp auf, was zur Ausschüttung einer erhöhten Menge an T-Zell-abhängigen entzündlichen Zytokinen, wie IL-17, IL-22 und IP-10, führte. Die Aktivierung von $CD4^{+}$ T-Zellen mit Nlrp3-sufizienten DCs führte ebenfalls zu einem entzündlichen Phänotyp und lässt auf die Abhängigkeit von DCs gegenüber Nlrp3-basierten Effekten schließen.

Auf Nlrp3-defizienten DCs wurde eine erhöhte Expression von CD103 im Vergleich zu LP-DCs in Nlrp3-suffizienten Mäusen nach Kolitisinduktion beobachtet. Die erhöhte Expression von CD103 auf Nlrp3-defizienten DCs korrelierte mit einer erhöhten Expression von FLT3L und einer reduzierten Expression von GM-CSF. Die Ko-Kultivierung von Nlrp3-defizienten DCs mit T-Zellen führte zu einer gesteigerten FLT3L-Produktion von T-Zellen. Umgekehrt führte die Ko-Kultivierung von Nlrp3-suffizienten DCs mit T-Zellen zu einer vermehrten GM-CSF-Produktion von T Zellen.

Es wurde auch gezeigt, dass die T-Zell-vermittelte Entzündung negativ durch den IL-18R-Signalweg der adoptiv transferierten T-Zellen reguliert wird. Der Defekt in der IL-18R-Expression führte zu einer stärkeren Kolonentzündung, einer erhöhten Expression von pro-inflammatorischen Zytokinen und einer erhöhten Immunzelleninfiltration in das Kolon. Ein wesentlicher, diese Effekte überlagernder Einfluss der Mikrobiota von Nlrp3-suffizienten versus Nlrp3-defizienten Mäusen wurde durch eine PCR-basierte mikrobielle Analyse nach Zusammensetzung der beiden Stämme ausgeschlossen.

Zusammengefasst ergeben sich aufgrund dieser Arbeit Hinweise darauf, dass Nlrp3-induziertes IL-1β eine Th-17/Th1-abhängige Darmpathologie begünstigt. Zusätzlich führt die Antigenpräsentation von Nlrp3-defizienten DCs zu einer Verschiebung des Gleichgewichts zwischen den beiden Wachstumsfaktoren FLT3L und GM-CSF in Richtung FLT3L. Dies könnte der entscheidende Faktor bei der Induktion von tolerogenen $CD103^{+}$ DCs, sowie T-Zellen mit einem nicht-entzündlichen Phänotyp sein, und stellt somit einen potentiellen therapeutischen Ansatz für die Behandlung der CED dar.

3 Introduction

3.1 Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic, recurring inflammatory disorder of the gastrointestinal tract (Puren, Fantuzzi et al. 1999; Podolsky 2002). The highest incidence rates and prevalence have been observed in northern Europe, the United Kingdom and America (Baumgart and Carding 2007). Nevertheless, rising rates in low incident areas have been documented in recent studies (Ng, Bernstein et al. 2013). The two main entities of IBD are Crohn's disease (CD) and ulcerative colitis (UC). Despite similarities between the two IBD forms, they are characterised by certain differences in the location and the nature of inflammatory modifications. Crohn's disease is a relapsing transmural inflammatory disease that can potentially extend to any part of the gastrointestinal tract. On the other hand, ulcerative colitis is a non-transmural chronic inflammation restricted to the colon (Baumgart and Sandborn 2007). IBD symptoms differ depending on the location and severity of inflammation; however, common symptoms include diarrhoea, rectal bleeding, abdominal pain and weight loss.

The pathophysiology of IBD is not yet fully understood, but studies have shown that incorrect immune reaction to gut microbiota in a genetically susceptible host drives intestinal inflammation (Abraham and Cho 2009). Understanding the interplay between environmental factors and genetic disposition have been intensified through genome-wide association studies, which have highlighted the importance of microbe sensing in intestinal immunity (Vermeire and Rutgeerts 2005; Cho 2008; Gregersen and Olsson 2009; Van Limbergen, Wilson et al. 2009). Additionally, studies have shed light onto the importance of intestinal epithelium in shaping mucosal immunity.

The epithelial barrier is a selectively permeable interface that regulates the balance between tolerance and immunity to bacteria and non-self antigens. Increasing evidence has shown that deterioration of the mucosal barrier with increased epithelial permeability allows translocation of antigens to the lamina propria, leading to uncontrolled inflammation (Mankertz and Schulzke 2007; Jager, Stange et al. 2013; Antoni, Nuding et al. 2014). Specialised epithelial cells like goblet cells are indispensable in regulating the epithelial barrier. These cells secrete mucin glycoproteins (MUC2), which regulate mucus production, leading to reduced bacterial adhesion to the epithelium (Van der Sluis, De Koning et al. 2006; Johansson, Phillipson et al. 2008).

3.2 Animal models of inflammatory bowel disease

Animal models of IBD are classified into different categories depending on the nature of inflammation and the mode of induction (Elson, Cong et al. 2005). These categories comprise of chemically-induced models, spontaneous models, genetically engineered models and adoptive T cell transfer models. Despite the fact that none of the IBD animal models fully mirror IBD pathogenesis in humans, they allow important new insights into the pathogenesis of gut inflammation. The most widely used experimental models are chemically-induced models using 2,4,6-trinitrobenzene sulfonic acid (TNBS) or dextran sodium sulphate (DSS). Although both chemicals act by damaging the epithelial barrier, TNBS-induced colitis is believed to closely mimic CD, while DSS-induced colitis might mimic certain aspects of UC (Alex, Zachos et al. 2009). Despite the simplicity of chemically-induced models, and the fact that these models are ideal for studying mucosal damage and innate effector mechanisms, they are limited in the investigation of the adaptive immune system.

Adoptive T cell transfer colitis is an IBD model, in which T cells are adoptively transferred to immunocompromised mouse strains, such as recombinant activating gene (RAG) knock out or severe combined immunodeficiency (SCID) mice, leading to the disruption of T cell homeostasis and colitis induction. The classical model in this group is characterised by adoptive transfer of $CD4^{+}CD45Rb^{High}$ T cells (naïve T cells) from healthy mice into immunoincompetent mice lacking T and B cells (Powrie 1995; Powrie 2004). Adoptive transfer of $CD4^{+}CD45Rb^{High}$ T cells is widely used because it is clearly more compatible to human IBD than the erosive self-limiting models. Additionally, this model is ideal for studying immunological mechanisms responsible for induction as well as regulation of gut inflammation.

3.3 NLR family, pyrin domain containing (Nlrp3) inflammasome

Not only have recent studies demonstrated the importance of the Nlrp3 inflammasome in regulating intestinal homeostasis, but they have also emphasised on the consequences of single nucleotide polymorphisms, which affect the expression of Nlrp3 components (Villani, Lemire et al. 2009; Chen and Nunez 2011; Zhang, Wang et al. 2014). IL-1β and IL-18 are important inflammatory cytokines, which significantly contribute to intestinal inflammation and are activated by caspase-1, a component of Nlrp3 inflammasome (Siegmund 2002).

The Nlrp3 inflammasome, which is the inflammasome that has been studied most extensively to date, is a large protein complex consisting of three sub-units; Nlrp3, the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) and caspase-1 (Agostini, Martinon et al. 2004). This inflammasome senses pathogens and

danger signals like bacterial toxins, external ATP or molecules associated with stress. Upon activation Nlrp3 oligomerises through corresponding interactions between NACHT domains; the PYD on Nlrp3 interacts with the PYD domain of ASC. CARD domain of ASC then recruits the CARD domain of caspase-1, leading to cleavage of active caspase-1 (**fig. 2-1**). The cleaved caspase-1 leads to maturation of proinflammatory cytokines IL-1β and IL-18, which mediate immune responses.

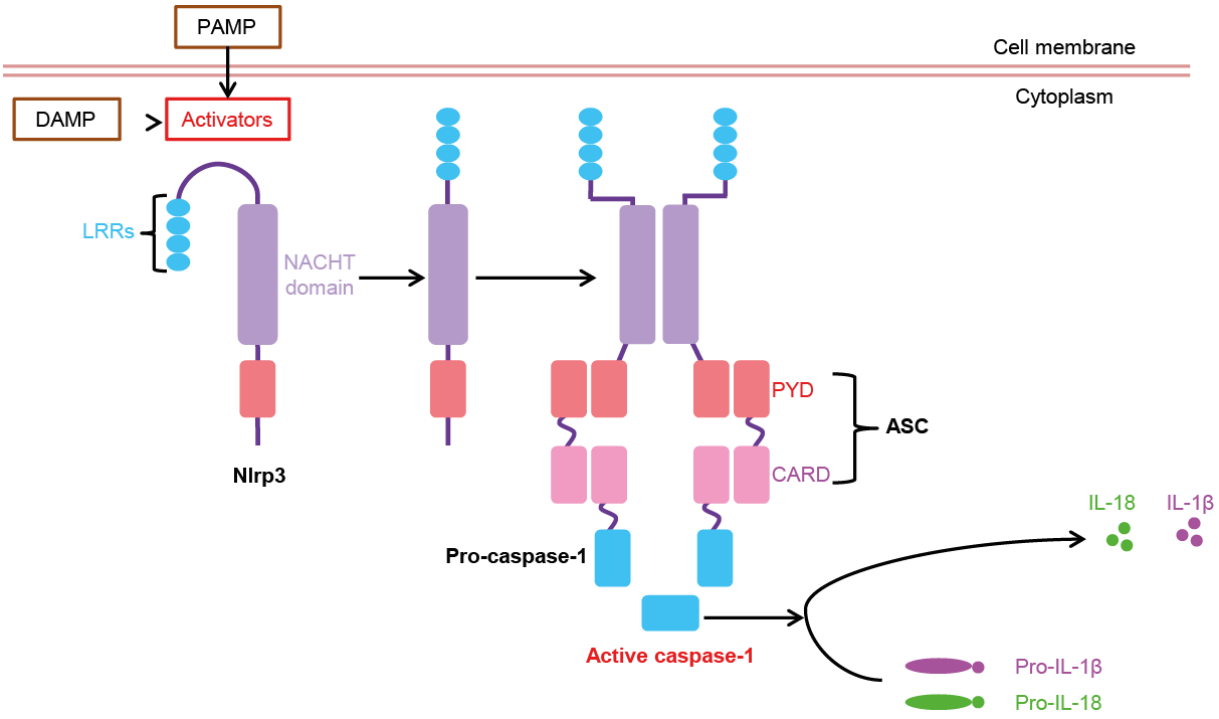

Figure 3-1: Activation of Nlrp3 inflammasome.

Upon detection of cellular stress caused by danger signals, e.g. bacterial toxins (nigericin), external ATP or molecules associated with stress (e.g. crystalline structures), Nlrp3 oligomerises through a corresponding interaction between NACHT domains. PYD domain of the oligomerised Nlrp3 subunit then binds PYD domains of ASC subunit thereby allowing binding of CARD domains of pro-caspase-1 subunit leading to cleavage of caspase-1. Active caspase-1 then cleaves inactive forms of IL-1β and IL-18. Adapted from (Schroder, Zhou et al. 2010).

3.4 IL-1β and IL-18 in intestinal inflammation

Proinflammatory cytokines are indispensable for fighting infections and establishing immunity. The two main proinflammatory cytokines IL-1β and IL-18 are closely related not only because they belong to the IL-1 family, but also because their immature forms are inactive until cleaved by the protease caspase-1, a subunit of Nlrp3 inflammasome.

IL-1β, primarily produced by innate leucocytes for example neutrophils, macrophages and dendritic cells has a broad spectrum of systemic and local effects. This cytokine has the ability to not only stimulate dendritic cells (DCs), macrophages and neutrophils (Dinarello

1996; Dinarello 2009), but also to promote antigen-dependent proliferation and differentiation (Ben-Sasson, Hu-Li et al. 2009). IL-1 receptor (IL-1R1), which is expressed on several types of cells, binds mature IL-1β and initiate IL-1R1 signalling (Sims and Smith 2010). The significance of IL-1β in intestinal immune regulation was confirmed by recent work, which showed its importance in mediating chronic gut inflammation. IL-1β was essential in initiating the infiltration of IL-17A-producing innate lymphocytes and $CD4^+$ T cells to the colon (Coccia, Harrison et al. 2012). Concordantly, numerous studies have described an enhanced secretion of IL-1β in the colon of IBD patients (Mahida, Wu et al. 1989; Ligumsky, Simon et al. 1990; Brynskov, Tvede et al. 1992; Dionne, D'Agata et al. 1998). Correlation of high colonic IL-1β secretion with increased disease intensity suggests the importance of IL-1β in promoting IBD. Furthermore, high levels of this cytokine have been reported in animal models of colitis (Cominelli, Nast et al. 1990; Okayasu, Hatakeyama et al. 1990). Blockage of IL-β was able to reverse IBD-induced inflammation (Cominelli, Nast et al. 1992; Siegmund, Lehr et al. 2001).

IL-18, another IL-1 family cytokine also pivotal for intestinal inflammation, was originally described as “IFN-γ-inducing factor”, but termed IL-18 in 1995 after purification (Okamura, Nagata et al. 1995). Despite regulation and signalling similarities that IL-18 shares with IL-1β, biologic functions differ substantially. While IL-1β is barely detectable in healthy humans and mice, IL-18 precursor is detected in blood monocytes, peritoneal macrophages, mouse spleen and in the epithelial cells of the entire gastrointestinal tract in healthy subjects (Puren, Fantuzzi et al. 1999).

The role of IL-18 has been very controversial, depending on the cytokine milieu: IL-18 can either be antiinflammatory or proinflammatory. In concert with IL-12, IL-18 drives Th1 differentiation by inducing the production of IFN-γ (Seki, Tsutsui et al. 2001). In agreement with this, neutralisation of IL-18 in chemically-induced models of colitis proved to be protective and was linked to reduction of IFN-γ production (Siegmund, Lehr et al. 2001; Ten Hove, Corbaz et al. 2001). Additionally, IL-18 was detected in inflamed intestines of CD patients as a mature protein, but its inactive form was detected in healthy intestinal tissue (Pizarro, Michie et al. 1999). Defective inflammasome-dependent epithelial integrity has been linked to decreased levels of IL-18 (Zaki, Boyd et al. 2010).

Nevertheless, contradicting results have shown that administration of exogenous IL-18 restores mucosal healing in caspase-1 deficient mice (Dupaul-Chicoine, Yeretssian et al. 2010). Attempts have been made to reconcile these conflicting observations. Siegmund proposed that the type of effect induced by IL-18 is site-dependent (Siegmund 2010). It was argued that IL-18 activation within the epithelium leads to the preservation of the intestinal

barrier by inducing epithelial cell proliferation, therefore regenerating the damaged epithelial barrier. Nevertheless, hyperactive IL-18 intercepts the transcriptional program controlling goblet cell development, leading to depletion of goblet cells, therefore promoting DSS-induced colitis (Nowarski, Jackson et al. 2015). A recent study adding more debate to the effect of IL-18 reported that IL-22 directly promotes the expression of IL-18 in intestinal epithelial cells, hence contributing to inflammation (Munoz, Eidenschenk et al. 2015).

Effects of IL-18 on T cells was also described in a previous study, which showed that IL-18 is a key epithelial-derived cytokine that regulates the differentiation of distinct subsets of $CD4^+$ T cells during both homeostatic and inflammatory conditions (Harrison, Srinivasan et al. 2015). They showed that IL-18, which is constitutively produced by intraepithelial cells (IEC) acted directly on IL-18R1 expressed on $CD4^+$ T cells by limiting Th17 differentiation in part by neutralising IL-1R signalling. Additionally, it was also shown that IL-18R signalling was critical for $FoxP3^+$ regulatory T cells (T_{reg}) cell-mediated regulation of gut inflammation.

3.5 Dendritic cells and intestinal immune regulation

The intestinal immune system maintains a fragile balance between immunogenicity against foreign pathogens and tolerance of commensal bacteria. This critical immune response is initiated by DCs, a subset of innate immune cells, which are responsible for antigen uptake and presentation to T cells. Depending on the type of antigen sensed, DCs can either induce an inflammatory or a tolerogenic immune response.

The regulatory function of DCs is indispensable in the gut, where the immune system is not only constantly challenged by non-harmful antigens and commensal bacteria, but also by pathogens. Intestinal DCs have the ability to react towards signals received in their local environment, enabling them to discriminate between commensal microorganisms and potentially dangerous pathogens, therefore maintaining the balance between tolerance and active immunity (Chirdo, Millington et al. 2005; Hart, Al-Hassi et al. 2005).

The crossroad between tolerance initiation and an active immune response relies on the sub-populations of DCs characterised by their specific surface receptors, and factors present in the tissue environment during activation of DCs and T cell priming. Numerous subsets of DCs have been characterised in the mesenterial lymph node (MLN), Peyer's patches and in the primary effector site lamina propria (LP) (Iwasaki and Kelsall 2001; Johansson-Lindbom, Svensson et al. 2005; Siddiqui and Powrie 2008; Rescigno 2009). Of all the subpopulations of DCs found in the intestine, recent research has put special interest on the expression of Integrin α_E (CD103) on DCs. α_E integrin is expressed together with β_7 as a heterodimer,

forming the $\alpha_E\beta_7$ complex (Kilshaw and Murant 1990; Teixido, Parker et al. 1992). This integrin is not only found on a subset of DCs but also on $CD4^+$, effector memory $CD8^+$ and $CD8^+$ regulatory T cells (Lehmann, Huehn et al. 2002; Uss, Rowshani et al. 2006). The best-known ligand of integrin αE is E-cadherin expressed by epithelial cells, which allows the adhesion of $CD103^+$ cells on the epithelial layer (Siddiqui, Laffont et al. 2010).

3.6 $CD103^+$ and $CD103^-$ dendritic cells

The study of intestinal DCs has been intensified over the past years, and there is a better understanding regarding their phenotype und function (Bogunovic, Ginhoux et al. 2009; Yuan, Dee et al. 2015; Muzaki, Tetlak et al. 2016). $CD103^+$ and $CD103^-$ DC subsets have been described in the intestine. Despite the fact that both phenotypes prime and promote the expression of gut homing receptors on naïve T cells, the fate of T cells they activate differs. $CD103^-$ DCs have been described to cause a rapid generation of effector T cells in the gut, while $CD103^+$ DCs induce differentiation of naïve $CD4^+$ T cells into regulatory T cells (Coombes, Siddiqui et al. 2007; Sun, Hall et al. 2007; Cerovic, Houston et al. 2013; Scott, Bain et al. 2015). An increased expression of transforming growth factor-β (TGF-β) and retinaldehyde dehydrogenase (RALDH2), which supports the differentiation of $FoxP3^+$ T_{regs}, has also been observed in $CD103^+$ DCs.

In the absence of pathogen recognition (steady state), a small population of $CD103^+$ DCs is believed to migrate from the LP to the intraepithelial compartment, where they survey the gut content (Farache, Koren et al. 2013). At steady state, a minimal release of inflammatory signals or an inherent differentiation programme of DCs in the absence of TLR signalling (Buza, Benjamin et al. 2008), induces an essential CCR7-dependent intestinal DC migration from the LP to the MLN (Jang, Sougawa et al. 2006; Worbs, Bode et al. 2006; Stagg 2007). In the MLN, $CD103^+$ DCs metabolise vitamin A into retinoic acid (RA) using the key enzyme RALDH2, which together with TGF-β converts naïve T cells into $FoxP3^+$ T_{regs} (Coombes, Siddiqui et al. 2007; Svensson, Johansson-Lindbom et al. 2008; Agace and Persson 2012). Additionally, $CD103^+$ DCs induce the expression of gut homing receptors CCR9 and $\alpha_4\beta_7$ on T cells (Johansson-Lindbom, Svensson et al. 2005; Johansson-Lindbom and Agace 2007). Furthermore, increased expression of additional factors like indoleamine 2,3-dioxygenase (IDO) and thymic stromal lymphopoietin (TSLP) boost the ability of $CD103^+$ DCs to inhibit effector cells (Matteoli, Mazzini et al. 2010; Spadoni, Iliev et al. 2012).

Murine intestinal DC populations are further classified into $CD11b^+$ and $CD11b^-$ subsets (Bogunovic, Ginhoux et al. 2009; Schulz, Jaensson et al. 2009; Varol, Vallon-Eberhard et al. 2009). $CD103^+CD11b^-$ DCs are equivalent to classical splenic CD8α DCs (Liu, Victora et al.

2009) stemming from pre-conventional DCs (pre-cDCs) lineage (Bogunovic, Ginhoux et al. 2009; Varol, Vallon-Eberhard et al. 2009), which are dedicated to give rise to cDCs (Liu, Victora et al. 2009). On the other hand, $CD103^+CD11b^+$ DCs display classical DC activities, characterised by their ability to migrate to MLN, where they are able to present digested antigen to T cells (Johansson-Lindbom, Svensson et al. 2005; Jaensson, Uronen-Hansson et al. 2008; Bogunovic, Ginhoux et al. 2009; Schulz, Jaensson et al. 2009).

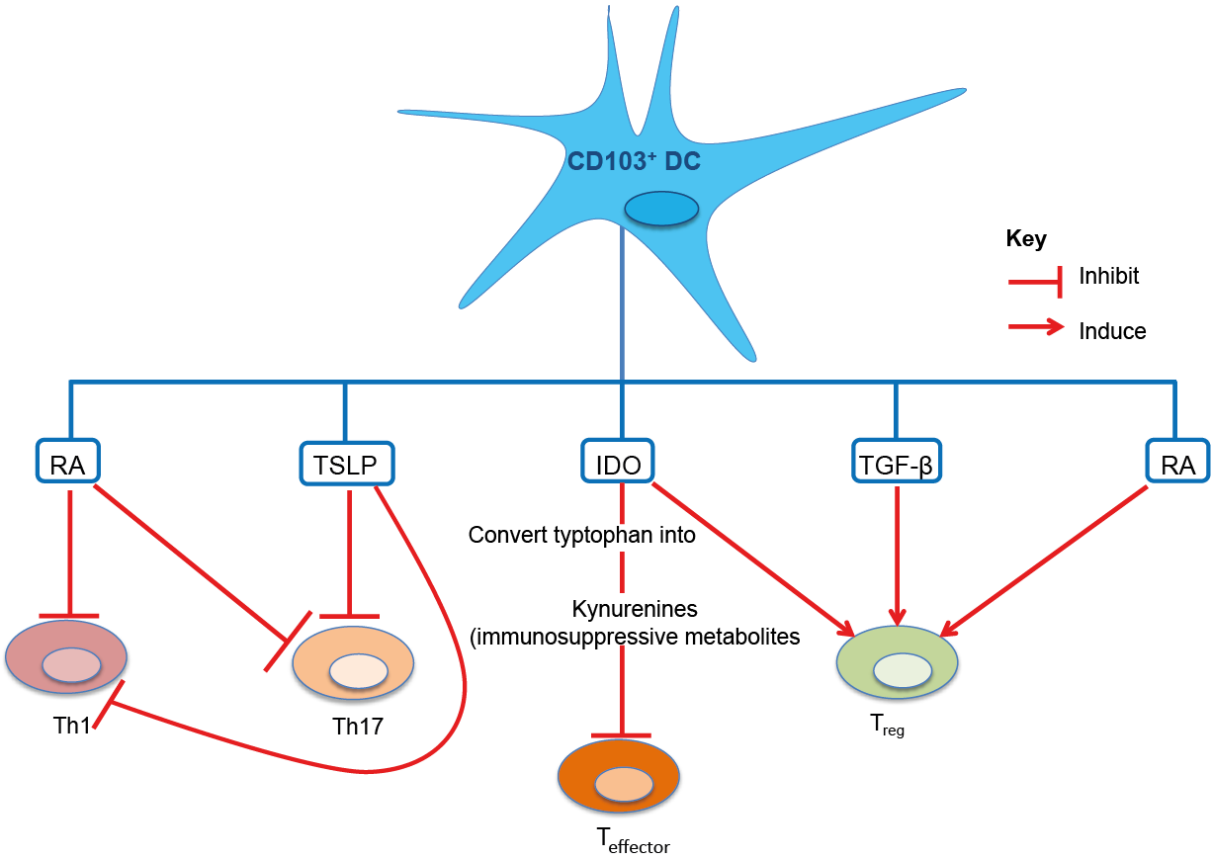

Figure 3-2: Tolerogenic $CD103^+$ dendritic cells in the mesenterial lymph nodes.

In the MLN, $CD103^+$ DCs metabolise vitamin A into retinoic acid (RA) using the key enzyme retinal aldehyde dehydrogenase. In concert with TGF-β and IDO, RA converts naïve T cells into $FoxP3^+$ T_{regs} and inhibits the development of effector T cells. Additionally, thymic stromal lymphopoietin (TSLP) also boosts the ability of $CD103^+$ DCs to inhibit effector T cells.

Despite the fact that $CD103^+$ DCs are believed to be mainly tolerogenic at steady state, they also have the potential to convert naïve T cells into effector T cells. Under inflammatory conditions, $CD103^+$ DCs (unlike their steady-state counterparts), displayed lower expression of RALDH2, and induced an inflammatory Th1 response in a TLR- and chemokine-dependent manner (Laffont, Siddiqui et al. 2010; Farache, Koren et al. 2013).

In contrast to $CD103^+$ DCs, studies have shown that $CD103^-$ DCs have an immunogenic phenotype in both steady state and inflammation (Siddiqui, Laffont et al. 2010). $CD103^-$ DCs

not only have the ability to migrate to the lymph node and to prime T effector cells, especially IFN-γ- and IL-17-producing T cells, but also produce factors like osteopontin that drive intestinal inflammation (Cerovic, Houston et al. 2013; Atif, Uematsu et al. 2014; Kourepini, Aggelakopoulou et al. 2014; Scott, Bain et al. 2015).

4 Objectives

Contact of bacterial components with immune cells of the lamina propria seems to be the key mechanism in regulating IBD pathogenesis. Different cell populations of the innate and adaptive immune system (e.g. DCs, macrophages and T cells) in lamina propria and mesenterial lymph nodes are involved in regulating the transition from steady state to inflammation. Further studies clarifying the mechanisms involved in the immune processes, which lead to intestinal inflammation, are needed. The four main objectives of this study were:

1) To investigate the role of Nlrp3-dependent cytokines IL-18 and IL-1β in a T cell transfer model of colitis, particularly at the early phase of colitis induction; 2) To characterise intestinal DCs as the cellular platform of Nlrp3 effects, resulting in the regulation of T cell plasticity; 3) To investigate the role of T cell IL-1R and IL-18R signalling and its imbalance as a mechanism of tolerogenic versus inflammatory outcome after $CD4^+$ T cell transfer into immunoincompetent mice; 4) To rule out biasing effects of differences in microbiota of Nlrp3-sufficient and Nlr3-deficient mice through cohousing experiments and PCR-based microbial analysis of the intestinal microbiome.

Previous work of our group has shown that Nlrp3 plays a major role in the pathogenesis of DSS-induced colitis, a chemically-induced inflammation (Bauer, Duewell et al. 2010). Reduced IL-1β production in the macrophages of $Nlrp3^{-/-}$ mice after oral DSS administration and protection from the DSS colitis was observed. Despite several advantages of the DSS model, such as simplicity, high reproducibility and almost immediate induction of mucosal inflammation, it has certain limitations in studying the adaptive immune response. In order to overcome these limitations, $CD4^+CD45Rb^{High}$ T cell transfer colitis model was employed in this study. This model was used to investigate the earliest immunological events that initiate intestinal inflammation. The questions addressed were: Do Nlrp3-deficient mice after adoptive T cell transfer have similar protection, as observed in DSS-induced colitis? Does Nlrp3-dependent inflammation correlate to levels of IL-1β and other associated proinflammatory cytokines, such as IL-17? Is the polarisation of transferred naïve T cells Nlrp3-dependent?

As it has been widely described, DCs are the first line of defence in intestinal immunity. They are able to discriminate between non-harmful and harmful antigens and present antigens to T cells, therefore inducing active immunity or tolerance. That is why it was important to investigate the effect of Nlrp3 inflammasome on the differentiation of DC subsets, and the role of the different subsets in the regulation of T helper cells differentiation.

IL-1R and IL-18R do not only share a downstream signalling pathway in T cells, but maturation of their ligands is also caspase-1-dependent (Thomassen, Bird et al. 1998; Lee, Kim et al. 2004). A deeper understanding of the effect of these two related yet different signalling pathways on the fate of T cell differentiation is inevitable for clarifying their role in IBD. With this in mind, it was important to investigate the role of IL-1R and IL-18R signalling in T cells in the regulation of gut inflammation.

It has been shown both in animal models of intestinal inflammation and in IBD patients that microbiota is one of the key players that mediate intestinal inflammation. Several species have been described that either have inflammatory or antiinflammatory potential. In order to rule out biasing effects of differences in microbiota composition of Nlrp3-deficient and Nlrp3-sufficient mice, it was important to perform deep sequencing analysis of the microbial content in the colon of both mouse strains as well as studying the influence of cohousing, leading to the exchange of the microbiome, on microbiome content and colitis induction.

5 Materials

5.1 Equipments

Table 5-1 : Equipments

Name	Company
Blotting system	Bio-Rad, Germany
Cell culture CO_2 incubator (BD 6220)	Heraeus, Germany
Cell culture Laminar Flow	Thermo Scientific, Germany
Centrifuge (5424 and 5415R)	Eppendorf, Germany
Centrifuge (Multifuge 3L-R)	Thermo Scientific, Germany
Cover glass	VWR, Germany
Dissociator, gentle MACS Dissociator	MACS Miltenyi Biotech
ELSIA reader (Mithras LB940)	Berthold Technologies, Germany
FACSCanto II	BD Bioscience, Germany
Fine scale, MC1 Analytic AC 210 S	Sartorius, Germany
Gel blotting paper	Whatman Paper GmbH, UK
Gel electrophoresis system, Power-pac 3000	Biorad, Germany
Gel electrophoresis system, Power-pac P25	Biometra, Germany
Glass capillary pipette	Hirschmann, Germany
Insulin U-100 0.3 ml	BD Microfine, Germany
Lab-Tek® Chamber slide	Thermo Scientific, Germany
Lightcycler® 480 II	Roche, Germany
Microscope Axiovert25 and Axiovert200M	Zeiss, Germany
Microscope slides (Superfrost® Plus Menzel-Gläser)	Thermo Scientific, Germany
Microscope TCS SP5 II	Leica, Germany
Microscope,Nikom TMS-F	Nikon,Japan
NanoDrop® 2000c	Thermo Scientific, Germany
Nitrocellulose membrane (AmershamTM-HybondTM-ECL)	GE Healthcare, Germany
Oven, Mini Oven MKII	MWG Biotech, Germany

PCR machine, Biometra UNOII-Thermoblock	Biometra, Germany
pH meter	WTW, Germany
Power Pac Basic	Bio-Rad, Germany
Rotator, Assistent 348 RM5	Karl Hecht AG, Germany
Scale SBC21	Scale Tec, USA
Scalpel (No. 22)	Feather, Japan
Shaker,IKA-Schüttler MTS4	Janke & Kunkel IKA Labortechnik
Sutures (Prolene 5-0)	Ethicon, USA
Thermocycler T3	Biometra, Germany
Thermomixer 5436	Eppendorf, Germany
Vortex Genie 2	Scientific Industries, Germany
Vortex, Galxy Mini	Merck Eurolab, Germany
Water bath	Köttermann, Germany
Western Blot analyzer (LAS4000 mini)	FujiFilm, Germany

5.2 Chemicals and reagents

Table 5-2: Chemicals and reagents

Name	**Company**
1,4-Dithiothreitol (DTT)	Sigma-Aldrich, Germany
10x Cell Lysis Buffer	Cell Signalling, USA
3,3-diaminobenzidine (DAB)	Dako, USA
4-dimethylamino-benzaldehyde (Ehrlich's reagent)	Sigma-Aldrich, Germany
Alcian Blue solution (pH 2.5)	Sigma-Aldrich, Germany
Ammonium acetate	life technologies, Germany
Antisedan	Pfizer, USA
Bio-Rad DC^{TM} Protein Assay Reagent A	Bio-Rad, Germany
Bio-Rad DC^{TM} Protein Assay Reagent B	Bio-Rad, Germany
Bio-Rad DC^{TM} Protein Assay Reagent S	Bio-Rad, Germany
Bovine serum albumin	Roth, Germany

Brefeldin A, Ready Made Solution 10 mg/ ml in DMSO	Sigma-Aldrich, Germany
Catalase	Sigma-Aldrich, Germany
Cell lysis buffer (10x)	Cell Signalling Technology, USA
Chloroform	Roth, Germany
Collagenase	Sigma-Aldrich, Germany
Collagenase D	Roche, Germany
CountBrightTM absolute Counting Beads	life technologies, Germany
DC Protein Assay (Bradford)	Bio-Rad, Germany
Deoxyribonucleotide triphosphate (dNTP)-Mix	Invitrogen, Germany
Dimethyl sulfoxide	Roth, Germany
DNase I	Roche, Germany
dNTP-Mix, 10mM each	Thermo Scientific, Germany
dNTP-Mix, 10mM each	Thermo Scientific, Germany
Dorbene	Pfizer, USA
DPX	Merck, Germany
Dream Taq Green PCR Mastermix	Thermo Scientific, Germany
Dulbecco's PBS (1x)	Lonza, Belgium
Easy Coll solution (d=1.124g/l)	Biochrome, Germany
Eosin Y	Merck, Germany
Ethanol	Sigma-Aldrich, Germany
Ethylenediaminetetraacetic acid (EDTA)	Sigma-Aldrich, Germany
FACSFlow, FACSClean	BD Biosciences
Flumazenil	Inresa, Germany
Formal-FIXX	Thermo Shandon, UK
Glacial acetic acid	Merck, Germany
Heparin-Natrium Braun 25000 I.E./5 ml	Rathiopharm, Germany
Hydrogen peroxide (H_2O_2, 30%)	Merck, Germany
Ionomycin calcium salt	Sigma-Aldrich, Germany
Isoflurane-CP®	CP-Pharma, Germany

Isopropanol	Apotheke Uni Munich, Germany
Isopropanol	Applichem, Germany
KAPA PROBE FAST Universal qPCR Master Mix	peqlab, Germany
Larid-buffer pH 8.3	Apotheke Uni Munich, Germany
Lipofectamine RNAiMax	life technologies, Germany
Lipopolysaccheride-EK, ultrapure (LPS)	InvivoGen, USA
L-Tryptophan	Sigma-Aldrich, Germany
Mayer's Hemalum	Roth, Germany
Methanol	Merck, Germany
Midazolam	Ratiopharm, Germany
MolTaq	Molzym GmbH, Germany
Naloxone	Inresa, Germany
Oligo dT 18 Primer	Eurofins, Germany
PageRuler TM Plus	Thermo Scientific, Germany
PageRulerTM Plus Prestained Protein Ladder	Thermo Scientific, USA
Paraformaldehyde (PFA)	Merck, Germany
Phenol-chlorofrom isoamyl alcohol	Sigma-Aldrich, Germany
Pierce ECL Western Blotting Substrate	Thermo Scientific, Germany
PMA (Phorbol 12-myristate 13-acetate)	Sigma-Aldrich, Germany
Potassium hydrogenphosphate	Merck, Germany
Primer-probe mix, 10x conc.	Roche, Germany
Propidium iodide	Sigma-Aldrich, Germany
Proteinase Inhibitor Cocktail (Complet Mini)	Roche, Germany
Proteinase K	Sigma-Aldrich, Germany
Revert Aid H Minus RT (Reverse Transkriptase)	Thermo Scientific, Germany
Revert Aid H Minus RT (Reverse Transkriptase)	Thermo Scientific, Germany
RiboLock RI (RNAse Inhibitor)	Thermo Scientific, Germany
RiboLock RI (RNAse Inhibitor)	Thermo Scientific, Germany
Saponine	Sigma-Aldrich, Germany

Sodium ascorbate	Sigma-Aldrich, Germany
Sodium azide (NaN_3, 10%)	Sigma-Aldrich, Germany
Sodium chloride (NaCl 0.9%)	Baxter, UK
Sodium dodecyl sulfate (SDS)	Sigma-Aldrich, Germany
Sodium Hydroxide (NaOH)	Apotheke Uni Munich, Germany
Sulfuric acid (H_2SO_4, 2N)	Apotheke Uni Munich, Germany
Super Signal Western Maximum sensetive Signal	Thermo Scientific, Germany
Target antigen retrieval solution (10 x, pH 6.0)	Dako, USA
TEMED	Roth, Germany
Temgesic (Buprenorphin)	RB Pharmaceuticals, UK
TMB Substrate Reagent Set	BD Bioscience, Germany
Trichloroacetic acid	Roth, Germany
TRIS BASE Ultra Qualität	Roth, Germany
Trypan blue	Sigma-Aldrich, Germany
Trypsin-EDTA (10x)	PAA, Austria
Turbo-DNase	life technologies, Germany
UltraComp eBeads®	eBioscience, Affymetrix , USA
Vectashield mounting medium	Vector Laboratories, USA
Xylene	J.T. Baker, Netherlands

5.3 Buffers

5.3.1 Western blot

Laemmli buffer (6x)

347 mM SDS

299 µM Bromphenol blue

4.7 ml Glycerol

0.5 M Tris, pH 6.0

649 mM DTT 4.1 ml ultrapure water

Stacking buffer (4x, pH 6.8)

248 mM Tris

14 mM SDS

15 µM Bromphenol blue

in ultrapure water

Separating buffer (4x, pH 8.8)

1.5 M Tris

14 mM SDS

in ultrapure water

Running buffer (10x)

248 mM Tris

1.92 M Glycine

35 mM SDS

in ultrapure water

Transfer buffer (20x)

198 mM Tris

2 M Glycine

in ultrapure water

Transfer buffer (1x)

20x stock

10% MeOH

in ultrapure water

Blocking buffer

5% BSA

in TBST

Washing buffer (TBST)

165.9 mM Tris-HCl

44.5 mM Tris

1.5 M NaCl

0.5% Tween 20

in ultrapure water

5.3.2 Immunocytochemistry

Fixation buffer

4% PFA

in PBS

Permeabilisation buffer

0.2% TritonX-100

in PBS

Blocking buffer

2% BSA

in PBS

5.3.2.1 Flow cytometry

FACS buffer

2 mM EDTA

2% FBS

0.1% NaN_3

in PBS

Permeabilisation buffer

0.5% saponine

in PBS

Fixation buffer

1% PFA in PBS

5.3.3 T cell assay

Dyna/MACS-buffer

0.2% FBS

2mM EDTA in PBS

5.3.4 Cell culture reagents and media

Table 5-3: Cell culture reagents and media

Name	Company
4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid	PAA, Austria
DMEM High Glucose (4.5 g/l) without L-Glutamine	PAA, Austria
Dulbecco´s PBS (1x) without Ca^{2+} and Mg^{2+}	PAA, Austria
Dynabeads® Mouse T activator CD3/CD28	life technologies,Germany
Ethylenediaminetetraacetic acid (EDTA) DISOD.SALT 0.5 M,	Sigma-Aldrich, Germany
Fetal bovine serum (FBS)	life technologies,Germany
Hank's balance salt solution (HBSS) with Ca^{2+} and Mg^{2+}	PAA, Austria
Hank's balance salt solution (HBSS) without Ca^{2+} and Mg^{2+}	PAA, Austria
Hanks´Salt solution without Ca^{2+} and Mg^{2+}	Biochrome, Germany
L-glutamine (200 mM)	PAA, Austria
LPS-EB ultrapure	InvivoGen, USA
MEM-NEAA (non-essential amino acids)	life technologies, Germany

Opti-MEM	life technologies, Germany
OVA class II (H-ISQAVHAAHAEINEAGR-OH)	JPT, Germany
Penicilline/Streptomycin (100 x)	PAA, Austria
Roswell Park Memorial Institute (RPMI) 1640 medium	Biochrome, Germany
Sodium pyruvate	Biochrome, Germany
TRYPSIN-EDTA (10X) 100ML	PAA, Austria
VLE RPMI 1640 (very low endotoxin)	Biochrome, Germany
β-mercaptoethanol	Roth, Germany

Plastic materials for cell culture experiments were purchased from BD Bioscience (Germany), Corning (USA), Eppendorf (Germany), Greiner bio-one (Germany) or Sarstedt (Germany).

Tumour cell medium

10% FBS

2 mM L-glutamine

100 IU/ml penicillin

100 µg/ml streptomycin

in DMEM

T cell medium

10% FBS 10% FBS

2 mM L-glutamine

100 IU/ml penicillin

100 µg/ml streptomycin

1 mM sodium pyruvate

1% MEM-NEAA

50 µM β-mercaptoethanol

in RPMI 1640 in

DC medium

2 mM L-glutamine

100 IU/ml penicillin

100 µg/ml streptomycin

1 mM sodium pyruvate

1% MEM-NEAA

50 µM β-mercaptoethanol in VLE RPMI 1640

5.4 Breeding lines

Table 5-4: Mice breeding lines

Genotype	Origin
Nlrp3$^{-/-}$	Donation from Prof. Jurg Tschopp (Department of Biochemistry, University of Lausanne, Switzerland)
Rag1$^{-/-}$	Donation from Prof. Dr. Norbert Gerdes (Institute of Cardiovascular Prevention, University Hospital of Ludwig-Maximilians-Universität München)
Nlrp3$^{-/-}$Rag1$^{-/-}$	Generation by crossing Nlrp3$^{-/-}$ and Rag1$^{-/-}$mice. Embryo Transfer in ZVH (Zentrale Versuchstierhaltung, SPF room), University Hospital of Ludwig-Maximilians-Universität München)

5.5 Kits

Table 5-5: Kits

Name	Company
Bio-Plex Cell Lysis Kit	Bio-Rad, Germany
CD11c MicroBeads, mouse	Miltenyi Biotech, Germany
CD4^{+} T Cell Isolation Kit, mouse	Miltenyi Biotech, Germany
Cell TraceTM CFSE Cell Proliferation kit	life technologies, Germany
Dyna Mouse CD4 Negative isolation Kit	Invitrogen, Germany
KAPA PROBE FAST Universal 2X qPCR Master Mix	peqlab, Germany
Lamina Propria Dissociation Kit, mouse	Miltenyi Biotech, Germany
LS columns	Miltenyi Biotech, Germany
Mouse FLT3L Duoset ELISA Set	R&D Systems, Germany
Mouse GM-CSF Duoset ELISA Set	R&D Systems, Germany
Mouse IFN-gamma DuoSet ELISA	R&D Systems, Germany
Mouse IL-1 beta/IL-1F2 DuoSet ELISA,	R&D Systems, Germany
Mouse IL-12 (p70) DuoSet ELISA	R&D Systems, Germany
Mouse IL-18 Platinum ELISA	eBioscience, Germany
Mouse IL-22 ELISA Ready-SET-Go!®	eBioscience, Germany
Mouse IL-23 DuoSet ELISA	R&D Systems, Germany
Mouse TNF-alpha DuoSet ELISA	R&D Systems, Germany

peqGOLD RNA Lysis Buffer T	peqlab, Germany
peqGOLD Total RNA Kit (S-Line)	peqlab, Germany
RevertAidTM First strand cDNA Synthesis kit	Thermo Scientific, USA
TGF-β, murine (ELISA)	eBioscience, Germany
TNF-α, murine (ELISA)	R&D Systems, Germany

5.6 Antibodies

5.6.1 Primary conjugated antibodies

Table 5-6: Primary conjugated antibodies

Specificity	Fluorochrome	Host	Isotype	Reactivity	Concentration	Company
CD3	APC/Cy7	rat	IgG2b, κ	mouse	1/200	BioLegend, USA
CD3	PB	hamster	IgG	mouse	1/200	BioLegend, USA
CD4	PerCP	rat	IgG2a, κ	mouse	1/200	BD, Phamingen, Germany
CD4	PE	rat	IgG2b, κ	mouse	1/200	BD, Phamingen, Germany
CD8	APC	rat	IgG2a, κ	mouse	1/200	BioLegend, USA
CD8	APC/Cy7	rat	IgG2a, κ	mouse	1/200	BioLegend, USA
CD11b	PerCP/Cy5.5	rat	IgG2b, κ	mouse/human	1/200	BD, Phamingen, Germany
CD11c	APC/Cy7	hamster	IgG	mouse	1/200	BioLegend, USA
CD11c	PB	hamster	IgG	mouse	1/200	BioLegend, USA
CD25	APC	rat	IgG1	mouse	1/200	Caltag, Germany
CD44	FITC	rat	IgG2b, κ	human/mouse	1/200	eBioscience, Germany
CD45Rb	FITC	rat	IgG2a, κ	mouse	1/200	BD, Phamingen, Germany
CD62L	APC	rat	IgG2a, κ	mouse	1/200	BD, Phamingen, Germany
CD86	PE	rat	IgG2b, κ	mouse	1/200	BioLegend, USA
CD103	Alex Fluor 488	hamster	IgG	mouse	1/200	BioLegend, USA

F4/80	APC	rat	IgG2a, κ	mouse	1/200	eBioscience, Germany
Foxp3	PE	rat	IgG2a, κ	mouse	1/200	eBioscience, Germany
MHC-II	FITC	mouse	IgG2a, κ	mouse	1/200	BD, Phamingen, Germany
NK-1.1	PerCP	mouse	IgG2a, κ	mouse	1/200	BioLegend, USA

5.6.2 Primary unconjugated antibodies

Table 5-7: Primary unconjugated antibodies

Specificity	Host	Isotype	Reactivity	Company
CD103	hamster	IgG	mouse	BioLegend, USA
CD3	rat	IgG2b, κ	mouse	BioLegend, USA
CD4	rat	IgG2a, κ	mouse	BioLegend, USA
E-cadherin	mouse	IgG2a, κ	mouse	BD, Phamingen, Germany
IL-1β/IL-1F2	goat	IgG	mouse	R&D Systems, Germany

5.6.3 Secondary conjugated antibodies

Table 5-8: Secondary conjugated antibodies

Specificity	Fluorochrome	Host	Isotype	Reactivity	Company
Donkey anti-goat IgG (H+L) Alexa Fluor®488	AF488	donkey	IgG	goat	Invitrogen, Germany
Donkey anti-goat IgG-HRP	HRP	donkey	IgG	goat	Santa Cruz Biotechnology, USA
Donkey anti-rat IgG (H+L) Alexa Fluor®488	AF488	donkey	IgG	rat	Invitrogen, Germany
Goat anti-hamster IgG AF488 (H+L)	AF488	goat	IgG	hamster	life technologies, Germany
Goat anti-mouse IgG (H+L) Alexa Fluor®488	AF488	goat	IgG	mouse	Invitrogen, Germany
Goat anti-mouse IgG (H+L) Alexa Fluor®647	AF647	goat	IgG	mouse	Invitrogen, Germany
Goat anti-mouse IgG1-HRP (γ1 chain specific)	HRP	goat	IgG1	mouse	Southern Biotech, USA
Goat anti-mouse IgG2a-HRP	HRP	goat	IgG2a	mouse	Southern Biotech, USA

(γ2a chain specific)					
Goat anti-mouse IgG2c-HRP (γ2c chain specific)	HRP	goat	IgG2c	mouse	Southern Biotech, USA
Goat anti-mouse IgG-HRP	HRP	goat	IgG	mouse	Santa Cruz Biotechnology, USA
Goat anti-mouse IgG-HRP (γ chain specific)	HRP	goat	IgG	mouse	Southern Biotech, USA
Goat anti-rabbit Ig FITC	FITC	goat	Ig	rabbit	BD Bioscience, Germany
Goat anti-rabbit IgG (H+L) Alexa Fluor®488	AF488	goat	IgG	rabbit	Invitrogen, Germany
Goat anti-rabbit IgG (H+L) Alexa Fluor®546	AF546	goat	IgG	rabbit	Invitrogen, Germany
Goat anti-rabbit IgG (H+L) Alexa Fluor®555	AF555	goat	IgG	rabbit	Invitrogen, Germany
Goat anti-rabbit IgG-HRP	HRP	goat	IgG	rabbit	Santa Cruz Biotechnology, USA
Goat anti-rat IgG (H+L) Alexa Fluor®546	AF546	goat	IgG	rat	Invitrogen, Germany
Goat anti-rat IgG (H+L) Alexa Fluor®647	AF647	goat	IgG	rat	Invitrogen, Germany
Goat anti-rat IgG AF546 (H+L)	AF546	goat	IgG	rat	life technologies, Germany
Goat anti-rat IgG-HRP	HRP	goat	IgG	rat	Santa Cruz Biotechnology, USA
Rabbit anti-goat IgG (H+L) Alexa Fluor®555	AF555	rabbit	IgG	goat	Invitrogen, Germany

5.7 Recombinant cytokines and proteins

Table 5-9: Recombinant cytokines and proteins

Name	Company
Recombinant murine FLT3L	Peprotech, Germany
Recombinant murine GM-CSF	Peprotech, Germany
Recombinant murine IL-1β	Peprotech, Germany
Recombinant murine IL-12	Peprotech, Germany
Recombinant murine IL-18	Biovision incoporated
Recombinant murine IL-2	Peprotech, Germany
Recombinant murine IL-23	Peprotech, Germany
Recombinant murine IL-4	Peprotech, Germany
Recombinant murine IL-6	Peprotech, Germany

5.8 Primers

5.8.1 Primer sequences for genotyping PCR

Table 5-10: Primer sequences for genotyping PCR

Gene	Sequence 5´-> 3´
Nlrp3 common	aaatcgtgctgcttcatgt
Nlrp3 wild-type	tcaagctaagagaactttctg
Nlrp3 mutant	acactcgtcatcttcagca
Rag1 common	ccggacaagtttttcatcgt
Rag1 wild-type	gaggttccgctacgactctg
Rag1 mutant	tggatgtggaatgtgtgcgag

5.8.2 Primer sequences for rt-qPCR

Table 5-11: Primer sequences for rt-qPCR

Gene mRNA	Species		Sequence 5´-> 3´	Probe No.
BCL2 Left	mouse	left	agtacctgaaccggcatctg	
BCL2 Right	mouse	right	ggggccatatagttccacaaa	75
Caspase-1 Left	mouse	left	ttggtcttgtgacttggaggac	

Caspase-1 Right	mouse	right	agaaacgttttgtcagggtca	105
Caspase-11 Left	mouse	left	tctccagagcgagtttcttctt	
Caspase-11 Right	mouse	right	tgttttctgaccggctgac	17
CCL2 Left	mouse	left	catccacgtgttggctca	
CCL2 Right	mouse	right	gatcatcttgctggtgaatgagt	62
CCR9 Left	mouse	left	catccacgtgttggctca	
CCR9 Right	mouse	right	gatcatcttgctggtgaatgagt	105
CD103 Left	mouse	left	cctggaccactacaaggaacc	
CD103 Right	mouse	right	ttgcagtccttctcgtaggg	11
CD11c Left	mouse	left	atg gag cct caa gac agg ac	
CD11c Right	mouse	right	gga tct ggg atg ctg aaa tc	20
CD3 Left	mouse	left	cttgtacctgaaagctcgagtg	
CD3 Right	mouse	right	tgatgattatggctactgctgtc	10
CD4 Left	mouse	left	agggctgtggcagtgtctac	
CD4 Right	mouse	right	gccaggaacactgtctggtt	109
FLT3L Left	mouse	left	aggcctgccagaatttctct	
FLT3L Right	mouse	right	gcttctagggctatgggactc	25
FoxP3 Left	mouse	left	tca gga gcc cac cag tac a	
FoxP3 Right	mouse	right	tct gaa ggc aga gtc agg aga	78
GM-CSF Left	mouse	left	gcatgtagaggccatcaaaga	
GM-CSF Right	mouse	right	cgggtctgcacacatgtta	79
GM-CSFR Left	mouse	left	cagacggacggacacagac	
GM-CSFR Right	mouse	right	ggtgatgttcatggcatgtg	4
IDO 1 Left	mouse	left	ttgctactgttttgaattgtaatgtg	
IDO 1 Right	mouse	right	aagctgcccgttctcaatc	96
IDO 2 Left	mouse	left	tgcacctggaattacgacac	
IDO 2 Right	mouse	right	gcaagagatcttggcagca	1
IFN-γ Left	mouse	left	atctggaggaactggcaaaa	
IFN-γ Right	mouse	right	ttcaagacttcaaagagtctgaggta	21

IL 27 Left	mouse	left	catggcatcacctctctgac	
IL 27 Right	mouse	right	aagggccgaagtgtggta	38
IL-12(p35) Left	mouse	left	ccaggtgtcttagccagtcc	
IL-12(p35) Right	mouse	right	gcagtgcaggaataatgtttca	62
IL-17 Left	mouse	left	catgagtccagggagagctt	
IL-17 Right	mouse	right	gctgagctttgagggatgat	74
IL-18 Left	mouse	left	caaaccttccaaatcacttcct	
IL-18 Right	mouse	right	tccttgaagttgacgcaaga	46
IL-1β Left	mouse	left	agttgacggaccccaaaag	
IL-1β Right	mouse	right	agctggatgctctcatcagg	38
IL1R1 Left	mouse	left	attgttgaacatcgccactg	
IL1R1 Right	mouse	right	aaatgagccccagtagcactt	2
IL-22 Left	mouse	left	tttcctgaccaaactcagca	
IL-22 Right	mouse	right	tctggatgttctggtcgtca	17
IL-22BP Left	mouse	left	acaacagcatctactttgtgcag	
IL-22BP Right	mouse	right	cccccagcagtcaactttat	21
IL-22R Left	mouse	left	tgctctgttatctgggctacaa	
IL-22R Right	mouse	right	tcaggacacgttggacgtt	9
IL-23 Left	mouse	left	tccctactaggactcagccaac	
IL-23 Right	mouse	right	agaactcaggctgggcatc	19
IL-6 Left	mouse	left	gctaccaaactggatataatcagg	
IL-6 Right	mouse	right	ccaggtagctatggtactccagaa	6
IP-10 Left	mouse	left	gctgccgtcattttctgc	
IP-10 Right	mouse	right	tctcactggcccgtcatc	3
IRF4 Left	mouse	left	ggagtttccagaccctcaga	
IRF4 Right	mouse	left	ctggctagcagaggttccac	6
NLRC4 Left	mouse	right	gaagaatcctgtgatctccaagag	
NLRC4 Right	mouse	left	gatcaaattgtgaagattctgtgc	40
Nlrp3 Left	mouse	right	cccttggagacacaggactc	

Nlrp3 Right	mouse	right	ggtgaggctgcagttgtcta	82
Nlrp6 Left	mouse	left	ccagcttctgcatctgagagt	15
Nlrp6 Right	mouse	right	ctcccttgccactgcatc	
PUMA Left	mouse	left	tacagcggagggcatcag	79
PUMA Right	mouse	right	ttctccggagtgttc	
RALDH2 Left	mouse	left	catggtatcctccgcaatg	33
RALDH2 Right	mouse	right	gcgcatttaaggcattgtaac	
RORγT Left	mouse	left	agagacaccaccggacatct	71
RORγT Right	mouse	right	caagggatcacttcaatttgtg	
Smad1 Left	mouse	left	tgaaaacaccaggcgacata	25
Smad1 Right	mouse	right	tgaggcattccgcatacac	
Smad2 Left	mouse	left	aggacggttagatgagcttgag	9
Smad2 Right	mouse	right	gtccccaaatttcagagcaa	
Smad3 Left	mouse	left	tccgtatgagcttcgtcaaa	32
Smad3 Right	mouse	right	ggtgctggtcactgtctgtc	
Smad5 Left	mouse	left	catggattcgaggctgtgta	32
Smad5 Right	mouse	right	gtactggtgacgtcctgtcg	
SOCS3 Left	mouse	left	atttcgcttcgggactagc	83
SOCS3 Right	mouse	right	aacttgctgtgggtgaccat	
SPP1 Left	mouse	left	gaggaaaccagccaaggac	52
SPP1 Right	mouse	right	tgccagaatcagtcactttca	
β-Actin Left	mouse	left	ctaaggccaaccgtgaaaag	64
β-Actin Right	mouse	right	accagaggcatacagggaca	
Stat3 Left	mouse	left	gttcctggcaccttggatt	71
Stat3 Right	mouse	right	caacgtggcatgtgactctt	
Stat5b Left	mouse	left	cgagctggtctttcaagtca	77
Stat5b Right	mouse	right	ctggctgccgtgaacaat	
TGF-β Left	mouse	left	tggagcaacatgtggaactc	72
TGF-β Left	mouse	right	cagcagccggttaccaag	

TNF-α Left	mouse	left	ctgtagcccacgtcgtagc	25
TNF-α Right	mouse	right	tttgagatccatgccgttg	

5.9 Software

Table 5-12: Software

Name	**Company**
Adobe Illustrator CS4	Adobe Systems, USA
Adobe Photoshop CS4	Adobe Systems, USA
Axiovision Rel.4.4	Zeiss, Germany
EndNote X4	Thomson Reuters, USA
FACSDiva	BD Bioscience, Germany
FlowJo 7.6.5	Tree Star, USA
Graphpad Prism 5.0	Graphpad Software, USA
Image J	Image J Software, USA
LAS AF V2.2.1	Leica, Germany
Lightcycler 480 SW 1.5	Roche, Germany

6 Methods

6.1 Cell culture

Cells were cultivated at 37°C with 5% CO_2 and 95% humidity. Cell number and viability were determined by hemocytometer using 0.5% trypan blue in PBS. Cell culture experiments were performed under a sterile laminar flow hood unless stated otherwise.

6.2 Immunological methods

6.2.1 Enzyme-linked immunosorbent assay (ELISA)

Detection of chemokines and cytokines by ELISA kits was performed according to the manufacturer's instructions.

6.2.2 Western blot

Cells were harvested and then lysed in an appropriate volume of lysis buffer for 30 min on ice. Debris was pelleted for 10 min at 14 000 g at 4°C, and protein concentration was determined by Bradford assay. Samples were then diluted with Laemmli buffer and denatured for 5 min at 95°C. Appropriate amount of protein samples were loaded on 10-15% sodium dodecyl sulphate (SDS) gel depending on the size of the protein of interest. Protein samples and 5 µl PageRulerTM plus prestained Protein Ladder were separated for 90 min at 100 V. Proteins were then transferred to a nitrocellulose membrane using Trans-Blot® Electrophoresis Transfercell for 60 min at 350 mA at RT. The membrane was either blocked with 5% BSA/TBST or 5% fat free milk for 60 min at RT. Afterwards, protein samples were stained with the first antibody overnight at 4°C, followed by a secondary antibody staining for 60 min at RT. The membrane was washed three times for 10 min after every antibody staining and then developed using chemiluminescence substrate ECL according to the manufacturer's instructions. The membrane was then exposed using Western Blot analyser LAS4000 mini.

6.3 Molecular biology methods

6.3.1 Polymerase chain reaction

Different mouse genotypes developed were verified using polymerase chain reaction (PCR). Mouse genomic DNA samples were prepared from 2 mm tail tips, which were incubated with 75 µl alkaline lysis buffer (25mM NaOH/0.2 Mm EDTA) for 30 min at 95°C. After incubation, samples were cooled to 15 °C and then neutralised by 75 µl 40mM Tris HCl (pH 5.5). PCR

reactions was performed using either Nlrp3-specific or Rag1-specific primer pairs with the following programs; Nlrp3 (94°C, 3 minutes; 94°C, 30 seconds, 58°C 30 sec, 72°C, 1 min for 39 cycles) and then 72°C, 10 min or Rag1 (94°C, 15 min; 94°C, 30 sec, 63°C 30 sec, 72°C, 1 min for 35 cycles and then 72°C, 10 min).

6.3.2 Quantitative analysis of mRNA

6.3.2.1 RNA isolation

RNA isolation was performed using the peqGOLD Total RNA isolation kit from peqlab according to the manufacturer's instructions. A highly denaturing guanidine-thiocyanate containing lysis buffer, which inactivates RNAases, and an Ultra Turrax instrument were used to lyse and homogenise tissue or cells. Lysed samples were loaded on a column and centrifuged for 1 min at 12,000 g. Flow through was mixed with an identical volume of 70% methanol and vortexed carefully, and then loaded on a PerfectBind RNA column. Contaminants were washed with two different washing buffers. RNA was eluted with RNase free water and the concentration was determined via a photometrical method by Nano Drop®.

6.3.2.2 cDNA transcription

RNA was reverse transcribed into cDNA using RevertAID™ First stranded cDNA Synthesis kit from Thermo Scientific according to manufacturer's instructions. The kit uses RevertAID™ reverse transcriptase with a lower RNase H activity and RiboLock™, which inhibits all eukaryotic RNases, therefore protecting the RNA from degradation. Additionally, a synthetic single-stranded 18-mer primer oligonucleotide (Oligo $(dt)_{18}$), which allows selective reverse transcription of RNA through its 3'-end poly (A) was used to enable selective annealing to poly (A) tailed mRNA.

For the cDNA synthesis, 2 µg isolated RNA was incubated for 60 min at 42°C for amplification with 1 µl Oligo$(dT)_{18}$ primer, 1 µl RiboLock™ (20 U/µl), 4 µl Reaction buffer (5x), 2 µl dNTP mix (10mM), 1 µl RevertAid™ M-MuLV (200 U/µl) and nuclease free water to a final volume of 20 µl. The reaction was completed by heating at 70°C for 10 min then cooled down at 4°C.

6.3.2.3 Quantitative real time polymerase chain reaction

Quantitative real time PCR is a very sensitive method used to quantify copy numbers of PCR templates such as cDNA. KAPA PROBE FAST qPCR Kit from peqlab was used. The appropriate gene primers were designed with respect to Roche Library and the matching probes were purchased from Roche. The procedure was performed according to the

manufacturer's instructions except for the total volume that was scaled down from 20 µl to 10 µl (5 µl KAPPA PROBE FAST UNIVERSAL qPCR Maste Mix (2x), 0.2 µl forward primer, 0.2 µl reverse primer, 0.1 µl probe and then scaled to 10 µl by 1.5 µl water). β-actin was used as housekeeping gene, and target transcripts were quantified by 2^{-ddCT} relative quantification, which relates the PCR signal of the target transcript in a treatment group to an untreated control.

6.4 Polymerase chain reaction-based microbial analysis

Fresh stool samples were collected from single-housed Nlrp3-deficient and Nlrp3-sufficient mice (both $Rag1^{-/-}$) and then shock-frozen in liquid nitrogen. The same mice were then cohoused for three weeks, after which fresh stool samples were collected again and shock-frozen in liquid nitrogen. Microbial communities in the stool samples were then analysed by high-throughput 16S ribosomal RNA gene sequencing at the Technical University of Munich in Freising-Weihenstephan in cooperation with Dr. Thomas Clavel (Zentralinstitute für Ernährung- und Lebensmittelforschung) as described in their previous work (Schaubeck, Clavel et al. 2016).

6.5 Animal experiments

6.5.1 Animals

$Nlrp3^{-/-}$ and $Rag1^{-/-}$ mice were bred and maintained under specific pathogen free (SPF) conditions in an accredited animal facility at the University Hospital of LMU Munich. IL-$18R^{-/-}$ ($Il18r1tm1^{Aki}$) and IL-$1R^{-/-}$ ($Il1r1^{tm1Imx}$) mice were provided by PD Dr. med. Gerald Denk (Medizinische Klinik II, LMU Munich). OT II mice (Tg(TcraTcrb)425Cbn) were provided by Prof. Dr. Thomas Brocker, (Institute of Immunology, LMU Munich) and wild-type mice were purchased from Janvier laboratory (St. Berthevin Cedex, France). Mice were fed standard mice chow pellets and had access to autoclaved tap water supplied in bottles. All experiments were approved by the regional animal study committee and are in agreement with the guidelines for the proper use of animals in biomedical research. Mice used for experiments were more than 8 weeks of age and were anesthetised with isoflurane for blood withdrawal, subcutaneous (s.c.) tumour cell inoculation and adoptive T cell transfer.

6.6 Organ and single cell preparation

6.6.1 Isolation of spleen cells

Spleen was homogenised into a single cell suspension by gentle dissociation through a 40 µm cell strainer wetted by cell isolation buffer (PBS supplemented with 2% foetal bovine serum (FBS)). Splenocytes were pelleted at 400 g for 5 min at RT and erythrocytes were

then lysed with 2 ml prewarmed ammonium chloride-Tris (ACT) buffer for 5 min at RT. Lysed cells were washed off and then cell count and viability was determined.

6.6.2 Isolation of mesenterial lymph nodes

Mesenterial lymph nodes (MLN) were gently homogenised through a 100 µm cell strainer wetted with cell isolation buffer. Erythrocytes were lysed before MLN-derived cells were further used.

6.6.3 Isolation of murine T cells

For isolation of mouse T cells (untouched $CD4^+$ T cells), MACS T cell isolation kit from Miltenyi Biotech was used according to manufacturer's instructions. The principle of this system is isolation of untouched $CD4^+$ T cells by depleting non $CD4^+$ T cells using a biotin-conjugated antibody cocktail against CD8a, CD11b, CD11c, CD19, CD45R (B220), CD49b (DX5), CD105, Anti-MHC-class II, Ter-119 and TCRγ/δ. Further labelling with magnetic anti-biotin MicroBeads allows the retention of unwanted cells in the magnetic field, while unlabelled target cells pass through the column.

Lysed splenocytes were incubated with 10 µl biotin-conjugated antibody cocktail in 40 µl MACS buffer per 10^7 cells for 5 min at 8°C. 20 µl anti-biotin MicroBeads in 30 µl MACS buffer per 10^7 cells was added to the splenocytes and then further incubated for 10 min under rotation at 8°C. Unbound antibody was washed with MACS buffer for 5 min at 400 g, 4°C. Cells were resuspended with MACS buffer and loaded on an LS column attached to a magnetic field. Unlabelled $CD4^+$ T cells passed through the column (negative fraction) and their purity was controlled by flow cytometry.

6.6.4 Isolation of intraepithelial cells and lamina propria

Intraepithelial lymphocytes (IELs) and lamina propria (LP) cells were isolated using Mouse Lamina Propria Dissociation Kit from Miltenyi Biotec. This method is based on a combination of mechanical dissociation and enzymatic degradation of extracellular adhesion proteins using gentleMACS™ Dissociation.

Colon tissue was cleaned with 1x Hank's balanced salt solution without calcium and magnesium (1x HBSS w/o) and then cut longitudinally. Colon sections were then cut into small fragments and incubated with 20 ml predigestion solution (1× HBSS w/o containing 5 mM EDTA, 5% FBS and 1 mM DTT) for 20 min at 37°C. Incubated colon tissue suspension was passed through a 70 µm cell strainer and LP tissue, which was retained on the cell strainer, was predigested again.

Flow through containing epithelial and subepithelial cells as well as intraepithelial lymphocytes was removed and the remaining LP tissue was collected into a gentleMACS™ C tube containing 2.35 ml preheated digestion solution (1×HBSS with calcium and magnesium, containing 5% FBS). LP tissue was dissociated using gentleMACS Dissociator (m_intestine_01 program). Finally, debris was discarded by passing the dissociated tissue through a 100 µm cell strainer and the flow through containing LP-derived cells was washed before further analysis.

6.7 Generation of bone marrow-derived dendritic cells

Bone marrow cells were isolated from murine femur and tibia. Bones were sterilised with 70% ethanol and then dried. Epiphyses were cut off and bone marrow was flushed out using culture medium. Contents of the bone marrow were then filtered through a 40 µm cell strainer and centrifuged for 5 min at 400 g. Erythrocytes were lysed for 3 min at RT and isolated cells were differentiated with either 20 ng/ml IL-4 and 20 ng GM-CSF for 7 days or with 100 ng/ml FLT3L for 9 days in RPMI complete medium. Medium on the GM-CSF/IL-4-differentiated DCs was changed on the second and fifth day.

6.8 Adoptive T cell transfer colitis

Adoptive T cell transfer colitis is a well-characterised model of chronic colitis, in which inflammation is induced by disruption of T cell homeostasis. In this model, naïve precursors of T effector cells ($CD4^+CD45Rb^{High}$ T cells) are isolated from healthy donor mice and reconstituted into immunoincompetent mice (in our case $Rag1^{-/-}$ mice). This protocol was established by Fiona Powrie and colleagues (Powrie, Leach et al. 1993; Powrie, Correa-Oliveira et al. 1994). $CD4^+$ T cells were purified from splenocytes using Dynabeads® Untouched™ Mouse CD4 Cells Kit according to manufacturer's instruction.

Splenocytes were incubated for 20 min at 8°C with 140 µl antibody mix solution (20 µl antibody mix, 100 µl Dyna buffer and 20 µl FBS) per 10^7 cells. Unbound antibody was washed off and then cells were incubated for 15 min at RT with 1 ml Dynabeads suspension (800 µl Dyna buffer and 200 µl Dynabeads®) per 10^7 cells. 1 ml Dyna buffer was added to the cell suspension and then placed on a magnet for 2 min.

The supernatant containing $CD4^+$ T cells was then collected. Isolated cells were washed with FACS buffer and stained with appropriate antibodies against CD4 and CD45Rb. $CD4^+CD45Rb^{High}$ cells were then FACS purified using the BD FACSAria™ III. $CD4^+CD45Rb^{High}$ population was identified as the 40% of $CD4^+$ cells exhibiting the brightest CD45Rb staining.

Purified naïve T cells were washed with PBS to discard FBS and then 0.4 x 10^6 cells resuspended in 100 µl were adoptively transferred into $Rag1^{-/-}$ mice via i.p. injection. Disease progression and development was monitored for a period of 4 weeks.

6.9 Histological and clinical score

Blinded investigators monitored body weight change, presence of blood in stool and stool consistency. For stool consistency, normal-formed stool-pellets were scored 0, soft and not well-formed stool-pellets were scored 2 and severe diarrhoea was scored 4. For haemoccult test, 0 points were given to negative test results, 2 points for positive results, and mice with rectal bleeding were given 4 points. An overall clinical score ranging from 0 for healthy mice to 4 for severe colitic mice was determined by dividing the total of both stool consistency and haemoccult test score and then divided by two. Colon weight per length, a parameter widely used as an indicator of inflammation in the colon, was determined by weighing the entire colon from caecum to anus and measuring its length. 1 cm of distal colon was used for histological analysis and the rest was used for further analysis ex vivo. 4% formalin was used to fix distal colon tissue rings, which were then embedded in paraffin. Haematoxylin and eosin (H&E) was used to stain 4 µm colon tissue sections and analysed in a blinded fashion. For sub-score of inflammatory cell infiltration, 0 scores were given to scarce inflammatory cells in the lamina propria, 1 scores were given to elevated frequency of infiltrating cells, 2 scores were given to increased number of inflammatory cells infiltrating the submucosa, and 3 scores were given to inflammatory cells extending to the transmural layer. The level of epithelial damage was determined by the following scores; 0 points for lack of mucosal damage, 1 point for minimal focal lymphoepithelial lesions, 2 points for erosion or ulceration of the mucosal layer, and 3 points for to severe mucosal injury extending to the structures of the intestinal wall. Both the inflammatory cell infiltration score and epithelial damage score were added up, with healthy mice getting 0 total sub-scores and mice with maximal colitis-induced inflammation getting 6 total sub-score.

6.10 T cell proliferation/polarisation assay

$CD4^+$ T cells were isolated using $CD4^+$ T Cell Isolation Kit mouse (Miltenyi Biotec, Germany) according to manufacturer's instruction. MACS-isolated untouched $CD4^+$ T cells were stained with fluorescence markers allowing the selection of T cells. Murine $CD4^+$ T cells were then purified by sorting on a FACSAria III. 1 x 10^5 purified $CD4^+$ T cells were cultured in 200 µl complete RPMI medium in 96- well U-bottom plates. Sorted naïve $CD4^+$ T cells were labelled with 2.5 µM CFSE, stimulated with 5 µg of plate-bound anti-CD3 and 2 µg of soluble CD28 for 72 hrs. For stimulation of T cells, different exogenous cytokines were added to

CD3/CD28-stimulated $CD4^+$ T cells (20 ng/ml IL-1β, 10 ng/ml IL-18 or 10 ng/ml IL-18 and 2 ng/ml IL-12).

2 x 10^4 of either MACS isolated splenic DCs or BM-DCs were stimulated with 0.5 µg/ml LPS overnight. DCs were then pulsed with 25 µg/ml MHC-II-specific peptide OVA class II (ISQAVHAAHAEINEAGR). 2 x 10^4 LPS-stimulated and peptide-loaded DCs were cocultured with 10^5 purified $CD4^+$ T cells for 5 days.

6.11 Flow cytometry

The flow cytometer used in this study was BD FACSCanto II which uses three different lasers; a blue laser (488 nm, air-cooled, 20 mW solid state), a red laser (633 nm, 17 mW HeNe) and a violet laser (405 nm, 30 mW solid state). The advantage of these lasers is their adequate filter bands, which allow simultaneous detection of different emissions of different fluorochromes. In order to omit spectral overlap, compensation of different emissions was done using single-stained UltraComp eBeads.

For extracellular staining, cells were incubated with appropriate antibody concentration for 20 min at RT in the dark. After incubation, cells were washed with 500 µl FACS buffer and then analysed by BD FACSCanto II. In order to stain intracellular proteins, stained surface molecules were fixed and then the cells were permeabilised in order to allow intracellular antibodies to pass through the membrane. FoxP3 Fix/Perm buffer set was used for the fixation and permeabilisation of cells. After surface staining, cells were fixed with fix buffer for 30 min at 4°C, then washed and permeabilised for 30 min at 37°C with perm buffer. Finally, cells were stained with intracellular antibodies for 25 min at room temperature.

Analysis of flow cytometry data was performed using the FlowJo software. Data is represented as pseudocolour plots, contour plots or histograms.

Table 6-1: Fluorescence characteristics of used fluorochromes, wavelength of the excitation lasers and detection filters using the LSR-II flow cytometry

Fluorochrome	Absorption maximum (nm)	Emission maximum (nm)	Excitation wave length (nm)	Detection filter (nm)
7-AAD	543	648	543	670/14
Alexa-Fluor-488	495	519	488	695/40
Alexa-Fluor-647	650	665	633	660/20
APC	650	660	633	660/20
APC-CY7	650	785	633	780/60
FITC	495	525	488	695/40
Pacific Blue	410	455	405	450/50
PE	564	575	488	575/26
PE-Cy7	564	767	543	780/60
PerCP	490	675	488	575/26
PI	493	619	543	610/20

6.12 Statistical analysis

Data are expressed as means ± SEM. Statistical significance of differences between different groups and controls was determined by Student t test. Differences were considered statistically significant at $p < 0.050$. Statistical analysis was conducted using GraphPad Prism software (version 5.02).

7 Results

7.1 Establishment of breeding lines

Three breeding lines of immunodeficient mouse strains, all stemming from a C57BL/6 genetic background, were established: Nlrp3$^{-/-}$, Rag1$^{-/-}$ and Nlrp3$^{-/-}$Rag1$^{-/-}$. A modified breeding strategy using breeding pairs of Nlrp3 heterozygous and homozygous mice (Nlrp3$^{+/}$Rag1$^{-/-}$ x Nlrp3$^{-/-}$Rag1$^{-/-}$) was applied. This strategy resulted in offspring that exhibited in 50% a Nlrp3$^{+/-}$Rag1$^{-/-}$ genotype and in 50% a Nlrp3$^{-/-}$Rag$^{-/-}$ genotype, but was otherwise of identical genetic background. Genotyping confirmed successful establishment of these breeding lines (**fig. 6-1 a and b**).

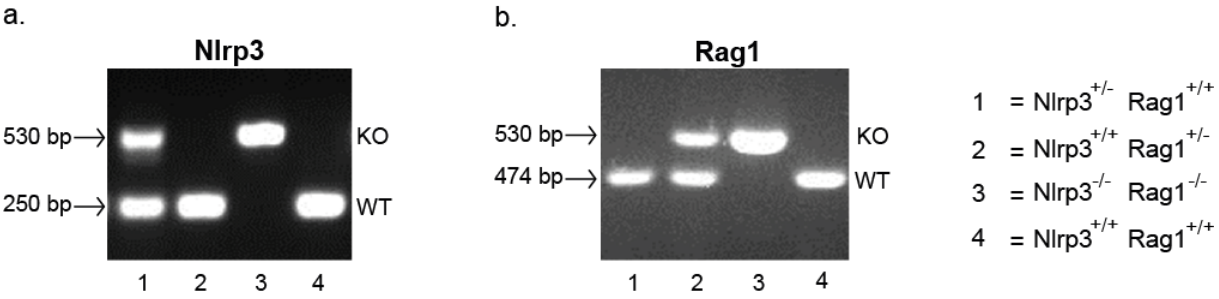

Figure 7-1: Generation and genotyping PCR of Nlrp3$^{-/-}$Rag1$^{-/-}$ double knock-out mice.

Analysis of Nlrp3 (a) and Rag1 (b) genotypes by PCR from tail DNA. Results obtained from; wild-type Nlrp3 (a lane 2 and 4) and Rag1 (b lane 1 and 4), heterozygous Nrlp3 (a lane 1) and Rag1 (b lane 2), and knock-out Nlrp3 (a lane 3) and Rag1 (b lane 3). Rag1 wild-type band and Rag1 knock out (KO) band have 474 bp and 530 bp respectively and Nlrp3 wild-type and Nlrp3 knock out (KO) have 250 bp and 530 bp respectively.

7.2 Nlrp3-deficient Rag1$^{-/-}$ mice are protected from CD45Rb^{High} T cell transfer colitis

The T cell transfer colitis model is based on adoptive transfer of naive, wild-type CD4^{+}CD45Rb^{High} T cells into Rag1$^{-/-}$ mice lacking functionally active T and B cells. Adoptively transferred CD4^{+} T cells encounter their respective antigens in a contact-dependent manner with host myeloid cells, particularly DCs. Mice develop clinical signs of colitis within four weeks when 0.4 x 10^{6} CD4^{+}CD45Rb^{High} T cells are transferred, but not after transfer of CD45Rb^{High} and CD45Rb^{Low} CD4^{+} T cells containing regulatory T cells (data not shown).

Eight to twelve week old Nlrp3-sufficienct and Nlrp3-deficient Rag1$^{-/-}$ mice were reconstituted with sorted CD4^{+}CD45Rb^{High} T cells (purity 98%) (**fig. 6-2 a**) isolated from wild-type mice. Weight loss was significantly reduced in Nlrp3-deficient compared to Nlrp3-sufficient mice (**fig. 6-2 b**). Concordantly, Nlrp3-sufficient mice displayed an aggravated clinical and histological colitis score four weeks after colitis induction (**fig. 6-2 c-e**). Higher colon weight

per length of Nlrp3-sufficient mice was indicative of increased colonic inflammation (**fig. 6-2 f**). Histological scoring of inflammatory infiltration and epithelial damage correlated with higher CD3 mRNA expression in colonic tissue (**fig. 6-2 g**). Collectively, these findings suggest a protective effect of Nlrp3-deficiency during the early phase of adoptive T cell transfer colitis.

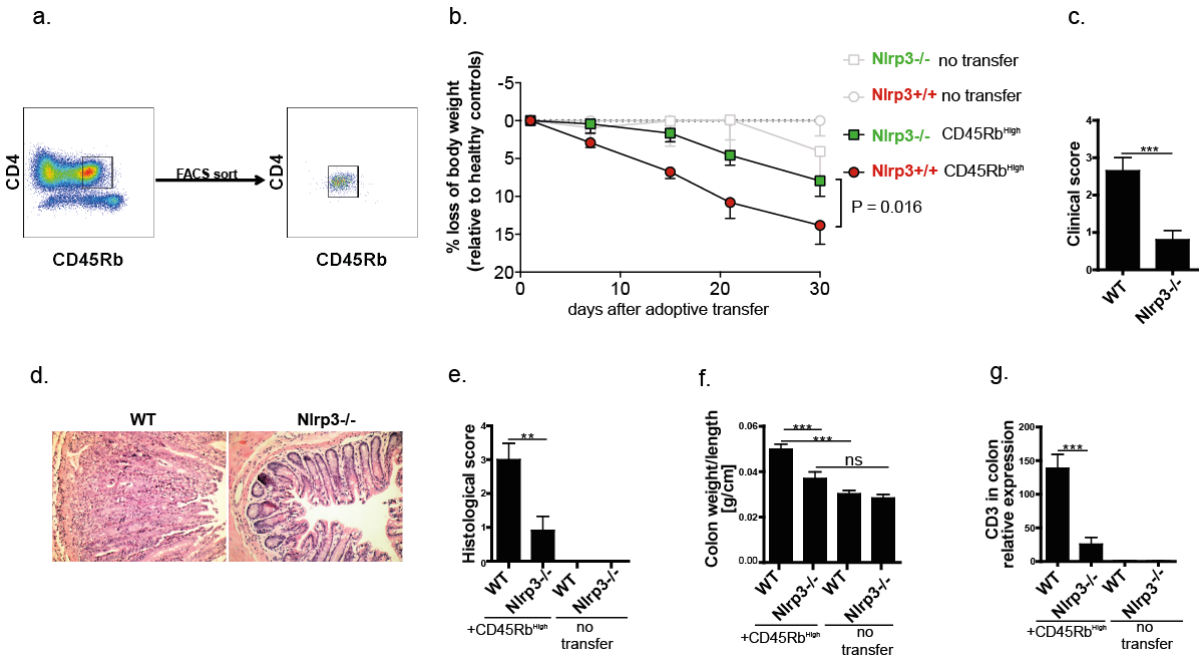

Figure 7-2: Nlrp3-deficiency protects mice from colitis after CD45Rb^{High} T cell transfer.

Nlrp3-deficient and Nlrp3-sufficient mice were reconstituted i.p. with CD4^{+}CD45Rb^{High} T cells (a) from C57BL/6 mice. Percentage body weight change (b, n = 16-18) and clinical score (c, n = 11) after adoptive transfer were monitored for 29 days. Subsequently mice were sacrificed and intensity of inflammation was analysed by blinded histological scoring (d and e, n=11), as well as calculating colon weight per length (f, n=11). Expression of T cell marker CD3 was analysed by rt-qPCR (g, n = 11). Clinical score, histological score, colon weight per length and CD3 mRNA expression data are shown as mean ± SEM of one out of three independent experiments. *p<0.050, **p<0.010, ***p<0.001 as calculated by t test.

7.3 Nlrp3-dependent inflammation correlates with increased IL-1β levels and is associated with other proinflammatory cytokines

NF-κB signalling is a pivotal pathway involved in colonic inflammation. This pathway regulates expression of various cytokines and chemokines and modulates inflammatory processes involved in IBD (Schottelius and Dinter 2006). Activation of the NF-κB pathway induces transcriptional upregulation of pro-IL-1β and its active form induces TNF-alpha (TNF-α), a proinflammatory cytokine, which plays an integral role in the pathogenesis of IBD (Ikejima, Okusawa et al. 1990; Bethea, Gillespie et al. 1992; Saperstein, Chen et al. 2009).

In order to investigate the role of IL-1β and other proinflammatory cytokines in T cell-induced colitis, colon tissue was analysed 29 days post transfer for the expression of cytokines both on mRNA and protein level.

Protection of Nlrp3-deficient mice was associated with a significantly reduced level of TNF-α (**fig. 6-3 a**). It has been described that Nlrp3 expression is induced by TLR agonists in a NF-κB-dependent manner (Qiao, Wang et al. 2012). As expected, unreconstituted Nlrp3-sufficient mice expressed low levels of Nlrp3 at steady state, whereas adoptive T cell transfer strongly induced Nlrp3 expression (**fig. 6-3 b**). This was not the case for other inflammasome components, such as Nlrp6 or NLRC4 (**fig. 6-3 i and j**)**.** As anticipated, no upregulation of Nlrp3 mRNA was found in $Nlrp3^{-/-}$ mice. Similarly, Nlrp3-sufficient mice strongly upregulated IL-1β mRNA expression after colitis induction, which was significantly reduced in $Nlrp3^{-/-}$ mice (**fig. 6-3 c**).

The role of IL-18 expression was more complex. Unexpectedly, IL-18 mRNA expression was significantly suppressed in diseased Nlrp3-deficient mice after T cell transfer and highest expression levels were found in healthy animals (no T cell transfer) (**fig. 6-3 d**). Thus, IL-18 mRNA expression under steady state appeared to be independent on Nlrp3 inflammasome signalling. IL-6, an inflammatory cytokine produced by immune cells infiltrating the inflamed gut during IBD (Jones, Crabtree et al. 1994; Baumgart and Carding 2007; Bernardo, Vallejo-Diez et al. 2012), plays an important role in supporting T cell survival and apoptosis resistance in the LP at the inflamed site (Atreya, Mudter et al. 2000; Neurath, Finotto et al. 2001). IL-6 mRNA expression followed the expected pattern of significantly increased expression in diseased Nlrp3-sufficient mice (**fig. 6-3 e**). In accordance with mRNA levels, protein expression of TNF-α, IL-1β and IL-6 in colon tissue were significantly higher in Nlrp3-sufficient as compared to Nlrp3-deficient mice (**fig. 6-3 f-h**).

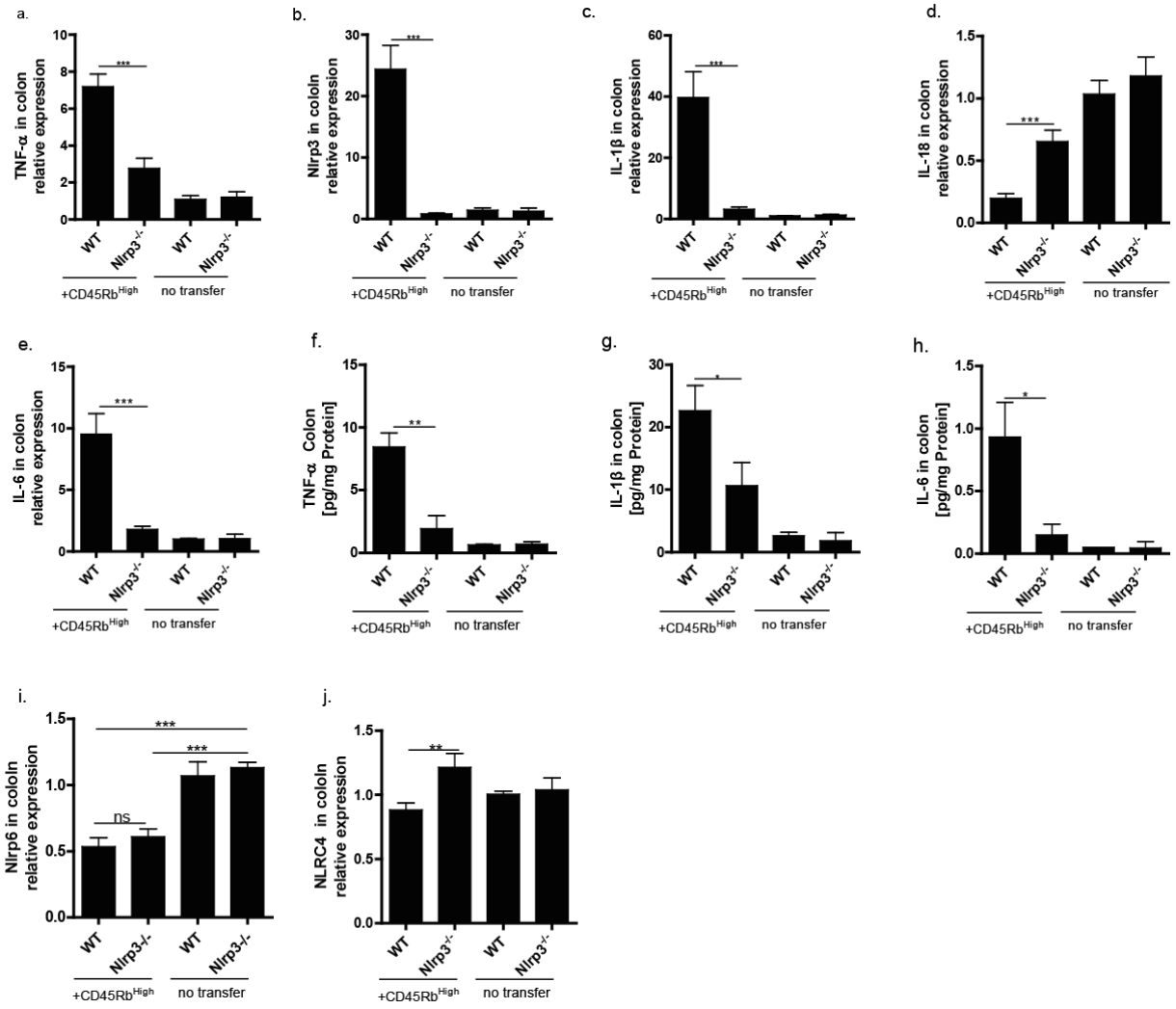

Figure 7-3: Expression of proinflammatory cytokines in colonic tissue is reduced in Nlrp3-deficient mice after CD45Rb^{High} T cell transfer.

Nlrp3-deficient and Nlrp3-sufficient Rag1$^{-/-}$ mice were reconstituted i.p. with CD4^{+}CD45Rb^{High} T cells from C57BL/6 mice to induce colitis. At sacrifice four weeks later, expression of proinflammatory cytokines (a, c, d and e; n=11) and inflammasome subunits (b, i and j, n=11) were analysed on mRNA level by rt-qPCR; cytokine levels were also measured on protein level by ELISA (f, g and h, n=5). Data from one out of three independent experiments are shown represented as means ± SEM. *p<0.050, **p<0.010, ***p<0.001 as calculated by t test.

7.4 Nlrp3 inflammasome plays a role in Th1/Th17 polarisation of adoptively transferred CD4^{+} T cells

Next, the role of the Nlrp3-inflammasome in T cell polarisation during colitis induction was assessed. Four weeks after adoptive T cell transfer, single cell suspensions were isolated from the LP; cells were stained for T cell markers and analysed by flow cytometry. Frequency of CD4^{+} T cells in the LP was higher in Nlrp3-sufficient than in Nlrp3-deficient mice, correlating with a more severe clinical score (**fig. 6-4 a**). Concordantly, mRNA expression of CD4 in the colon tissue of Nlrp3-suffiecient mice was higher (**fig. 6-4 b**). A significant

increase in the frequency of $CD4^+$ T cells was also observed in the MLN (**fig. 6-5 a and b**), as well as in the in the spleen (**fig. 6-5 e and f**). A similar trend was also observed in blood (**fig. 6-5 i and j**).

Intracellular IFN-γ expression of isolated $CD4^+$ T cells was slightly elevated in Nlrp3-sufficient mice (**fig. 6-4 c and e**), however this effect did not reach statistical significance. More pronounced effects were found when investigating chemokines and cytokines commonly associated with a Th1 phenotype of T cells. IP-10 (CXCL10) was significantly increased in Nlrp3-sufficient mice after adoptive transfer (**fig. 6-4 d**). IP-10 is a chemokine secreted by a wide range of tissue under proinflammatory conditions (Farber 1997; Neville, Mathiak et al. 1997) and preferably attracts Th1 lymphocytes to sites of inflammation (Taub, Lloyd et al. 1993; Taub, Longo et al. 1996). IL-12, primarily produced by antigen presenting cells (APCs) (Trinchieri 1998), is one of the key factors in the differentiation and expansion of Th1 cells. Its subunit IL-12(p35), which has been described among other functions to contribute to autoimmunity by negatively regulating IL-27 (Vasconcellos, Carter et al. 2011), was analysed. Expression of IL-12(p35) was significantly elevated in colon tissue of Nlrp3-sufficient mice, correlating with increased IP-10 levels (**fig. 6-4 f**); in line with this finding, levels of IL-27 were decreased (**fig. 6-4 g**).

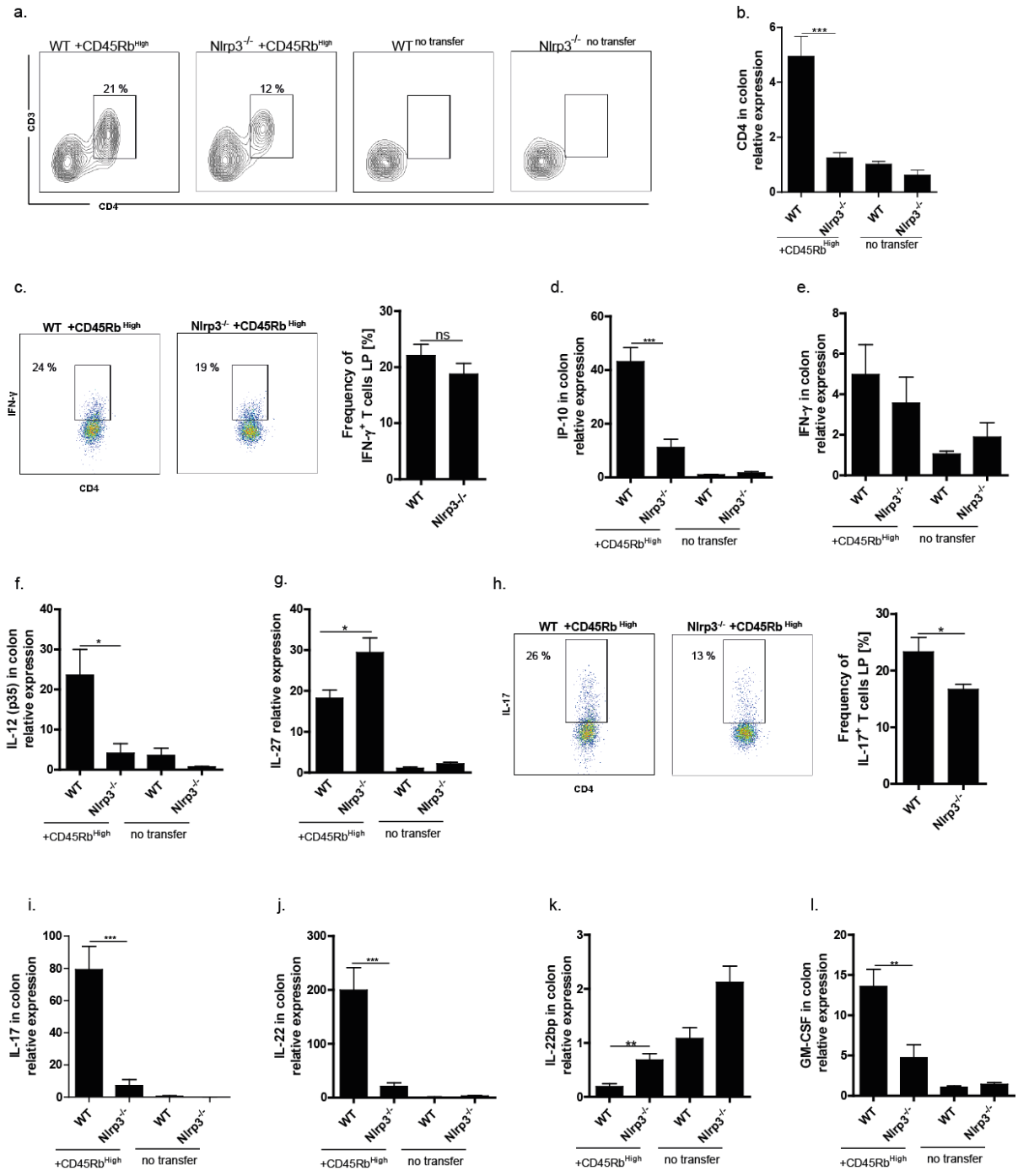

Figure 7-4: Nlrp3 inflammasome promotes Th1 and Th17 polarisation in the lamina propria.

Nlrp3-deficient and Nlrp3-sufficient Rag1$^{-/-}$ mice were reconstituted i.p. with CD4^{+}CD45Rb^{High} T cells from C57BL/6 mice to induce colitis. At sacrifice four weeks later, single cell suspensions isolated from the LP were characterised for CD4^{+} T cellular infiltration and CD4 expression in colon tissue (a and b respectively). Frequency of IL-17- and IFN-γ-expressing CD4^{+} T cells was analysed by flow cytometry (c and h) (n = 5 - 6 mice per group). mRNA expression of T cell-associated cytokines and chemokines in colonic tissue was analysed by rt-qPCR, measuring Th1 cytokines IP-10, IFN-γ, IL-12(p35) and IL-27 (d - g), Th17-associated cytokines IL-17, IL-22 and GM-CSF (i, j and l), as well as IL-22bp (k) a soluble inhibitor of IL-22. (n = 5 - 11). Flow cytometry and mRNA data are shown as means ± SEM of one out of three independent experiments. *p<0.050, **p<0.010, ***p<0.001 as calculated by t test.

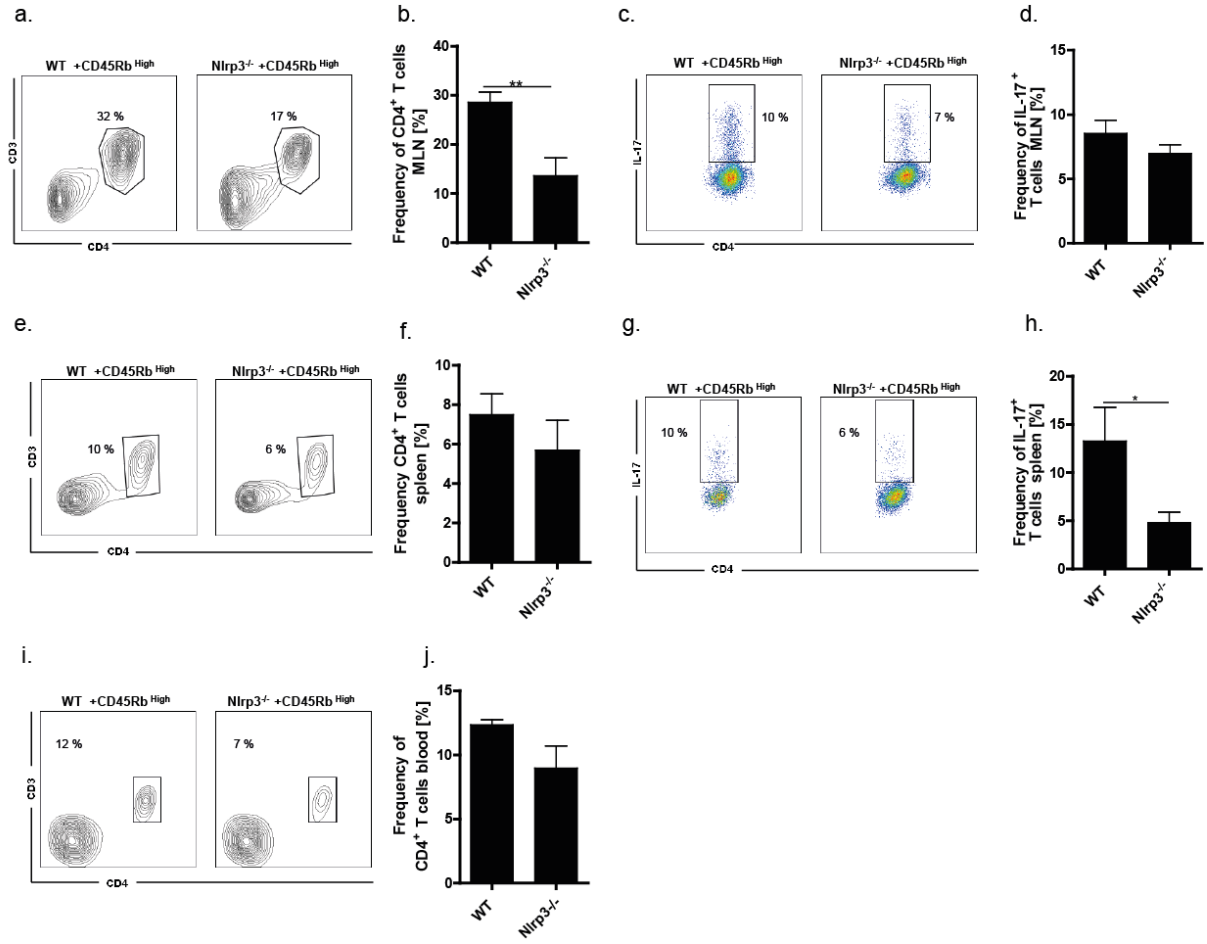

Figure 7-5: Increased CD4$^+$ T cell infiltration into MLN and spleen, and IL-17 production in spleen and MLN of Nlrp3-sufficient mice after adoptive T cell transfer.

Nlrp3-deficient and Nlrp3-sufficient Rag1$^{-/-}$ mice were reconstituted i.p. with CD4$^+$CD45Rb^{High} T cells from C57BL/6 mice to induce colitis. At sacrifice, frequency of MLN-derived CD4$^+$ T cells (a and b) and their IL-17 production (c and d) were analysed by FACS analysis. Frequency of CD4$^+$ T cells in the spleen (e and f) and IL-17 production (g and h) were analysed by FACS analysis. Frequency of CD4$^+$ T cells in peripheral blood (i – j) were analysed per FACS analysis. Data are presented as means ± SEM (n = 4 - 6). One out of three independent experiments are shown. *p<0.050, **p<0.010, as calculated by t test.

T cell activation and survival is promoted by IL-1β (Ben-Sasson, Hu-Li et al. 2009). Several studies have pointed out that IL-1β acts in concert with other proinflammatory cytokines to induce Th17 differentiation and autoinflammatory disorders (Horai, Saijo et al. 2000; Sutton, Brereton et al. 2006; Acosta-Rodriguez, Napolitani et al. 2007; Brydges, Mueller et al. 2009; Sutton, Lalor et al. 2009). Having this in mind, the effect of Nlrp3-deficiency in the development of Th17 polarisation in adoptive T cell transfer model was investigated.

Intracellular IL-17 expression of LP CD4$^+$ T cells was significantly higher in Nlrp3-sufficient than Nlrp3-deficient mice (**fig. 6-4 h**). Increased IL-17 levels were also observed in the MLN (**fig. 6-5 c and d**) and in the spleen (**fig. 6-5 g and h**). Accordingly, mRNA expression of IL-

17 in the colon tissue of Nlrp3-sufficient mice was significantly higher than in the colon of Nlrp3-deficient mice (**fig. 6-4 i**).

Moreover, Th17-associated cytokines IL-22 and GM-CSF showed increased mRNA expression levels in Nlrp3-sufficient mice (**fig. 6-4 j and l**); in contrast, expression of IL-22bp, a soluble inhibitor neutralising IL-22 by binding to its receptor, was reduced (**fig. 6-4 k**).

7.5 Intestinal dendritic cell infiltrate is increased after adoptive T cell transfer and consists predominantly of CD103^{+} dendritic cells in Nlrp3-deficient mice

DCs as key initiators and regulators of adaptive immune responses play a critical role in regulating the balance between tolerance and immunity in the intestinal mucosa. Phenotypic and functional characteristics of DCs are partly defined by signals they receive within the local microenvironment or from their immediate precursors. Depending on the type of DCs and their activation state, DC/T cell interaction leads to initiation of either immunity or tolerance.

For a better understanding of the role DCs play in the induction of colitis, cells derived from the LP were isolated and CD103^{+} and CD103^{-} DC subpopulations were analysed by flow cytometry. Adoptive transfer of CD4^{+}CD45Rb^{High} T cells resulted in an increased DC infiltration into LP as compared to untreated Rag1$^{-/-}$ mice, irrespective of Nlrp3 expression (**fig. 6-6 a**). In Nlrp3-suficient mice the DC infiltrate was dominated by proinflammatory CD103^{-}, whereas in Nlrp3-deficient mice the fraction of CD103^{+} DCs was significantly increased (**fig. 6-6 b and c**).

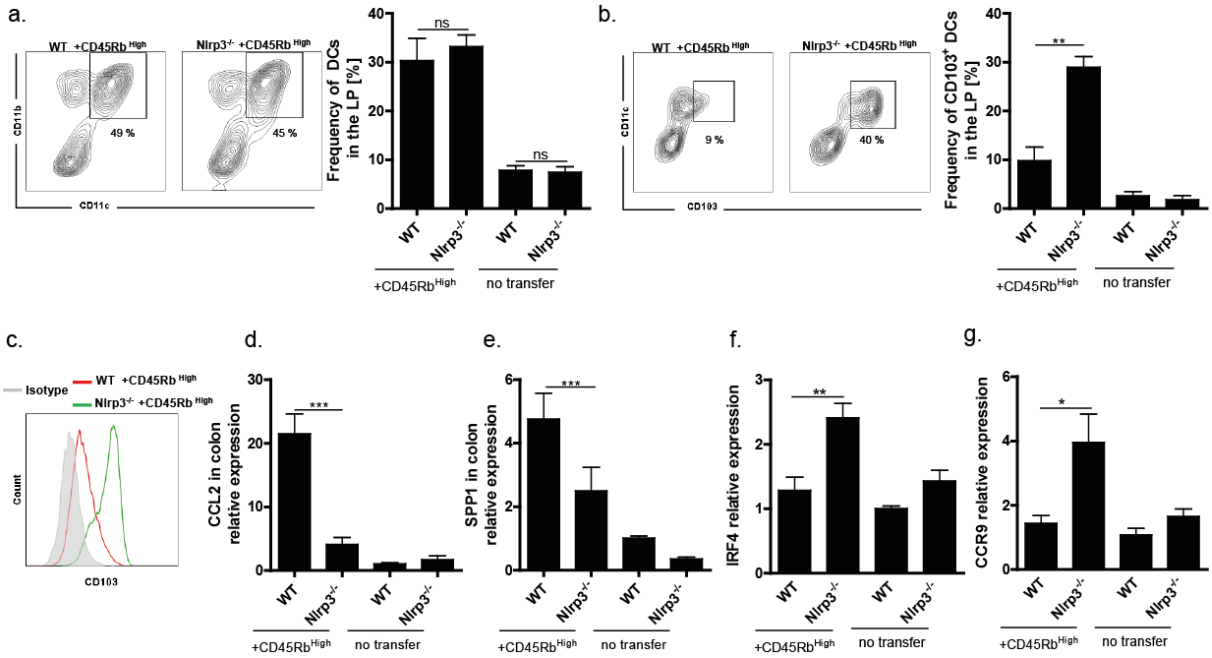

Figure 7-6: Intestinal DC infiltrate is induced by CD4$^+$CD45Rb^{High} T cell transfer and is shifted towards CD103$^+$ DCs in the absence of Nlrp3 inflammasome.

Nlrp3-deficient and Nlrp3-sufficient Rag1$^{-/-}$ mice were reconstituted i.p. with CD4$^+$CD45Rb^{High} T cells from C57BL/6 mice to induce colitis. At sacrifice, LP-derived DCs were analysed by flow cytometry and DC-associated marker genes were analysed by rt-qPCR. A representative FACS plot and a bar diagram (a) show frequency of CD11c$^+$ DCs in LP. Frequency (b) and expression level (c) of CD103$^+$ DCs amongst CD11c$^+$MHCII$^+$ cells was analysed per FACS. Relative expression of CCL2 (d), osteopontin (SPP1) (e), IRF4 (f) and CCR9 (g) in colon tissue was analysed by rt-qPCR. Data are presented as means ± SEM (n = 11 per transfer group, and n = 6 per control group). One experiment out of three independent experiments is shown. *p<0.050, **p<0.010, ***p<0.001 as calculated by t test.

CCL2 expression in colon tissue was significantly increased in Nlrp3-sufficient mice after adoptive T cell transfer as compared to Nlrp3-deficient mice (**fig. 6-6 d**). This observation is in line with previous work, which described CCL2 expression as a driver of intestinal inflammation (Reinecker, Loh et al. 1995; Mazzucchelli, Hauser et al. 1996; Popivanova, Kostadinova et al. 2009).

Recent studies suggest a proinflammatory role of osteopontin (SPP1) in TNBS- and DSS-induced colitis (Zhong, Eckhardt et al. 2006; Oz, Zhong et al. 2012). Its expression in DCs has been associated with disease severity (Shinohara, Jansson et al. 2005; Murugaiyan, Mittal et al. 2008; Shinohara, Kim et al. 2008; Murugaiyan, Mittal et al. 2010). As expected, expression of osteopontin was significantly higher in Nlrp3-sufficient mice (**fig. 6-6 e**). This finding is concordant with recent reports about excessive levels of osteopontin in CD103$^-$ DCs, driving intestinal inflammation (Kourepini, Aggelakopoulou et al. 2014). In contrast, expression analysis of IRF4 and CCR9 in colonic tissue showed lower levels in Nlrp3-sufficient mice (**fig. 6-6 f and g**).

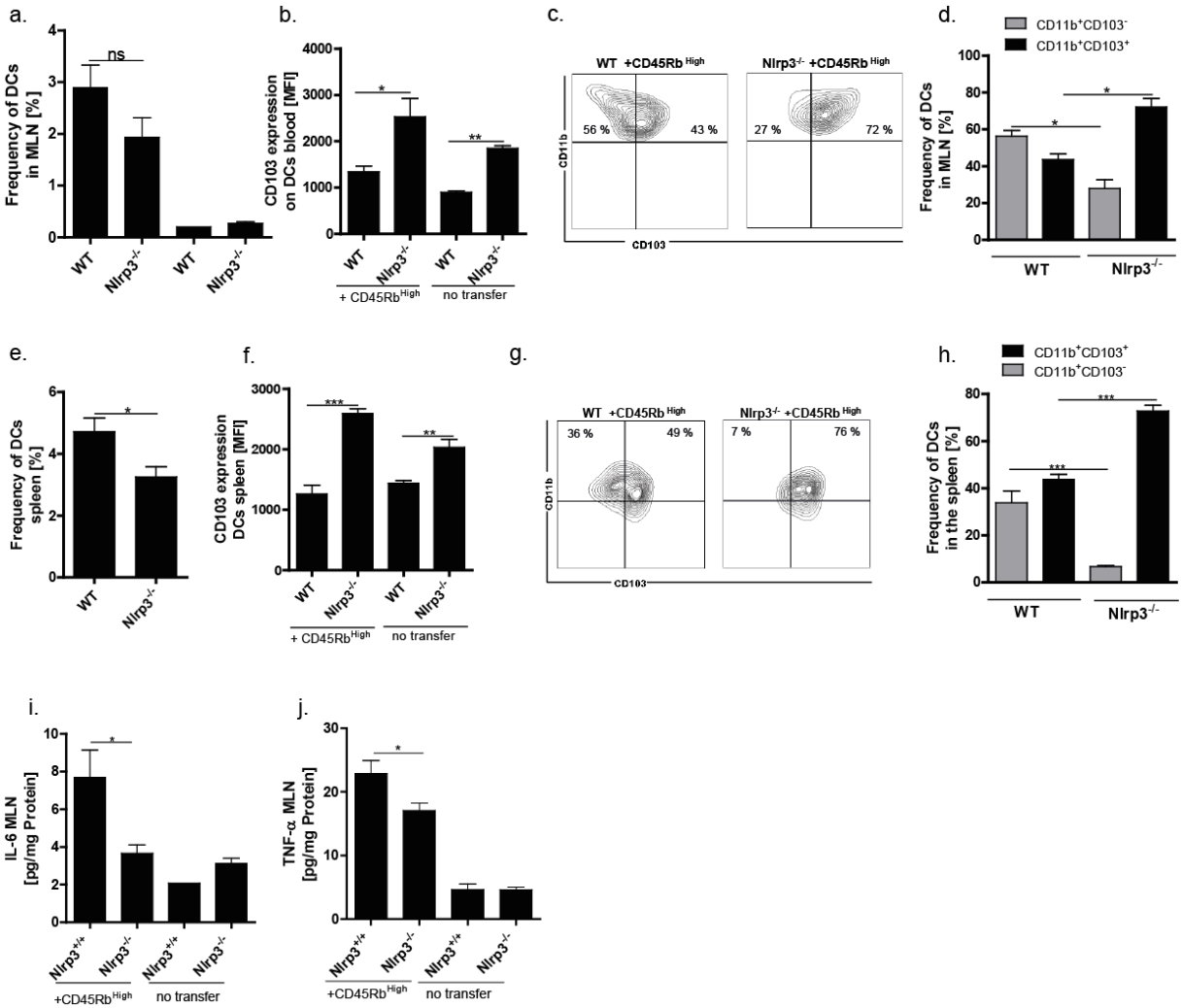

Figure 7-7: Increased expression of CD103^{+} by DCs in the MLN, spleen and blood of Nlrp3-deficient mice.

Nlrp3-deficient and Nlrp3-sufficient Rag1$^{-/-}$ mice were reconstituted i.p. with CD4^{+}CD45Rb^{High} T cells from C57BL/6 mice to induce colitis. At sacrifice, MLN-derived DCs (a and b) and spleen DCs (e and f) were analysed for CD103 expression by flow cytometry. MLN (c and d) and spleen (g and h) CD11c^{+}MHCII^{+} DCs were further characterised for expression of CD11b and CD103. Secretion of proinflammatory cytokines was determined in MLN tissue by ELISA (i and j). Data are presented as means ± SEM (n = 4 - 6). One of three independent experiments is shown. *p<0.050, **p<0.010, ***p<0.001 as calculated by t test.

Infiltration of DCs in the MLN, a destination where DCs from the LP migrate to was analysed. DC infiltration of MLN was increased after adoptive transfer of T cells (**fig. 6-7 a**). Total DCs infiltration of MLN differed only slightly between Nlrp3-sufficient and Nlrp3-deficient mice. However, DC phenotype was significantly shifted towards CD103^{+} DCs in Nlrp3$^{-/-}$ mice (**fig. 6-7 b-d**). Analysis of cytokine levels in MLN demonstrated lower secretion of the proinflammatory cytokines IL-6 and TNF-α in Nlrp3-deficient mice (**fig. 6-7 i and j**).

In addition, splenocytes were isolated 4 weeks after adoptive T cell transfer and DCs analysed by flow cytometry. Interestingly, the frequency of DCs was significantly higher in

Nlrp3-sufficient mice (**fig. 6-7 e**). However, similar to MLN, the expression of CD103 was significantly higher in splenic DCs of Nlrp3-deficient mice (**fig. 6-7 f**). Again, the total frequency of $CD11b^+$ DCs was comparable, but the frequency of $CD103^+$ DC among the $CD11b^+$ DCs was higher in $Nlrp3^{-/-}$ mice (**fig. 6-7 g and h**).

7.6 FLT3L and GM-CSF determine the phenotype of intestinal dendritic cells

FLT3L plays an indispensable role in regulating DC homeostasis in secondary lymphoid tissue (Maraskovsky, Brasel et al. 1996; Saunders, Lucas et al. 1996), while GM-CSF induces differentiation of inflammatory DCs (Blyszczuk, Behnke et al. 2013; Reynolds, Gibbon et al. 2016). $CD103^+$ DCs have been described to originate from macrophage DC precursors in a FLT3L-dependent manner, while $CD103^-$ $CD11b^+$ DCs have been described to originate from $Ly6C^{High}$ monocytes in response to GM-CSF (Varol, Vallon-Eberhard et al. 2009). Recent studies have shown that DC differentiation is partly dependent on T cells, especially $CD4^+$ T cells, which can produce GM-CSF as well as FLT3L (Saito, Boddupalli et al. 2013; Reynolds, Gibbon et al. 2016).

To assess whether the Nlrp3 inflammasome regulates the balance between GM-CSF and FLT3L, the phenotype of BM-DCs generated from Nlrp3-deficient or Nlrp3-sufficient mice were analysed *in vitro*. BM-derived cells were differentiated for one week with either GM-CSF and IL-4, or for nine days with FLT3L. FLT3L-differentiated DCs demonstrated a higher frequency of $CD103^+$ cells amongst resulting DCs, as compared to DCs differentiated with GM-CSF and IL-4. Interestingly, cells derived from Nlrp3-deficient mice and differentiated with FLT3L had a significantly higher expression of CD103 compared to Nlrp3-sufficient mice (**fig. 6-8 a and b**). BM-DCs were then stimulated overnight with LPS, and secretion of proinflammatory cytokines in supernatant was determined by ELISA. As expected, IL-1β secretion in DCs of Nlrp3-deficient mice was significantly reduced due to lack of IL-1β processing by the inflammasome (**fig. 6-8 c**). Secretion of TNF-α and IL-12(p70), which are IL-1β-associated proinflammatory cytokines, was also significantly reduced in Nlrp3-deficient BM-DCs (**fig. 6-8 d and e**). In contrast, secretion of IL-6 was comparable in both groups (**fig. 6-8 f**). Of note, cytokine secretion was most pronounced in GM-CSF and IL-4 generated DCs as compared to FLT3 DCs (**fig. 6-8 c-e**). The data confirm the reduced inflammatory properties of FLT3L-derived DCs in response to TLR4 stimulation.

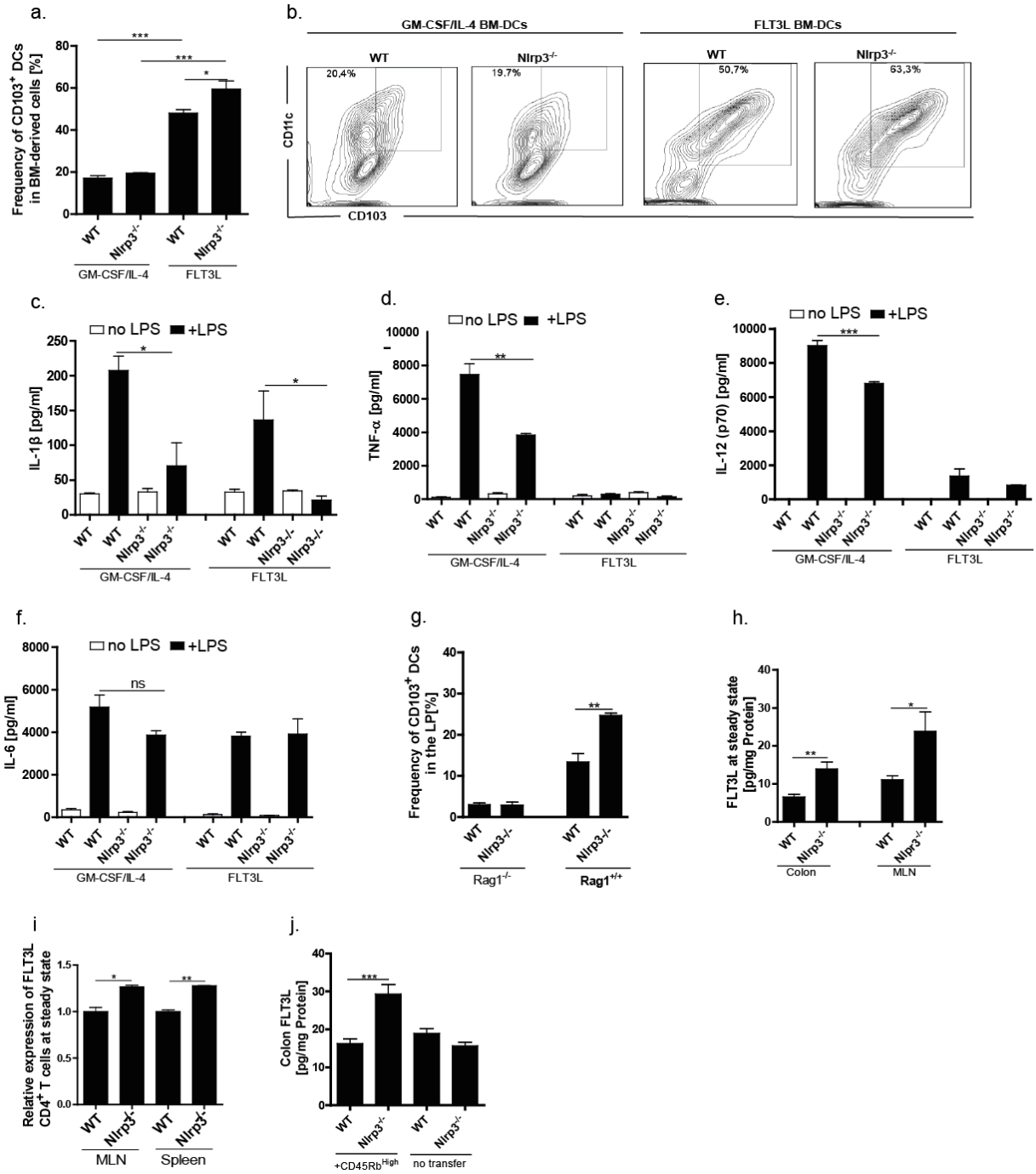

Figure 7-8: Nlrp3-deficient DCs express a less inflammatory phenotype and increased expression of FLT3L in Nlrp3-deficient CD4$^+$ T cells correlates with higher CD103 expression levels by lamina propria DCs.

BM-derived DCs generated from Nlrp3-deficient and Nlrp3-sufficient mice were cultured in the presence of either GM-CSF and IL-4, or FLT3L. At the end of culture period DCs were analysed by flow cytometry. A bar diagram displaying frequency of CD103$^+$ DCs (a) and a representative FACS plot (b) are shown. BM-derived DCs were stimulated for 12 hrs with LPS and secretion of proinflammatory cytokines IL-1β (c), TNF-α (d), IL-12(p70) (e) and IL-6 (f) was measured in supernatant by ELISA. Data are shown as means ± SEM (n = 4). Frequencies of CD103$^+$ DCs in the LP in T cell-deficient Rag1$^{-/-}$ and T cell-sufficient wt mice (Nlrp3-deficient versus Nlrp3-sufficient, respectively) were analysed at steady state by flow cytometry (g). Data are shown as means ± SEM of independent experiment (n = 9 to11), reproduced 3 times. Colon and MLN tissue from Rag1-sufficient

mice at steady state was analysed for FLT3L protein by ELISA (h). Data are shown as means ± SEM (g: n = 6-8, and h: n = 11). One out of three independent experiments are shown. Splenic $CD4^+$ T cells were sorted to 99% purity and analysed for expression of FLT3L by rt-qPCR (i). Data are shown as means ± SEM (n=4). $Rag1^{-/-}$ mice (either Nlrp3-sufficient or Nlrp3-deficient) were characterised for FLT3L production at steady state and after adoptive transfer of $CD4^+CD45Rb^{High}$ T cells (j). Data are shown as means ± SEM (n = 11). One of three independent experiments is shown. *p<0.050, **p<0.010, ***p<0.001 as calculated by t test.

To characterise the phenotype of steady state intestinal DCs, frequency of $CD103^+$ DCs in Nlrp3-deficient versus Nlrp3-sufficient $Rag1^{-/-}$ and wild-type ($Rag1^{+/+}$) mice was determined. Interestingly, LP infiltration of DCs was low in $Rag1^{-/-}$ mice at steady state (before having received T cell transfer) compared to steady state $Rag1^{+/+}$ mice (that also harbour a population of intestinal T cells). These data indicate that presence of T cells is mandatory for LP DC infiltration. Importantly, in the absence of T cells no difference between Nlrp3-deficient and Nlrp3-sufficient mice was found regarding frequency of $CD103^+$ DCs, whereas Rag1-sufficient, but Nlrp3-deficient mice had significantly increased numbers of $CD103^+$ DCs (**fig. 6-8 g**). These results confirmed literature reports (Saito, Boddupalli et al. 2013) and earlier findings of our group (Bauer, Duewell et al. 2012). In summary, presence of T cells in Nlrp3-deficient mice seemed mandatory for shifting intestinal DCs towards a $CD103^+$ phenotype.

FLT3L has been found indispensable for development of $CD103^+$ DCs (Waskow, Liu et al. 2008). In order to investigate the role of growth factors associated with DC phenotype in our model, FLT3L levels were determined in colon and MLN tissue of immunocompetent ($Rag1^{+/+}$) mice at steady state. Nlrp3-deficient mice had significantly higher levels of FLT3L than Nlrp3-sufficient mice (**fig. 6-8 h**). Accordingly, splenic $CD4^+$ T cells isolated from Nlrp3-deficient mice demonstrated a higher expression of FLT3L mRNA (**fig. 6-8 i**). Corroborating these findings, $Rag1^{-/-}$ mice, devoid of functionally active T cells exhibited low levels of FLT3L (**fig. 6-8 j**). Importantly, Nlrp3-deficient, but not Nlrp3-sufficient $Rag1^{-/-}$ mice exhibited increased FLT3L levels in colon tissue after adoptive transfer of $CD4^+CD45Rb^{High}$ T cells, possibly indicating that $CD4^+$ T cell-derived FLT3L mediates induction of $CD103^+$ DCs (**fig. 6-8 j**). In summary, Nlrp3-dependent differential expression of FLT3L may link the finding of increased frequency of $CD103^+$ DCs and reduced inflammatory phenotype of intestinal T cells in Nlrp3-deficient mice after T cell transfer.

Coculture of splenic OVA-peptide-loaded DCs with naive $CD4^+$ T cells from OT-II mice (**fig. 6-9 d**) demonstrated a shifted ratio of FLT3L and GM-CSF secretion, with Nlrp3-deficient DCs producing higher levels of FLT3 and Nlp3-sufficient DCs secreting more GM-CSF (**fig. 6-9 a**

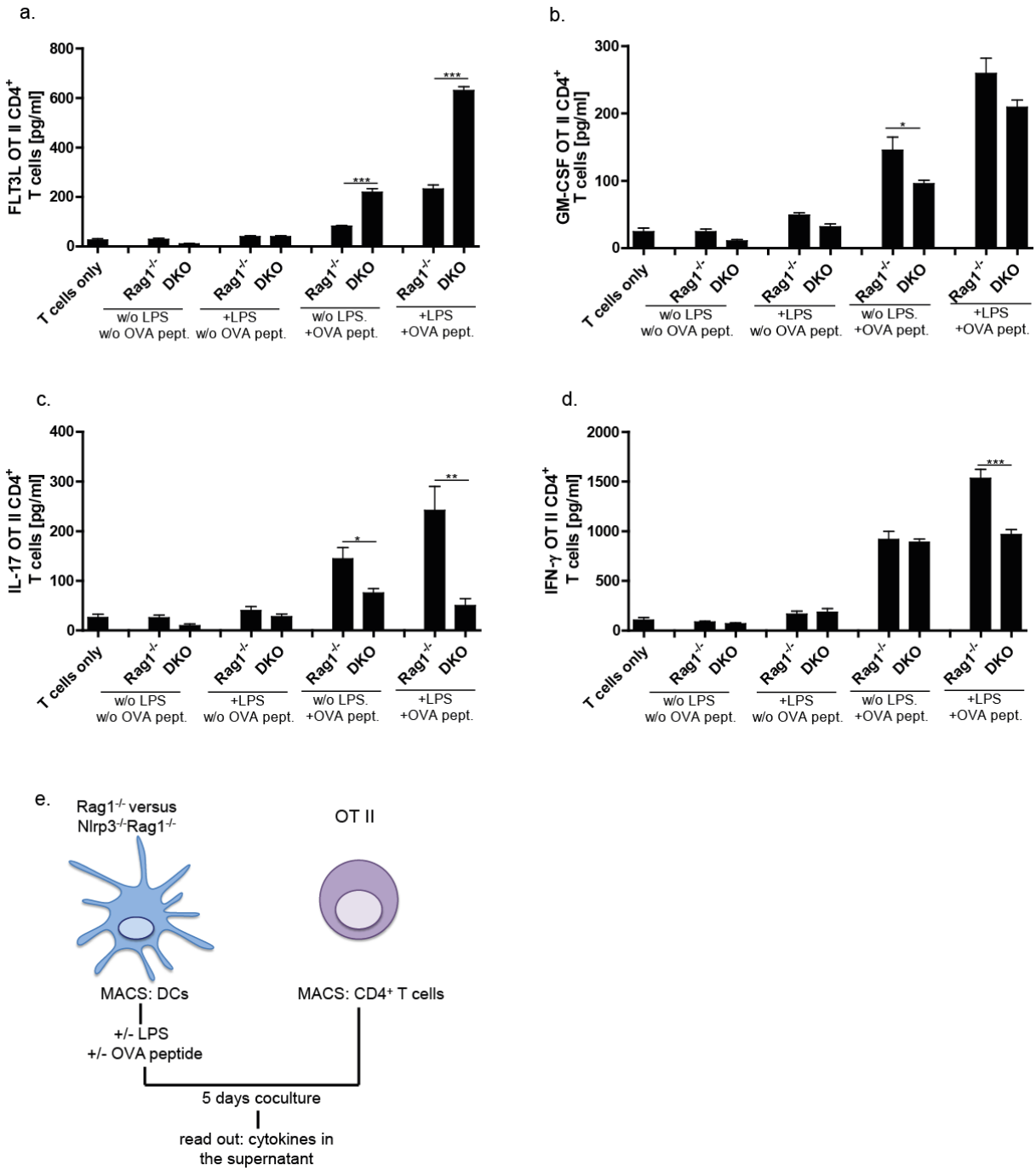

Figure 7-9: Nlrp3 inflammasome in DCs controls the balance of FLT3L and GM-CSF as well as proinflammatory cytokine production by OT-II CD4$^+$ T cells.

Rag1$^{-/-}$ and Nlrp3$^{-/-}$ Rag1$^{-/-}$ (DKO) splenic CD11c$^+$ DCs were MACS sorted and stimulated with LPS overnight. DCs were pulsed with MHC-II-restricted OVA-peptide and co-cultured with CD4$^+$ OT-II T cells for 5 days (e). Secretion of FLT3L, GM-CSF, IL-17 and IFN-γ was measured supernatants by ELISA (a-d). Data are shown as means ± SEM (n = 4). One of three independent experiments is shown: *p<0.050, **p<0.010, ***p<0.001 as calculated by t test.

and b). Accordingly, secretion of IL-17 and IFN-γ by the CD4$^+$ T cells were higher when cocultured with DCs from Nlrp3-sufficient mice (**fig. 6-8 c and d**). Thus, the Nlrp3

inflammasome controls the balance of FLT3L and GM-CSF production and as a consequence CD103 expression by DCs.

7.7 IL-1β induces CD4$^+$ T cell polarisation into Th17 cells

As host Nlrp3 status determines DC phenotype and shifts CD4$^+$ T cells towards a Th17/Th1 phenotype, differential contribution of Nlrp3-dependent cytokines IL-18 and IL-1β towards T cell plasticity was investigated. Under cell culture conditions, IL-1β, not IL-18, induced secretion of Th17-associated proinflammatory cytokines in splenic CD4$^+$ T cells, activated with CD3/CD28 mAb for 3 days. Proliferation of CD4$^+$ T cells was not affected by addition of IL-1β or IL-18 (**fig. 6-10 a**).

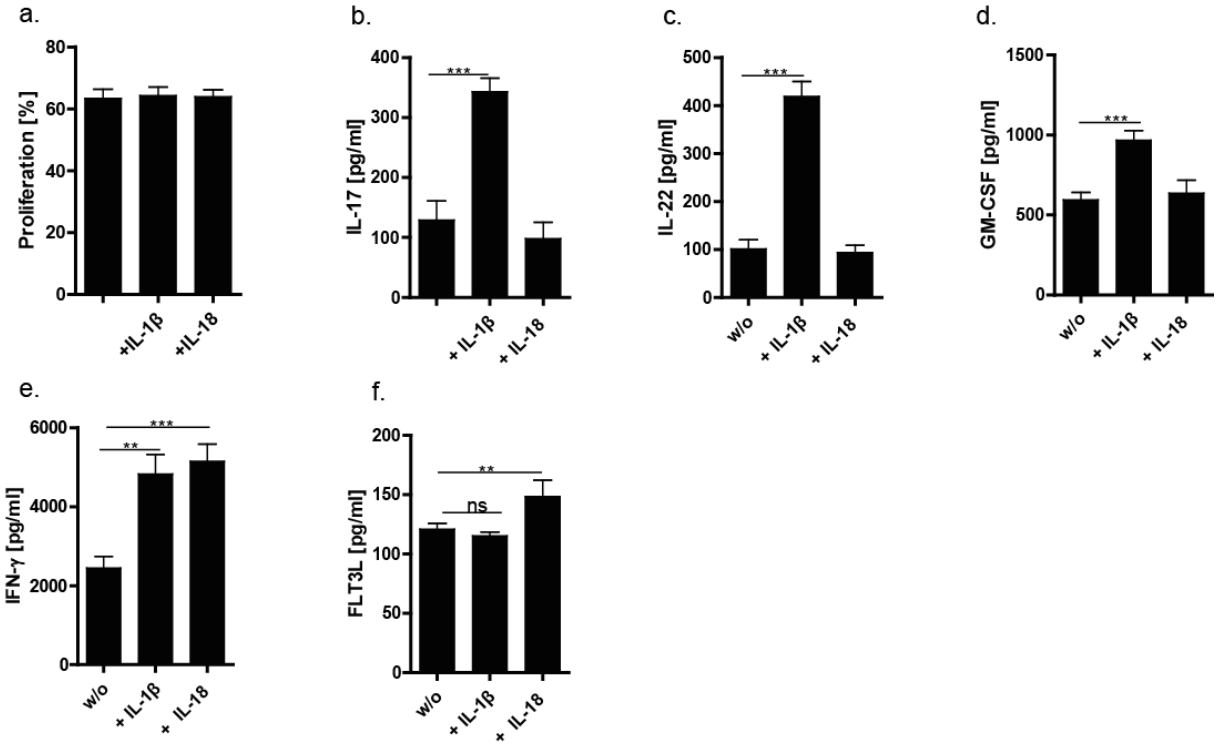

Figure 7-10: IL-1β induces secretion of Th17- and Th1-related cytokines in CD4$^+$ T cells.

Splenic CD4$^+$ T cells were polarised on anti-CD3/CD28 mAb-coated round bottom well plates in the presence of either 20 ng/ml IL-1β or 10 ng/ml IL-18. After 72 hrs, proliferation of T cells was analysed by the CFSE dilution (a) and secretion of IL-17, IL-22, GM-CSF IFN-γ and FLT3L was analysed by ELISA (b – f, n = 4, respectively). One experiment out of three independent experiments is shown. Data are shown as means ± SEM. One of three independent experiments is shown. **p<0.010, ***p<0.001 as calculated by t test.

CD4$^+$ T cells polarised in the presence of IL-1β demonstrated significantly higher secretion of IL-17 and IL-22 (**fig. 6-10 b and c**), as compared to IL-18. Both IL-1β and IL-18 induced secretion of the Th1 cytokine IFN-γ (**fig. 6-10 e**). IL-18 but not IL-1β slightly increased FLT3L production of CD4$^+$ T cells (**fig. 6-10 f**), whereas IL-1β induced secretion of GM-CSF (**fig. 6-10 d**). In summary, our data indicate that Nlrp3-dependent IL-1β mediates

proinflammatory effects in T cell transfer colitis. In addition, the balance between GM-CSF and FLT3L production by T cells is inversely regulated by IL-1β and IL-18.

7.8 Lack of IL-18R signalling in $CD4^+$ T cells promotes intestinal inflammation

It was shown in a recent study that, at steady state, IECs constitutively produce IL-18, which inhibits Th17 differentiation. IL-18 acts directly on IL-18R-expressing $CD4^+$ T cells, partly by antagonising MyD88-dependent signalling effectors downstream of IL-1R. In the same work authors showed that IL-18R expression of $FoxP3^+$ T_{reg} cells played an important role in prevention of experimental colitis (Harrison, Srinivasan et al. 2015).

As described above, inflamed colon tissue of mice receiving adoptively transferred $CD4^+$ T cells showed significantly reduced expression of IL-18 mRNA levels (**fig. 6-3 d**), strengthening the "*IL-18 protective effect*" hypothesis. To further clarify the role of T cell signalling of Nlrp3-dependent cytokines IL-1β and IL-18, $CD4^+CD45Rb^{High}$ T cells, derived from wild-type C57BL/6, IL-1R-deficient or IL-18R-deficient mice were adoptively transferred into $Rag1^{-/-}$ mice to assess colitis induction.

$Rag1^{-/-}$ mice receiving $IL\text{-}18R^{-/-}$ $CD4^+$ T cells lost more body weight, developed more pronounced symptoms of colitis and demonstrated exacerbated intestinal inflammation and epithelial damage on histological analysis (**fig. 6-11 a-d**) compared to mice reconstituted with WT $CD4^+$ T cells. Colonic CD3 expression was higher in mice reconstituted with $IL\text{-}18R^{-/-}$ $CD4^+$ T cells correlating with the increased colon weight per length as surrogate parameter of cellular infiltration and inflammation (**fig. 6-11 e and f**). In contrast, mice reconstituted with $CD4^+CD45Rb^{High}$ T cells from $IL\text{-}1R^{-/-}$ mice demonstrated no significant difference to mice reconstituted with WT T cells.

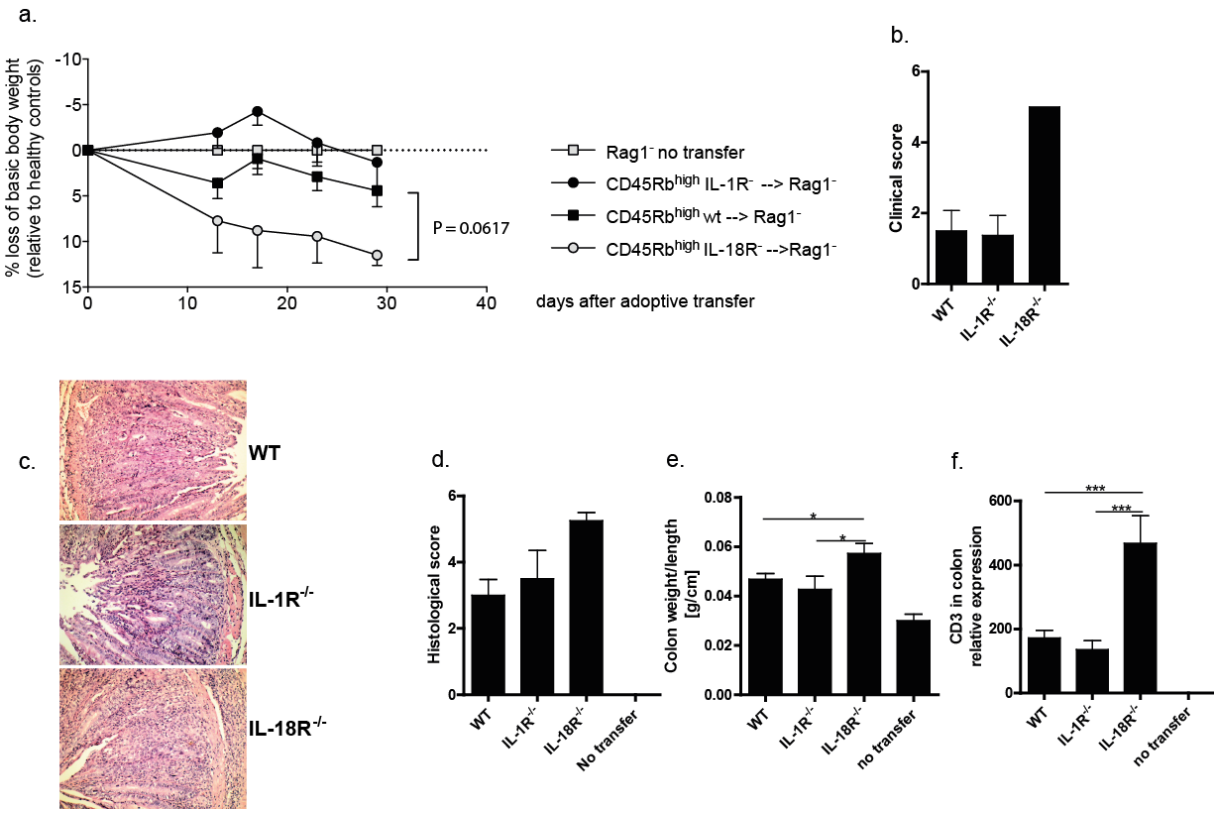

Figure 7-11: IL-18R signalling in adoptively transferred CD4$^+$ T cells dampens T cell-mediated colitis.

Rag1$^{-/-}$ mice were reconstituted i.p. with CD4$^+$CD45Rb^{High} T cells from either C57BL/6, IL-18R- or IL-1R-deficient mice and monitored for 4 weeks. Weight loss (a) and clinical score comprising of haematochezia and stool consistency (b) were determined. Histological analysis of colon tissue (c) was performed by blinded scoring (d). Colon weight per length (e) was calculated as surrogate parameter of inflammation. CD3 gene expression in colonic tissue was examined by rt-qPCR (f). Data are shown as means ± SEM (n = 12 to14). Data pooled from 3 experiments are shown. *p<0.050, ***p<0.001 as calculated by t test.

In addition, TNF-α, Nlrp3, IL-1β, IL-6 and IL-12p35 mRNA expression was significantly higher in colon tissue of mice reconstituted with IL-18R-deficient T cells (**fig. 6-12 a-d and f respectively**). Verifying earlier findings on downregulation of IL-18 mRNA expression levels after adoptive T cell transfer (**fig. 6-3 d**), IL-18 mRNA expression was significantly suppressed in all subgroups having received T cells (**fig. 6-12 e**). Expression of inflammatory markers SPP1 and CCL2 were highest in mice that received IL-18R$^{-/-}$ T cells (**fig. 6-12 g-h**).

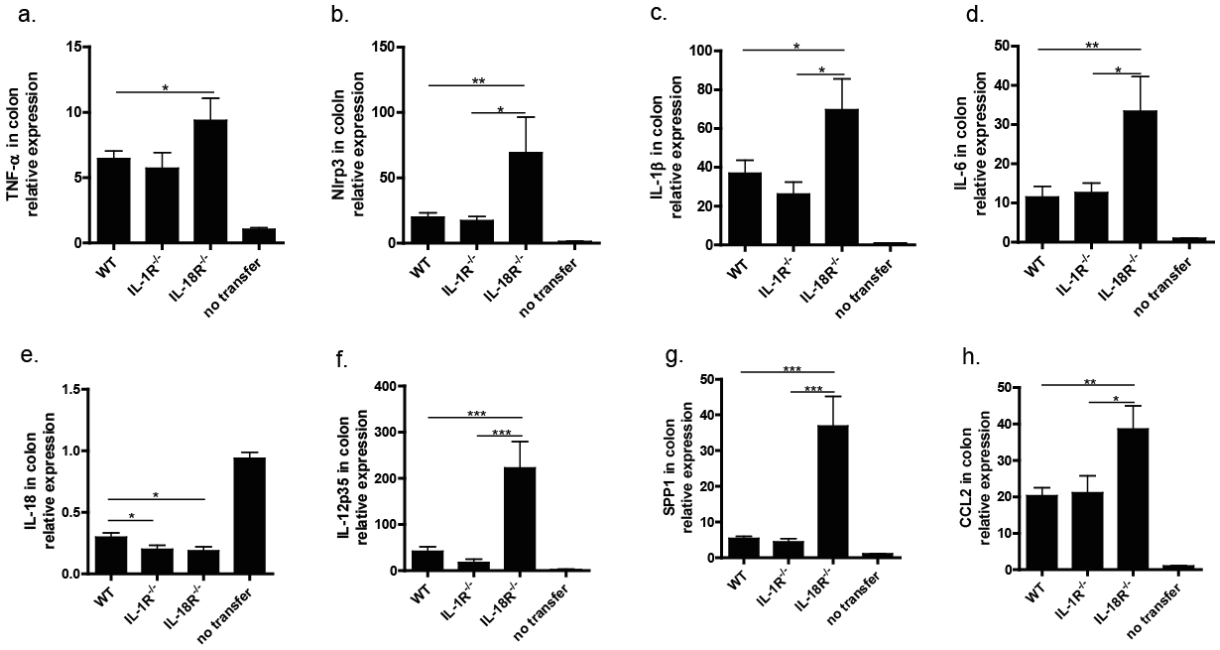

Figure 7-12: IL-18R signalling in adoptively transferred CD4$^+$ T cells regulates proinflammatory cytokine expression in colon tissue.

Rag1$^{-/-}$ mice were reconstituted i.p. with CD4$^+$CD45Rb^{High} T cells from either IL-1R$^{-/-}$, IL-18R$^{-/-}$ or C57BL/6 WT mice. Mice were sacrificed 29 days after adoptive T cell transfer and relative mRNA expression levels of TNF-α (a), Nlrp3 (b), IL-1β (c), IL-6 (d), IL-18 (e), IL-12p35 (f), SSP1 (g) and CCL2 (h) was analysed by rt-qPCR, Data are shown as mean ± SEM (n = 12-14). Data pooled from 3 experiments are shown.. *p<0.050, **p<0.010, ***p<0.001 as calculated by t test.

Recruitment of CD4$^+$ T cells into the LP was significantly increased after transfer of IL-18R$^{-/-}$ CD4$^+$CD45Rb^{High} T cells, as compared to transfer of WT or IL-1R$^{-/-}$ T cells (**fig. 6-13 a and b**). Flow cytometry analysis of LP-derived cells showed no difference between mice that received WT or IL-18R$^{-/-}$ T cells in terms of IFN-γ or IL-17 production, as assessed by intracellular cytokine staining (**fig. 6-13 c and d**). Expression of Th1 cytokine mRNA for IP-10, but not IFN-γ, was higher in mice that received IL-18R$^{-/-}$ CD4$^+$ T cells, compared to mice that had received WT or IL-1R$^{-/-}$ CD4$^+$ T cells (**fig. 6-13 e and f**). Analysis of Th17-associated cytokine mRNA expression revealed no significant effects of IL-18R signalling on IL-17, GM-CSF or IL-22, however, mice that had received IL-1R$^{-/-}$ T cells showed reduced expression of IL-17 mRNA. No significant differences were observed for GM-CSF, IL-22 and IL22bp (**fig. 6-13 g-j**).

Systematic mRNA expression analysis of cytokines, chemokines and growth factors relevant to the pathogenesis of T cell transfer colitis, clinical scores and infiltration of immune cells strengthened the concept of IL-18-mediated protective effects in T cell transfer colitis.

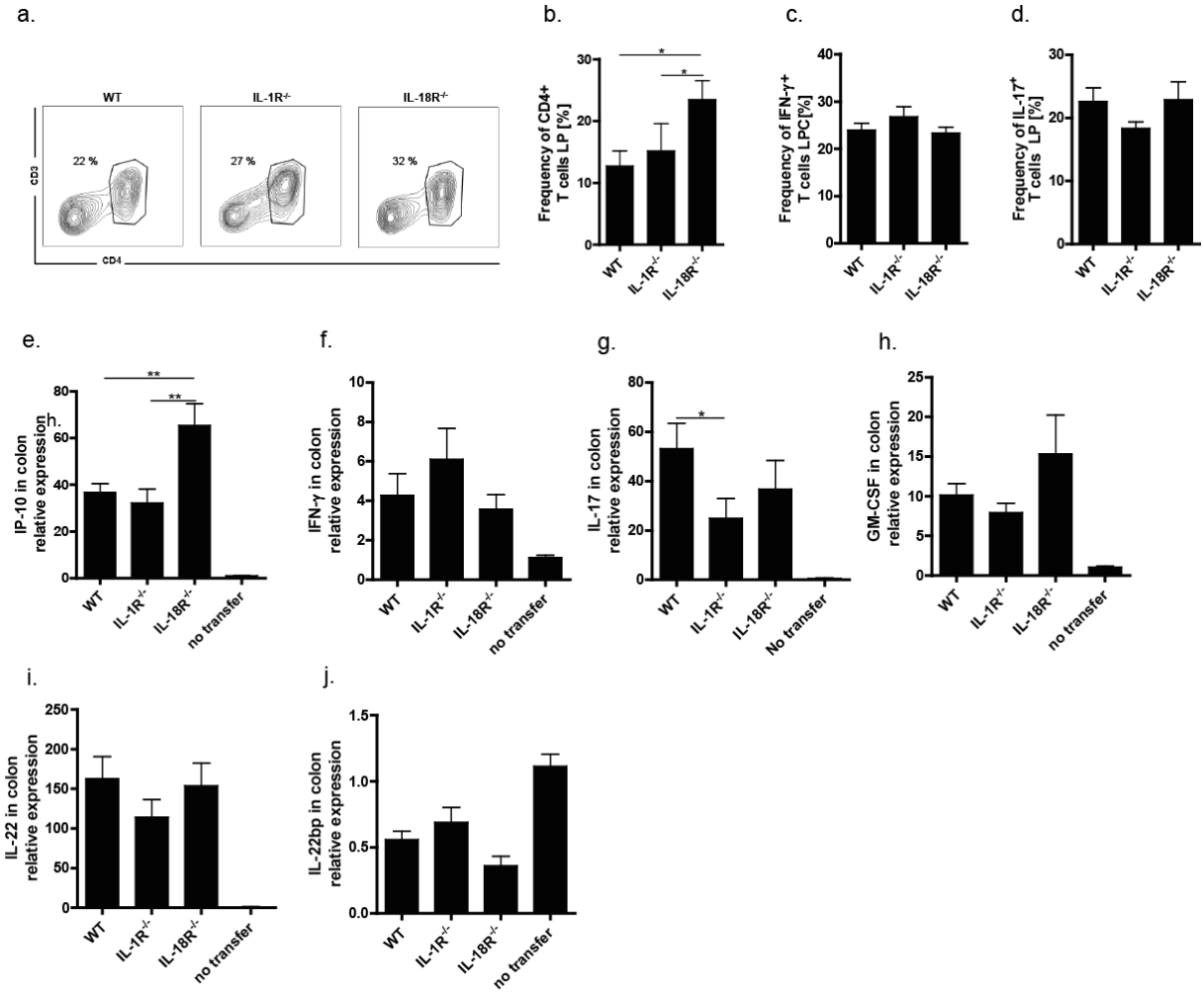

Figure 7-13: IL-18R signalling regulates T cell-mediated colitis.

Rag1$^{-/-}$ mice were reconstituted with CD4^{+}CD45Rb^{High} T cells from either IL-1R$^{-/-}$, IL-18R1$^{-/-}$ or C57BL/6 WT mice. Experimental mice were sacrificed 29 days after adoptive T cell transfer and infiltration of CD4^{+} T cells in the lamina propria (a and b), as well production of Th1 and Th17 associated cytokines were analyse per FACS analysis (c and d). Expression of Th1 (e and f) and Th17 (g-j) related cytokines were analysed per rt-qPCR. Data are shown as mean ± SEM (n = 12-14). Data pooled from 3 experiments are shown. *p<0.050, **p<0.010, as calculated by t test.

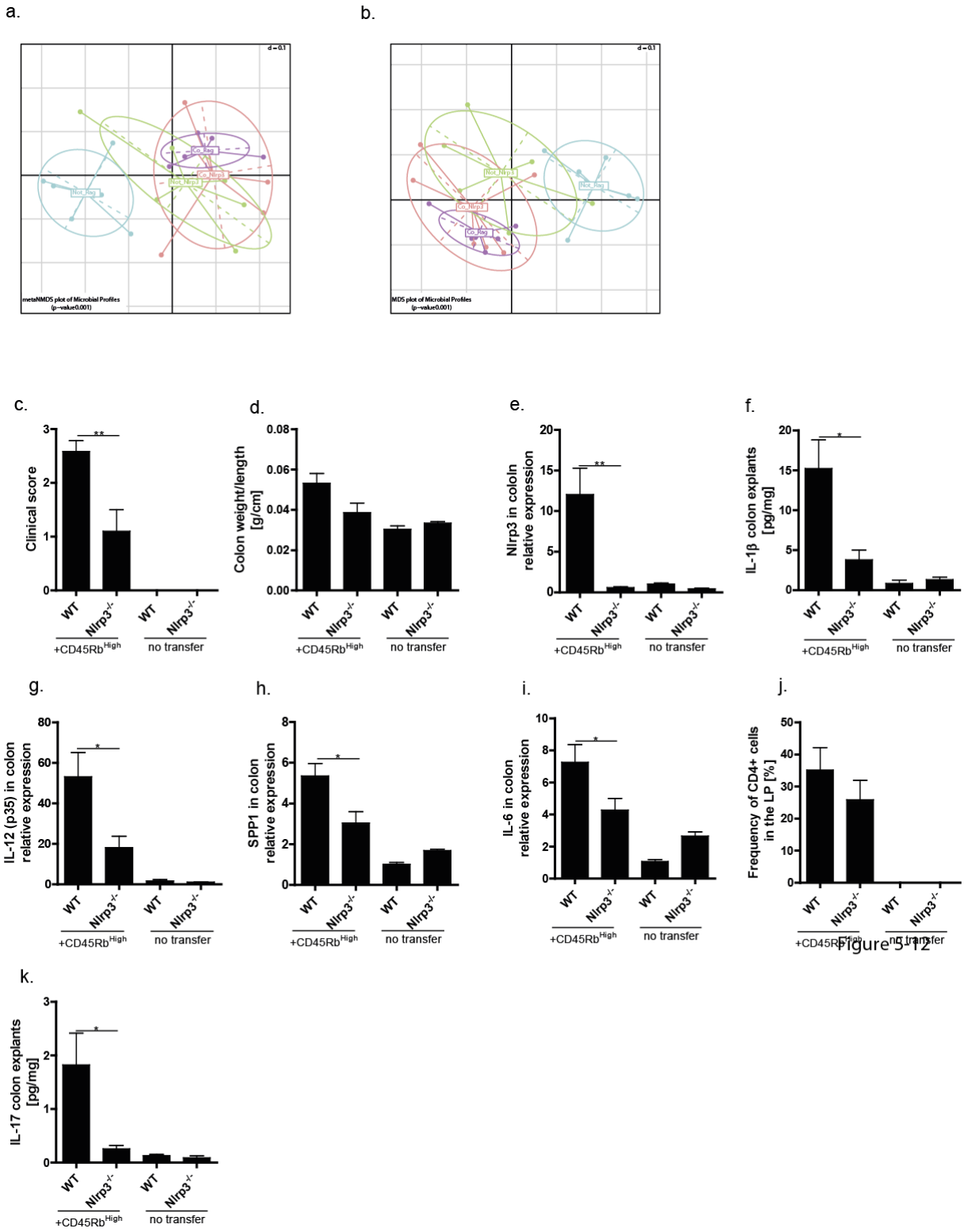

Figure 7-14: Co-housing of Nlrp3-sufficent and -deficient mice leads to assimilation of microbial content without influencing reduced colonic inflammation in Nlrp3-deficient mice.

Composition of the intestinal microbiome was investigated by 16S rRNA gene sequence analysis under steady state conditions in Nlrp3-deficient versus Nlrp3-sufficient Rag1$^{-/-}$ mice that had been housed individually or cohoused for four weeks. Then, fecal samples were collected, 16S rRNA gene sequence analysis was performed and data were assessed using a non-parametrical multiple dimensional scaling analysis at the Technical University of Munich in Freising-Weihenstephan.

Microbiota data are depicted in a beta diversity plot (a and b) Cohoused Nlrp3-deficient versus Nlrp3-sufficient mice were investigated for phenotypical differences after colitis induction. Mice were co-housed for 6 weeks, after which mice were reconstituted i.p. with $CD4^{+}CD45Rb^{High}$ T cells from C57BL/6 mice. Clinical score (c) after adoptive transfer were monitored for 29 days (n = 6). After four weeks mice were sacrificed. Intensity of inflammation was assessed by measuring colon weight per length (d). Expression of Nlrp3 (e) and inflammatory markers was analysed by rt-qPCR (f, g, k and i, n = 6). Frequency of LP-derived T cells was analysed by flow cytometry (j) and colon explants were cultivated overnight at 37°C in complete medium and IL-17 secretion was measured by ELISA (k). Data are represented as means ± SEM. **p*<0.050, ***p*<0.010, ****p*<0.001 as calculated by t test.

7.9 Protection of $Nlrp3^{-/-}$ mice from T cell-mediated colitis is maintained under cohousing conditions

To investigate potential differences in microbiota in Nlrp3-deficient and –sufficient mice that might impact the extent of T cell-mediated colitis, stool samples were collected from single-housed Nlrp3-deficient and Nlrp3-sufficient mice for 16S rRNA gene sequence analysis. The same mice were then cohoused for 3 weeks, after which stool samples were collected again for 16S rRNA gene sequence analysis (**fig. 6-14 a and b**). Microbiota analysis of non-cohoused stool demonstrated that Nlrp3-deficient mice (**fig. 6-14 a**, green circles) and Nlrp3-sufficient mice (**fig. 6-14 a**, blue circles), both with a $Rag1^{-/-}$ genotype, differed in composition of their intestinal flora. However, after three weeks of cohousing, the microbiota of Nlrp3-sufficient mice (circles in dark pink) had adjusted to the microbiota of Nlrp3-deficient mice (circle in magenta). In particular, the role of segmented filamentous bacteria (SFB) was examined, as SFB have been associated with Th17 responses (Ivanov, Atarashi et al. 2009; Goto, Panea et al. 2014). No significant differences were found between the subgroups (data not shown).

Mice were co-housed for three more weeks, when colitis was induced by transfer of $CD4^{+}CD45Rb^{High}$ T cells from C57BL/6 mice. 29 day after colitis induction, mice were sacrificed and the intensity of colon inflammation was analysed through clinical score and colon weight per length (**fig. 6-14 c and d**). Protection of $Nlrp3^{-/-}$ mice from colitis was still observed. Proinflammatory genes, such as Nlrp3, IL1β, IL-6, IL-12 and SPP1, were analysed per rt-qPCR. Reduced expression of pro-inflammatory genes (**fig. 6-14 e-i**) correlated with protection observed in the clinical score, slightly reduced numbers of $CD4^{+}$ T cells infiltrating the LP (**fig. 6-14 j**), as well as reduced secretion of IL-17 in cultured colon explants (**fig. 6-14 k**). Together, the results from the cohousing experiments disqualify biasing effects resulting from microbiota composition as explanation for the protection observed in $Nlrp3^{-/-}$ mice after adoptive transfer of $CD4^{+}CD45Rb^{High}$ T cells.

8 Discussion

8.1 Proinflammatory role of Nlrp3 in T cell transfer colitis

Despite the importance of Nlrp3 inflammasome in a number of inflammatory and autoimmune diseases, its exact role in IBD is still heavily disputed. Contradicting studies have described either a beneficial or a detrimental effect of Nlrp3 inflammasome (Bauer, Duewell et al. 2010; Dupaul-Chicoine, Yeretssian et al. 2010; Zaki, Boyd et al. 2010). These contradicting results were reconciled by previous work in our group (Bauer, Duewell et al. 2012), which showed that the composition of intestinal microflora significantly influenced disease severity in IBD models comparing wild-type and Nlrp3$^{-/-}$ mice. Notably, most of those studies describing the role of Nlrp3 inflammasome in IBD used erosive and self-limiting chemically-induced models, such as the DSS model. Despite the suitability of these models for investigating mucosal damage and innate immunity, they lack important aspects of IBD-induced inflammation in humans. In this study, the role of Nlrp3 inflammasome was investigated using T cell transfer colitis, a model that is significantly more compatible to immunological aspects of human IBD, and is instrumental in studying T cell-driven cellular interactions responsible for the onset as well as regulation of intestinal inflammation.

Nlrp3-sufficient Rag1$^{-/-}$ mice adoptively transferred with CD4^{+}CD45Rb^{High} T cells showed increased inflammation mirrored by clinical and histological scores (**fig. 6-2 b-f**). A significant 30-fold increase of Nlrp3 mRNA expression in colon tissue of diseased mice confirms induction of Nlrp3 during inflammation (**fig. 6-3 b**). These results were concordant with published work showing the ability of TLR agonist to induce Nlrp3 inflammasome in an NF-κB-dependent manner (Qiao, Wang et al. 2012; Boaru, Borkham-Kamphorst et al. 2015). Although a role of the Nlrp6 inflammasome has been described in intestinal inflammation (Elinav, Strowig et al. 2011; Seregin, Golovchenko et al. 2016), there was no increase of Nlrp6 mRNA expression during T cell-induced inflammation. Its expression was even lower in mice with colitis (**fig. 6-3 i**). Likewise, in our model, colonic expression of NLRC4 another member of the NLR family barely changed after colitis induction (**fig. 6-3 j**), contradicting studies describing its role in regulating intestinal inflammation (Carvalho, Nalbantoglu et al. 2012; Franchi, Kamada et al. 2012; Nordlander, Pott et al. 2014). These results could be partly explained by the fact that Nlrp6 and NLRC4 are of major importance for epithelial repair mechanisms, but probably less important for regulation of T cell-induced inflammation. Most studies showing the role of Nlrp6 and NLRC4 in intestinal inflammation were carried out in chemically-induced models, which focus more on epithelial damage and innate immunity.

Synthesis, maturation and release of IL-1β are tightly regulated and require two signals; NF-κB signalling and assembly of Nlrp3 inflammasome. NF-κB signalling resulting from TLR stimulation, leads to translation of an inactive 31 kDa IL-1β precursor (pro-IL-1β). Caspase-1 (Thornberry, Bull et al. 1992), a component of Nlrp3 inflammasome then cleaves pro-IL-1β into its active 17 kDa form. As anticipated, IL-1β mRNA expression as well as IL-1β protein level were reduced in Nlrp3-deficient mice reconstituted with $CD4^{+}CD45Rb^{High}$ T cells, correlating with reduced disease levels (**fig. 6-3 c and g**). This observation corroborates our hypothesis that lack of Nlrp3 inflammasome, which is the main regulator of bioactive IL-1β, leads to reduced levels of active IL-1β in T cell transfer colitis. Consistently, these results were not only in line with studies linking excessive secretion of Nlrp3-dependent IL-1β to intestinal inflammation (Seo, Kamada et al. 2015; Higashimori, Watanabe et al. 2016), but also corroborate studies showing high levels of IL-1β in IBD patients and in animal models of colitis (Satsangi, Wolstencroft et al. 1987; Mahida, Wu et al. 1989; Cominelli, Nast et al. 1990; Arai, Takanashi et al. 1998).

It has been shown that IL-1β not only induces TNF-α gene expression (Ikejima, Okusawa et al. 1990; Bethea, Gillespie et al. 1992), but also enhances TNF-α-mediated inflammatory responses (Saperstein, Chen et al. 2009). Notably, the importance of TNF-α in driving intestinal inflammation is mirrored in IBD therapies targeting TNF-α (Hanauer, Feagan et al. 2002; Atzeni, Doria et al. 2007). Additionally, IL-6, which is also induced by IL-1β (Tosato and Jones 1990; Yamaguchi, Matsuzaki et al. 1990; Cahill and Rogers 2008), has received constant attention as a marker of intestinal inflammation in IBD (Jones, Crabtree et al. 1994; Bernardo, Vallejo-Diez et al. 2012). This cytokine supports T cell survival and apoptosis resistance in the LP (Atreya, Mudter et al. 2000). Corresponding to the observation of reduced IL-1β levels, TNF-α and IL-6 levels were lower in Nlrp3-deficient mice (**fig. 6-3 a, f, e and h**). Taken together, these results confirm the role of Nlrp3-dependent IL-1β in inducing inflammation-associated cytokines and inflammatory processes in our model.

On the contrary, high mRNA expression of IL-18, which is also a Nlrp3-dependent cytokine, did not correlate with disease intensity. Healthy mice had higher levels expression of IL-18 compared to diseased mice. In this line, colon tissue of adoptively transferred Nlrp3-sufficient mice, which showed most severe colitis, expressed the lowest IL-18 mRNA levels (**fig. 6-3 d**). In order to take a closer look at the role of IL-18 in adoptive T cell transfer colitis, mice were injected with IL-18bp two hours before transfer and then weekly after transfer for a period of 4 weeks. Since downregulation of IL-18 in T cell transfer colitis correlated to disease intensity, treatment with IL-18bp, which is a soluble inhibitor of IL-18, was expected to result in more inflammation. Surprisingly, intestinal inflammation mirrored by clinical score and histological scores was slightly lower in mice treated with IL-18bp (data not shown).

Unexpectedly, IL-18 secretion from colon explants cultivated overnight in medium was significantly higher in mice treated with IL-18bp compared to mice without treatment (data not shown). Corresponding to the disputes surrounding the effects of IL-18 in intestinal inflammation, secretion of IL-18 in colon explants showed that IL-18 was independent of Nlrp3 inflammasome (data not shown). These results confirmed a recent study showing independence of IL-18 on Nlrp3 inflammasome (Wilson, Duewell et al. 2014). Notably, colitis-induced destruction of the epithelial cells, may attempt to explain reduced IL-18 mRNA expression levels observed in diseased mice.

Together these data indicate Nlrp3-dependent IL-1β as the driving force of T cell-dependent inflammation and induction of inflammatory-associated cytokines. The quick detrimental effect of IL-1β has been emphasised in studies showing that blocking IL-1β alone was ineffective in treating DSS-induced colitis and IBD (Kojouharoff, Hans et al. 1997; Carter, Valeriano et al. 2003).

8.2 Tolerogenic versus inflammatory dendritic cells in colitis

Uninterrupted exposure of the intestine to a wide range of antigens and immunomodulatory stimulation for example, components in the diet, normal microflora and pathogens, calls for a strict regulation of the immune response (Mowat 2003). The intestinal immune system has to distinguish harmful from non-harmful antigens by inducing protective immune response against pathogens, and at the same time develop active tolerance to harmless antigens; failure of keeping this balance is believed to result in IBD. APCs like DCs and macrophages take centre stage in intestinal immune regulation, playing specific yet integral roles. While DCs have the ability to migrate to the lymph node after antigen uptake and prime naïve T cells, macrophages use their phagocytic ability to digest bacteria and damaged cells (Coombes and Powrie 2008; Schulz, Jaensson et al. 2009; Cerovic, Houston et al. 2013). The path taken by DCs at the juncture of induction of tolerance and active immunity is dependent on the DC subpopulations as well as activation via surface receptors and on tissue microenvironment.

Despite differences in severity of colitis symptoms, Nlrp3-sufficient and Nlrp3-deficient mice demonstrated equally increased LP DC infiltration (**fig. 6-6 a**). However, composition of DC pools differed significantly. Nlrp3-deficient mice had recruited three times as many $CD103^+$ DCs to the LP compared to Nlrp3-sufficient mice (**fig. 6-6 b**). Evidence in the literature has shown that the expression of CD103 on DCs shifts the direction of an immune response by influencing the balance between intestinal effector and regulatory T cell activity (Annacker, Coombes et al. 2005; Cerovic, Houston et al. 2013). Increased frequency of $CD103^+$ DCs in

Nlrp3-deficient mice correlating with reduced inflammation was in line with these findings, describing $CD103^+$ DCs as regulatory cells. These DCs constitutively traffic the draining lymph node, where they induce conversion of naïve T cells into induced T_{regs}, a mechanism that relies on TGF-β (Coombes, Siddiqui et al. 2007; Sun, Hall et al. 2007; Iliev, Spadoni et al. 2009*)*. mRNA expression of IL-27, a DC-derived cytokine described to downregulate Nlrp3-inflammasome by limiting pathogenic T cell response and development of immunity was also increased in Nlrp3-deficient mice (Mascanfroni, Yeste et al. 2013) (**fig. 6-4 g**). Additionally, Muzaki et al. found that absence of $CD103^+CD11b^-$ DCs aggravated DSS-induced colitis by diminished expression of IDO1 and IL-18bp in IECs during early stages of colitis. (Muzaki, Tetlak et al. 2016). Furthermore, $CD103^+$ DCs are an important source of retinoic acid (Iwata, Hirakiyama et al. 2004; Jaensson, Uronen-Hansson et al. 2008; Yokota, Takeuchi et al. 2009), allowing them to induce gut homing receptors CCR9 and α4β7 on T cells (Johansson-Lindbom, Svensson et al. 2005; Jaensson, Uronen-Hansson et al. 2008). In correlation with increased $CD103^+$ DCs in the LP of Nlrp3-deficient mice, the expression of gut homing receptor CCR9 was elevated in colonic tissue of (**fig. 6-6 g**).

On the contrary, LP-derived DCs isolated from inflamed colon of Nlrp3-sufficient mice barely expressed CD103 (**fig. 6-6 b and c**). This reduced expression of CD103 correlated with increased disease intensity and increased expression of myeloid cell-derived proinflammatory cytokines (**fig. 6-2 and fig. 6-3**). Osteopontin, another proinflammatory marker (Ashkar, Weber et al. 2000; O'Regan 2003; Uaesoontrachoon, Wasgewatte Wijesinghe et al. 2013), which has an inflammatory role in TNBS- and DSS-induced colitis (Zhong, Eckhardt et al. 2006; Oz, Zhong et al. 2012), and is mainly expressed by $CD103^-$ DCs (Kourepini, Aggelakopoulou et al. 2014) was elevated in the colon tissue of Nlrp3-sufficient mice compared to Nlrp3-deficient mice (**fig. 6-6 e**). Increased expression of osteopontin in diseased Nlrp3-sufficient mice supports previously published work demonstrating that Nlrp3 inflammasome upregulated chemotaxis-related proteins like SPP1 (Inoue, Williams et al. 2012). The exact nature of these LP-derived $CD103^-$ DCs is still disputed, with some groups describing them as macrophage-like cells (Panea, Farkas et al. 2015). However, Cerovic et al. showed that intestinal $CD103^-$ DCs are equally capable of migrating to MLNs and inducing T cell responses (Cerovic, Houston et al. 2013). Additionally, recent work has demonstrated that $CD103^-$ DCs constitutively express IL12/IL-23(p40) and are capable of inducing proinflammatory Th17 (Scott, Bain et al. 2015). In line with the work of Scott and his colleagues, expression of IL-12(p35), a subunit of IL-12 was significantly elevated in Nlrp3-sufficient mice (**fig. 6-4 f**). Further studies also identified $CD103^- CX3CR1^+$ LP cells as drivers of Th17 cell responses to certain commensal bacteria, such as segmented filamentous bacteria (SFB) (Panea, Farkas et al. 2015).

It appears that under steady state conditions, APCs possibly irrespective of CD103 expression status are essentially antiinflammatory. These cells express a high quantity of IL-10, which participates in restimulation of T regulatory cells in situ (Hadis, Wahl et al. 2011). In contrast, in an on-going inflammation, myeloid-derived LP cells develop proinflammatory properties. Infection with pathogenic bacteria is the typical situation, which adjusts steady state mechanisms towards inflammation and a more aggressive T cell-based immune response. In an infection model of Citrobacter rodentium, $CD103^+$ intestinal cells have been shown to mediate polarisation of T cell responses towards Th17 (Schreiber, Loschko et al. 2013). However, it is generally believed that $CD103^-$ DCs play the key role in driving intestinal inflammation. Rivollier et al. reported that during colitis, monocytic cells switch their differentiation program and develop into $CD103^-CX3CR1^+CD11b^+$ DCs, which massively infiltrate the LP and act in a proinflammatory manner, by producing IL-12, IL-23, and TNF-α (Rivollier, He et al. 2012). These DCs acquire migratory properties and after reaching the MLN, drive differentiation of IFN-γ-producing T cells. In the course of colitis, frequency of $CD103^+$ DCs dramatically decreases.

Results of this study are quite concordant with these paradigms, finding increased numbers of LP DCs, with a high percentage of $CD103^-$ DCs in Nlrp3-sufficient mice. Reduced levels of proinflammatory cytokines are associated with higher frequency of $CD103^+$ DCs, indicating that $CD103^-$ DCs are the cellular platform for induction of colitis in $CD45Rb^{High}$ T cell transfer colitis. In line with these results, Laffont et al. found that inflammation dampens the tolerogenic properties of MLN $CD103^+$ DCs, which was associated with lower expression of TGF-β2 and ALDH1A2 (Laffont, Siddiqui et al. 2010). Unfortunately, the origin and the fate of $CD103^+$ and $CD103^-$ DCs was not investigated, leaving the possibility open that $CD103^+$ DCs down-regulate CD103 expression under inflammatory conditions.

In summary, the balance between stimulatory DCs (primarily $CD103^-$DCs) and tolerogenic DCs ($CD103^+$ DCs) is critical for maintaining intestinal immune homeostasis under steady state conditions. In the absence of colitis, Nlrp3-deficient mice recruit higher levels of $CD103^+$ DCs in LP and MLN (Bauer, Duewell et al. 2012) . The Nlrp3 inflammasome could thus serve as a regulator of DC homeostasis in the gut and as a switch for colitis induction. Evolutionary conserved mechanisms shift this balance towards inflammation in case of challenge by pathogenic bacteria. T cell transfer into immunoincompetent hosts can mimic inappropriate activation of these mechanisms as found in IBD. However, phenotypical analysis of intestinal DC subsets based on surface marker expression has to be interpreted in the context of the intestinal inflammatory cytokine network. Despite their tolerogenic function, $CD103^+$ DCs can switch from a tolerogenic to an immune-promoting phenotype, when appropriately stimulated (Uematsu, Fujimoto et al. 2008; Siddiqui, Laffont et al. 2010; Fujimoto, Karuppuchamy et al.

2011; Semmrich, Plantinga et al. 2012). Most importantly, IL-1β holds a prominent position as a master regulator of inflammatory responses.

8.3 Association of host Nlrp3 inflammasome with the inflammatory phenotype of adoptively transferred intestinal T cells

This study links Nlrp3 and elevated IL-1β levels to increased intestinal inflammation, mediated by shifting adoptively transferred T cells towards an inflammatory phenotype. It could be demonstrated that IL-1β levels were higher in diseased Nlrp3-sufficient mice compared to Nlrp3-deficient mice. In Nlrp3-sufficient mice, IL-17 levels were increased, too. Importantly, Th17 cells were shifted towards a Th1 phenotype, as indicated by increased IFN-γ and IP-10 production.

IL-17's function in intestinal inflammation is highly disputed, very similar to the controversy surrounding detrimental versus protective effects of Nlrp3 and caspase-1. Increased inflammation as a result of neutralisation of IL-17 indicated a protective effect of IL-17 in a chemically-induced model of IBD (Ogawa, Andoh et al. 2004). However, in a model of Helicobacter hepaticus driven colitis, IL-17 had a pathogenic role (Buonocore, Ahern et al. 2010). Correspondingly, not only did blockage of IL-17 dampened inflammation in a genetic model of spontaneous colitis (Chaudhry, Rudra et al. 2009), but also adoptive transfer of ROR-γ-deficient T cells into immunoincompetent mice failed to induce colitis (Leppkes, Becker et al. 2009). Finally, the group of Fiona Powrie could demonstrate that IL-1β was indispensable in induction of chronic intestinal inflammation in both T cell-independent and T cells-mediated colitis (Coccia, Harrison et al. 2012).

In this study, the role of Nlrp3 and IL-1β are concordant with recent findings on a proinflammatory role of IL-1β in adoptive transfer colitis. Basu and colleagues characterised the role of IL-1 in homeostasis of Th17 cells and induced T_{reg} (iT_{reg}) (Basu, Whitley et al. 2015), thereby shedding some light on the contradictory results that have been documented. While TGF-β promotes differentiation of both iT_{reg} and Th17, IL-6 favours Th17 cell differentiation to the detriment of iT_{reg} differentiation. (Bettelli, Carrier et al. 2006; Mangan, Harrington et al. 2006; Veldhoen, Hocking et al. 2006; Littman and Rudensky 2010). Most importantly, IL-1 signalling augments the IL-6 pathway thereby reversing retinoic acid-dependent FoxP3 expression and inducing Th17 cell responses (Basu, Whitley et al. 2015). Considering these findings, the expression of FoxP3 in Th17 cells and in colonic tissue was analysed. Concordant with the paradigm of IL-1 effects on plasticity of CD4 T cells, Nlrp3-deficient mice had higher mRNA expression levels of colonic FoxP3 (data not shown). Supporting the findings of Basu and his colleagues, Meng et al., described an association

between Nlrp3 and Th17. Increased level of IL-1 observed in mice with a hyperactivating Nlrp3 mutation induced proinflammatory Th17 dominance (Meng, Zhang et al. 2009). Nlrp3-mutant DCs co-cultured with $CD4^+$ T cells induced IL-1-dependent Th17 differentiation. Interestingly, increased frequency of Th17 correlating with high IL-17 levels in serum was observed in patients with hyperactive Nlrp3 mutations (Lasiglie, Traggiai et al. 2011).

Not only has it been shown that Th17 cell-mediated inflammation is associated with neutrophil infiltration and increased GM-CSF production (Kroenke, Carlson et al. 2008), but also that IL-1β upregulated GM-CSF production by Th17 (Codarri, Gyulveszi et al. 2011; El-Behi, Ciric et al. 2011). Based on these findings, expression of colonic GM-CSF mRNA was analysed. As anticipated, expression of GM-CSF was increased in Nlrp3-sufficient as compared to Nlrp3-deficient mice (**fig. 6-4 l**). Another Th17-derived cytokine is IL-22, which is a member of the IL-10 family of cytokines. IL-22 has been implicated as an effector cytokine in defence against mucosal pathogens (Zheng, Danilenko et al. 2007; Ouyang, Rutz et al. 2011). It is involved in tissue homeostasis as well as epithelial repair (Sugimoto, Ogawa et al. 2008; Zenewicz, Yancopoulos et al. 2008). Beside these beneficial effects, IL-22 can be a potent inducer of inflammation (Sonnenberg, Fouser et al. 2011). Th17 cells have been described as major source of IL-22 (Chung, Yang et al. 2006; Liang, Tan et al. 2006). Analysis of colonic IL-22 expression after adoptive T cell transfer into $Rag1^{-/-}$ mice found increased IL-22 mRNA levels and Nlrp3-sufficient mice had a significantly higher expression of IL-22 mRNA compared to Nlrp3-deficient mice (**fig. 6-4 j**). All in all, these results are in line with published data, which identify IL-1β as the master regulator that shifts naïve T cells into proinflammatory Th17 cells. Reduced colitis associated with reduced Th17 was observed in Nlrp3-deficient mice. Reduced IL-17, which is the main cytokine produced by Th17 cells, and reduced GM-CSF and IL-22, inflammatory markers of Th17, were observed in Nlrp3-deficient mice. These results point out a clear role of IL-1β in this process in vivo, which was confirmed *in vitro*, where the addition of IL-1β to $CD4^+$ T cells induced secretion of the three main Th17-dependent inflammatory cytokines, IL-17, GM-CSF and IL-22 (**fig. 6-10 b, c and d**).

Due to the ambivalent feature of IL-22, its regulation is vital. This cytokine is strictly regulated by endogenous binding protein IL-22bp, which is a soluble IL-22 receptor that specifically binds to IL-22 and prevents binding of IL-22 to the membrane bound receptor, IL-22R1 (Kotenko, Izotova et al. 2001; Xu, Presnell et al. 2001; Wei, Ho et al. 2003). It has been shown that IL-22bp expression is down-regulated after intestinal tissue damage (Sonnenberg, Nair et al. 2010), possibly as a protection mechanism against invading bacteria. Recently, Huber et al. linked IL-22bp expression to activation of Nlrp inflammasomes. IL-22bp was highly expressed by colonic DCs at steady state conditions.

However, sensing of intestinal tissue damage via Nlrp3 or Nlrp6 inflammasome led to an IL-18 dependent down-regulation of IL-22bp, resulting in an increased ratio of IL-22/IL-22bp (Huber, Gagliani et al. 2012). An association between $CD103^+$ DCs and IL-22bp was shown by Martin et al. who described constitutive production of retionic acid-dependent IL-22bp in lymphoid- and gut-derived $CD103^+CD11b^+$ DCs (Martin, Beriou et al. 2013). Here, expression of IL-22bp in colon tissue of T cell-transferred $Rag1^{-/-}$ mice was investigated. IL-22bp expression was higher in healthy, mice than in mice that had received T cell transfer. In this line, IL-22bp expression was significantly higher in diseased Nlrp3-deficient mice compared to diseased Nlrp3-sufficient mice (**fig. 6-4 k**).

8.4 The ratio of T cell-derived FLT3L and GM-CSF as predictor of the inflammatory phenotype of dendritic cells

DCs have been shown to be the “ringleader” of the immune system, not only because of their antigen-presenting capacity, but also because they can migrate to the lymph node and initiate tolerance to self-antigens as well as induction of immune response (Steinman, Hawiger et al. 2003; Steinman 2012). Development of intestinal DC subsets is therefore tightly regulated. The growth factors GM-CSF and FLT3L regulate the two main populations in the LP. $CD103^+CD11b^+$ LP DCs are derived through a FLT3L-dependent pathway, whereas $CX3CR1^+CD103^-CD11b^+$ LP DCs are thought to derive through a CSF-dependent pathway (Bogunovic, Ginhoux et al. 2009; Varol, Vallon-Eberhard et al. 2009).

IL-1 has been shown to promote GM-CSF production by Th17 cells, indicating a key role of both cytokines in experimental autoimmune encephalomyelitis (EAE), a Th17-mediated model of an autoimmune disease (El-Behi, Ciric et al. 2011). Coccia et al. from the Powrie group found that colonic $IL\text{-}1R^{-/-}$ $CD4^+$ T cells isolated after T cell transfer secreted significantly less GM-CSF than colonic wild-type $CD4^+$ T cells, developing more severe colitis than IL-1R-sufficient mice (Coccia, Harrison et al. 2012). Both non hematopoietic-derived and T cell-derived FLT3L have been shown to mediate local DCs replenishment at steady state and during inflammation (Saito, Boddupalli et al. 2013). Saito and his colleagues were able to show that T cells generally produce FLT3L, but $CD4^+$ T cells were the more potent producers of FLT3L. Concordant with the literature, FLT3L differentiated a population of bone marrow-derived DCs that mainly expressed CD103, while GM-CSF and IL-4 differentiated DCs with a reduced expression CD103 (**fig. 6-8 a and b**). FLT3L-differentiated DCs, which had higher expression of CD103, secreted lower levels of proinflammatory cytokines after stimulation with LPS (**fig. 6-8 c-f**), confirming their tolerogenic property (Annacker, Coombes et al. 2005; Coombes, Siddiqui et al. 2007).

Since it has been shown that DC differentiation is partly dependent on T cell-derived growth factors, the effect of presence versus absence of T cells in DC development was analysed. $Rag1^{-/-}$ mice lacking T cells at steady state had a significantly lower frequency of $CD103^+$ DCs in the LP compared to T cell-sufficient mice at steady state (**fig. 6-8 g**). Like already shown in our previous work (Bauer, Duewell et al. 2012), Nlrp3-deficient mice had a lower frequency of $CD103^+$ DCs at steady state (**fig. 6-8 g**). Correspondingly, presence of T cells was indispensable for intestinal DCs in general as well as for $CD103^+$ and $CD103^-$ DCs in particular (**fig. 6-6 a and b**).

Increased level of FLT3L in the MLN and in the colon of healthy Nlrp3-deficient mice suggested a link to increased frequency of $CD103^+$ DCs observed in these mice (**fig. 6-8 h**). Accordingly, FACS-sorted splenic and MLN-derived $CD4^+$ T cells from Nlrp3-deficient mice had a significantly higher expression level of FLT3L compared to their Nlrp3-sufficient counterparts (**fig. 6-8 i**). Furthermore, the level of FLT3L was significantly increased in Nlrp3-deficient mice after T cell transfer (**fig. 6-8 j**). Saito et al. showed that DC homeostasis during inflammation is maintained by T cell-produced FLT3L, which provides a feedback loop to DCs to induce DC differentiation (Saito, Boddupalli et al. 2013). The significant increase of FLT3L secretion by OT II $CD4^+$ T cells cocultured with LPS-stimulated and OVA peptide-pulsed Nlrp3-deficient DCs indicated the role of Nlrp3 expression by DCs in inducing FLT3L secretion by T cells (**fig. 6-9 a**). On the contrary, Nlrp3-sufficient DCs cocultured with OT II $CD4^+$ T cells under the same conditions induced an elevated secretion of GM-CSF, IL-17 and IFN-γ, which are inflammatory markers of effector T cells (**fig. 6-9 b-d**). It can be speculated for Nlrp3-deficient mice, based on the correlation of FLT3L expression (**fig. 6-8 h, i and j**) and CD103 frequency (**fig. 6-6 b and c**), that increased expression of CD103 on DCs of Nlrp3-deficient mice is dependent on T cell-derived FLT3L. These findings are concordant with data in FLT3L-deficient mice, where a marked effect on mucosal $CD4^+$ T cell priming with decreased numbers of $FoxP3^+$ T_{regs} was described (Darrasse-Jeze, Deroubaix et al. 2009; Panea, Farkas et al. 2015).

Intestinal DCs might be the cellular platform integrating proinflammatory and antiinflammatory cytokine effects. At steady state, intestinal DCs, which are primarily tolerogenic, migrate to the MLN and activate naïve T cells into regulatory T cells (Coombes, Siddiqui et al. 2007; Agace and Persson 2012). However, in a situation of harmful bacterial challenge, DCs migrate to the MLN, where they present bacterial antigen to T cells and induce the activation of naïve T cells (Annacker, Coombes et al. 2005; Coombes, Siddiqui et al. 2007; Scott, Bain et al. 2015). Results of this study suggest that, DC-derived Nlrp3-dependent IL-1β predicts the fate of naïve T cells during inflammation. Nlrp3-sufficient DCs, which have a high level of IL-1β, are biased to activate naïve T cells into GM-CSF producing

T cells. These GM-CSF producing T cells are not only proinflammatory but also provide a feedback loop to DCs, supporting the differentiation of more $CD103^-$ DCs. On the contrary, Nlrp3-deficient DCs induce FLT3L-producing T cells, which are less inflammatory and similarly provide a feedback loop to DCs allowing differentiation of more $CD103^+$ DCs.

All in all these data indicate that FLT3L and GM-CSF are derived at least in part from adoptively transferred T cells and regulate recruitment of intestinal DC subpopulations during adoptive T cell transfer colitis. Whereas FLT3L promotes $CD103^+$ DCs, which retain certain tolerogenic aspects (at least in Nlrp3-deficient mice), GM-CSF promotes intestinal infiltration with $CD103^-$ DCs. In Nlrp3-sufficient mice this was associated with higher levels of proinflammatory cytokines, primarily IL-1β, and increased colitis severity.

8.5 IL-18R signalling in T cells plays a pivotal role in adoptive T cell transfer colitis

IL-18 is hardly a recapitulation of IL-1β, despite the fact that they share aspects of regulation and signalling pathways. IL-18 precursor is expressed in blood monocytes, peritoneal macrophages, mouse spleen and in the epithelial cells of the entire gastrointestinal tract in healthy subjects, as opposed to IL-1β (Puren, Fantuzzi et al. 1999). In agreement with literature reports, mRNA expression of IL-1β in steady state was barely present and almost a 40-fold increase was detected in colon tissue of inflamed Nlrp3-sufficient mice (**fig. 6-3 c**). On the other hand, mRNA expression of IL-18 was higher in colon of steady state mice and a significant decrease was detected in mice after adoptive T cell transfer colitis, with Nlrp3-sufficient mice showing the least expression level (**fig. 6-3 d**).

There is controversy about the role ofIL-18 in inflammatory processes; its proinflammatory or antiinflammatory effect is dependent on the cytokine milieu. In combination with IL-12, IL-18 is involved in Th1 differentiation leading to the production of IFN-γ (Nakanishi, Yoshimoto et al. 2001). Inhibition of IL-18 has been shown to induce protection against intestinal inflammation (Kanai, Watanabe et al. 2001; Ten Hove, Corbaz et al. 2001). On the other hand, protective effects have been observed in chemically-induced models of colitis and mature IL-18 has been detected in affected intestinal lesions from CD patients (Siegmund, Lehr et al. 2001; Ten Hove, Corbaz et al. 2001; Dupaul-Chicoine, Yeretssian et al. 2010). Furthermore, administration of exogenous IL-18 has been shown to restore mucosal healing in caspase-1-deficient mice (Dupaul-Chicoine, Yeretssian et al. 2010), and defective inflammasome-dependent epithelial integrity has been linked to decreased IL-18 (Zaki, Boyd et al. 2010).

A suggestion that the effect of IL-18 was dependent on the site of activation attempted to reconcile contradicting observations (Siegmund 2010). IL-18 is important in maintaining the

integrity of epithelium. Upon damage, IL-18 induces proliferation, therefore regenerating damaged epithelial cells. Conversely, lamina propria-derived IL-18 synergises with IL-12, creating an inflammatory milieu. Additionally, Dinarello et al. addressed this controversy by suggesting that the balance of IL-18 and its soluble receptor IL-18bp could be the deciding factor (Dinarello, Novick et al. 2013). Recently, Nowarski et al. showed that regardless of the cellular origin, IL-18 during DSS-induced colitis specifically targeted IL-18R signaling on epithelial cell and not hematopoietic or endothelial cells (Nowarski, Jackson et al. 2015). Furthermore, epithelial-derived IL-18 consequently inhibited maturation of goblet cell and hyperactive IL-18 completely depleted goblet cells. On the contrary, Harrison et al. showed that IL-18, which was mainly derived from the epithelial cells, not only drove Th1 differentiation, but also regulated different subgroups of intestinal $CD4^+$ T cells during both steady state and intestinal inflammation (Harrison, Srinivasan et al. 2015).

Adoptive T cell transfer of IL-$18R^{-/-}$ T cells resulted in a more intensive form of colitis compared to mice that received WT T cells or IL-$1R^{-/-}$ T cells (**fig. 6-11 a-f**). These results were in line with work published by Harrison et al. showing that IL-18R signalling in T cells promoted an antiinflammatory effect due to its ability to limit Th17 differentiation. Additionally, they showed that IL-18 was critical for $FoxP3^+$ T_{reg} cell-mediated control of intestinal inflammation. These results point out the important role of IL-18R signalling in the control of T cell-induced intestinal inflammation, because lack of IL-18R signalling on T cells resulted into an increase of not only proinflammatory cytokines (**fig. 6-12 a-d and f**), but also an increase in chemokines responsible for recruiting immune cells especially T cells to sites of inflammation (**fig. 6-12 g and h**). Although there was more infiltration of $CD4^+$ T cells in the LP after adoptive T cell transfer of IL-$18R^{-/-}$ T cells correlating with disease intensity (**fig. 6-13 a and b**), expression of Th17 cytokines was comparable to mice transferred with wild-type T cells (**fig. 6-13 g, h and i**). Possible explanation for this observation could be that the inflammatory effects of Th17 reached a plateau; alternatively, IL-18R-dependent reduction of inflammation was in fact Th17-indepent, but rather dependent on other T cell subsets, presumably T_{reg}.

Although the mechanism of IL-18R signalling on T cells in the regulation of T cell-dependent inflammation is still not fully clear, these results suggest an important role of this signalling pathway in the regulation of intestinal inflammation.

8.6 Role of intestinal microbiota in susceptibility to T cell-mediated colitis

The intestinal microbiota is instrumental in regulating homeostatic and pathogenic T cell responses in the gut. In a lymphopenic host, adoptively transferred with naive T cells, gut

flora will determine the phenotype of the induced T cell response. Since the group of Dan Littman described segmented filamentous bacteria, spore forming gram positive bacteria most closely related to the genus *Clostridium*, as inducers of intestinal Th17 responses in 2009, the role of microbiota in intestinal adaptive immune responses has been extensively investigated (Ivanov, Frutos Rde et al. 2008; Goto, Panea et al. 2014). Germ free mice, which lack Th17 cells in the colon, acquired Th17 cells after colonisation with intestinal microbiota. Treatment of new-born mice with vancomycin resulted in lower numbers of intestinal Th17 cells. Importantly, generation of Th17 cells required antigen presentation by MHC II in the periphery. Other commensal species, most prominently the *firmicutes* phylum and bifidobacteria, have been associated with the induction of intestinal T_{reg} and reduction of Th17 responses (Lopez, Gonzalez-Rodriguez et al. 2011; Round, Lee et al. 2011; Atarashi, Tanoue et al. 2013). Plasticity of the intestinal T cell pool towards a Th17/Th1 versus a T_{reg} phenotype might in turn regulate composition of steady state microbiota (Kumar, Moideen et al. 2016). Importantly, Th17 cells possess a remarkable plasticity, and inflammation is not induced by all Th17 cells. It has been shown that Th17-derived IL-10, which regulates Th17 plasticity, is dependent on either TGF-β1 and IL-6 or IL-23. Maintenance of Th17 with TGF-β1 and IL-6 favoured IL-10-producing Th17 cells, while stimulation with IL-23 inhibited production of IL-10 (McGeachy, Bak-Jensen et al. 2007). Basu and colleagues shed some light into the controversies surrounding Th17, by showing that Th17 and iT_{reg} actually stem from a shared developmental axis (Basu, Hatton et al. 2013). Importantly, Th17 cells possess a remarkable plasticity, and inflammation is not induced by all Th17 cells. It has been shown that Th17-derived IL-10, which regulates Th17 plasticity, is dependent on either TGF-β1 and IL-6 or IL-23. Maintenance of Th17 with TGF-β1 and IL-6 favoured IL-10-producing Th17 cells, while stimulation with IL-23 inhibited production of IL-10 (McGeachy, Bak-Jensen et al. 2007). Basu and colleagues shed some light into the controversies surrounding Th17, by showing that Th17 and iT_{reg} actually stem from a shared developmental axis (Basu, Hatton et al. 2013).

Colonisation of mice with SFB in an adoptive T cell transfer model caused severe intestinal inflammation, while mice colonised with other commensal bacteria excluding SFB had reduced colitis (Stepankova, Powrie et al. 2007), therefore disqualify the pathogenicity of Th17 per se. However, inflammation-inducing antigens can switch Th17 cells into proinflammatory cells. It remains unclear, which molecular mechanisms determine pathogenicity of adoptively transferred T cells, however, Nlrp3 is a strong candidate. T cells found in this model were not only producing more IL-17, but also more IFN-γ, when isolated from Nlrp3-sufficient mice. The transformation of Th17 cells into inflammation-inducing cells characterised by the production of IFN-γ has led to the term Th1-like cells. These Th1-like cells are capable of driving intestinal inflammation by releasing proinflammatory cytokines,

which boost inflammation (Feng, Qin et al. 2011; Sujino, Kanai et al. 2011). Importantly, IL-1β has been demonstrated to convert human IL-10-producing Th17 cells into proinflammatory IFN-γ-producing cells (Zielinski, Mele et al. 2012). Correspondingly, Basu and colleagues observed that that IL-1 was indispensable in fully overriding retinoic acid-mediated expression of FoxP3 and induced protective Th17 response (Basu, Whitley et al. 2015). All in all, Th17 cells are homeostatic and antiinflammatory per se in the presence of normal microflora; however stimulation with IL-1β and IL-12 or IL-23 converts these cells into proinflammatory Th17 cells capable of producing IFN-γ, therefore favouring intestinal inflammation.

Intestinal microflora might also determine the cytokine microenvironment that mediates T cell responses. In general, the role of microbiota in induction of intestinal inflammation is supported by reduction of colitis in various models after antibiotic treatment and in germ-free mice (Hudcovic, Stepankova et al. 2001; Garrett, Lord et al. 2007; Kirkland, Benson et al. 2012). Certain microbial strains are able to directly activate proinflammatory mechanisms. Escherichia coli isolated from inflammatory bowel diseases patients has been shown to activate the Nlrp3 inflammasome in macrophages (De la Fuente, Franchi et al. 2014).

It has been shown that Nlrp3 inflammasome status and resulting plasticity of the T cell pool might determine composition of the intestinal microflora (Elinav, Strowig et al. 2011). Amongst inflammasomes, Nlrp3 might be a particularly prominent key player. Polymorphisms in Nlrp3, found in CD patients, are associated with lower expression of Nlrp3 (Villani, Lemire et al. 2009). A weak inflammatory response towards E. coli might result in deficient bacterial clearance and sustained inflammation. Accordingly, absence of Nlrp3 signalling has been described to result in intestinal dysbiosis, with increased colonisation of certain pathogenic species, such as Enterobacteriaceae, Mycobacterium, and Clostridium (Hirota, Ng et al. 2011). Hirota et al. linked intestinal dysbiosis in $Nlrp3^{-/-}$ mice to increased susceptibility towards DSS- and TNBS-induced colitis. We previously described that cohousing of $Nlrp3^{-/-}$ mice with wild-type mice as well as treatment with antibiotics could minimize effects of Nlrp3-deficiency in colitis models based on innate immune phenomena, such as the DSS (Bauer, Duewell et al. 2012) model. Accordingly, susceptibility of $Aim2^{-/-}$ mice to DSS-induced colitis was associated with a dysregulated host response to the gut microbiota (Hu, Wang et al. 2015).

On the contrary, absence of Nlrp3 and IL-1β results in increased susceptibility to infection and mortality (Hasegawa, Kamada et al. 2012). Nlrp3-deficiency might therefore result in beneficial and detrimental effects: a protective role against pathogenic bacteria collides with detrimental effects on autoimmune phenomena. Whereas steady state activation of Nlrp3

regulates host defence, excessive activation results in inflammatory diseases mediated by proinflammatory cytokines including IL-1β. Seo et al. recently described that pathobiont Proteus mirabilis induces robust IL-1β via the Nlrp3 inflammasome (Seo, Kamada et al. 2015). Proteus mirabilis enhanced DSS-induced inflammation via Nlrp3 and IL-1R signalling. Importantly, in the absence of Nlrp3, Proteus mirabilis had no effect on DSS-induced colitis, indicating a proinflammatory role of IL-1β and IL-1R-signalling in DSS-induced colitis, confirming our results in the DSS model. Anakinra, an antagonist of IL-1R signalling, reversed the increase in DSS-induced colitis associated with Proteus mirabilis colonisation. In summary, differences in the intestinal microbiota might explain contradicting results obtained in Nlrp3$^{-/-}$, caspase-1$^{-/-}$ and IL-18$^{-/-}$ mice, however, when bias by differences in the intestinal microbiota is controlled, Nlrp3, and particularly Nlrp3-mediated IL-1β, has a proinflammatory role in colitis induction.

The simplest way to reduce bias by intestinal microbiota between genetically different mouse strains is performing cohousing experiments. Microbiota of Nlrp3-sufficient and Nlrp3-deficient Rag1$^{-/-}$ mice were analysed before and after cohousing at steady state, finding that the microbiota differed before cohousing and that Nlrp3-sufficient mice adjusted to the microbiota of Nlrp3-deficient mice three weeks after cohousing (**fig. 6-14 a and b**). Despite the fact that the microbiota of Nlrp3-sufficient mice adjusted to the microbiota of Nlrp3-deficient mice, no significant differences were found between the subgroups upon analysis of SFB. Nlrp3-deficient mice cohoused with Nlrp3-sufficient mice had reduced inflammation just like their non-cohoused counterparts (**fig. 6-14 c-k**). These data indicate that at least in the early phases of adoptive T cell transfer colitis, microbiota do not play a role in the observed phenotype of colitis protection in Nlrp3-deficient mice.

Results of this study show that Nlrp3-dependent IL-1β is instrumental in shaping T cell responses towards a proinflammatory Th17/Th1 phenotype. Proinflammatory, Th17-dependent cytokines, such as IL-17, IL-22 and GM-CSF, were upregulated. A prominent role of Nlrp3 in the plasticity of a homeostatic T cell response towards inflammation is concordant with recent literature. Under steady state conditions, gut microbiota constitutively primes LP macrophages to induce pro-IL-1β (Shaw, Kamada et al. 2012). Production of pro-IL-1β by stimulation of TLRs or members of the IL-1/IL-18 receptor family is tightly regulated and might involve epithelial and stromal cells (Franchi, Kamada et al. 2012). It remains unclear what kind of intestinal signal leads to the processing of pro-IL-1β into mature IL-1β. ATP, produced by microbiota, might play an important role (Mariathasan, Weiss et al. 2006; Atarashi, Nishimura et al. 2008; Iwase, Shinji et al. 2010; Hironaka, Iwase et al. 2013; Killeen, Ferris et al. 2013). Notably, Nlrp3 is a major candidate that controls the switch from homeostatic to proinflammatory Th17 cells. Control of this switch is mandatory to control

pathogenic bacteria on the one hand, but limit autoimmune responses on the other hand. Successful induction of inflammatory T cell responses would inhibit outgrowth of pathogenic bacteria.

Nlrp3 might not only be a key player in colitis models mediated by innate immune effects, but also in T cell-driven models of intestinal inflammation. Whereas the DSS model mimics effects of IBD on the epithelial barrier, with immunological phenomena being secondary to barrier malfunction, the T cell transfer model of colitis is a primarily immunological model, in which barrier function is, at least initially intact. Thus, early immunological responses in the T cell transfer model may mimic initial steps in the pathogenesis of Crohn's disease.

8.7 Conclusion

In summary, this study shows a clear role of Nlrp3 inflammasome in the initiation of T cell-induced intestinal inflammation. Nlrp3 inflammasome-dependent IL-1β does not only drive the differentiation of effector T cells, but also induces the secretion of key inflammatory cytokines, which regulate intestinal inflammation (**fig. 7-1a**). Results of this work also highlight the importance of maintaining the equilibrium between GM-CSF and FLT3L, two growth factors, which are crucial in regulating tolerogenic $CD103^+$ DCs and inflammatory $CD103^-$ DCs. In the presence of Nlrp3-dependent IL-1β, DCs activate $CD4^+$ T cells to secrete GM-CSF, therefore shifting the FLT3L/GM-CSF balance towards GM-CSF, an environment that favours the differentiation of $CD103^-$ DCs (**fig. 7-1a**). On the contrary, lack of IL-1β shifts FLT3L/GM-CSF balance to FLT3L, a milieu that is beneficial for $CD103^+$ DC development (**fig. 7-1b**). Importantly, although IL-18 production to be independent of Nlrp3 inflammasome, its signalling in $CD4^+$ T cells through IL-18R is critical for reducing T cell-induced intestinal inflammation.

The rapid and irreversible effects of IL-1β signalling induce a chain reaction of proinflammatory processes. This mechanism could explain the failure of anakinra in the treatment of an already established intestinal immune reaction, such as in human IBD (Lin, Hegarty et al. 2011). As effects of dysregulated IL-1β are potentiated so early and easily in IBD patients by IL-1β-dependent effector T cells and IL-1β-associated proinflammatory cytokines, targeting Nlrp3 inflammasome, which acts upstream of active IL-1β, might be a more promising therapeutic approach for controlling intestinal inflammation, than blocking IL-1R.

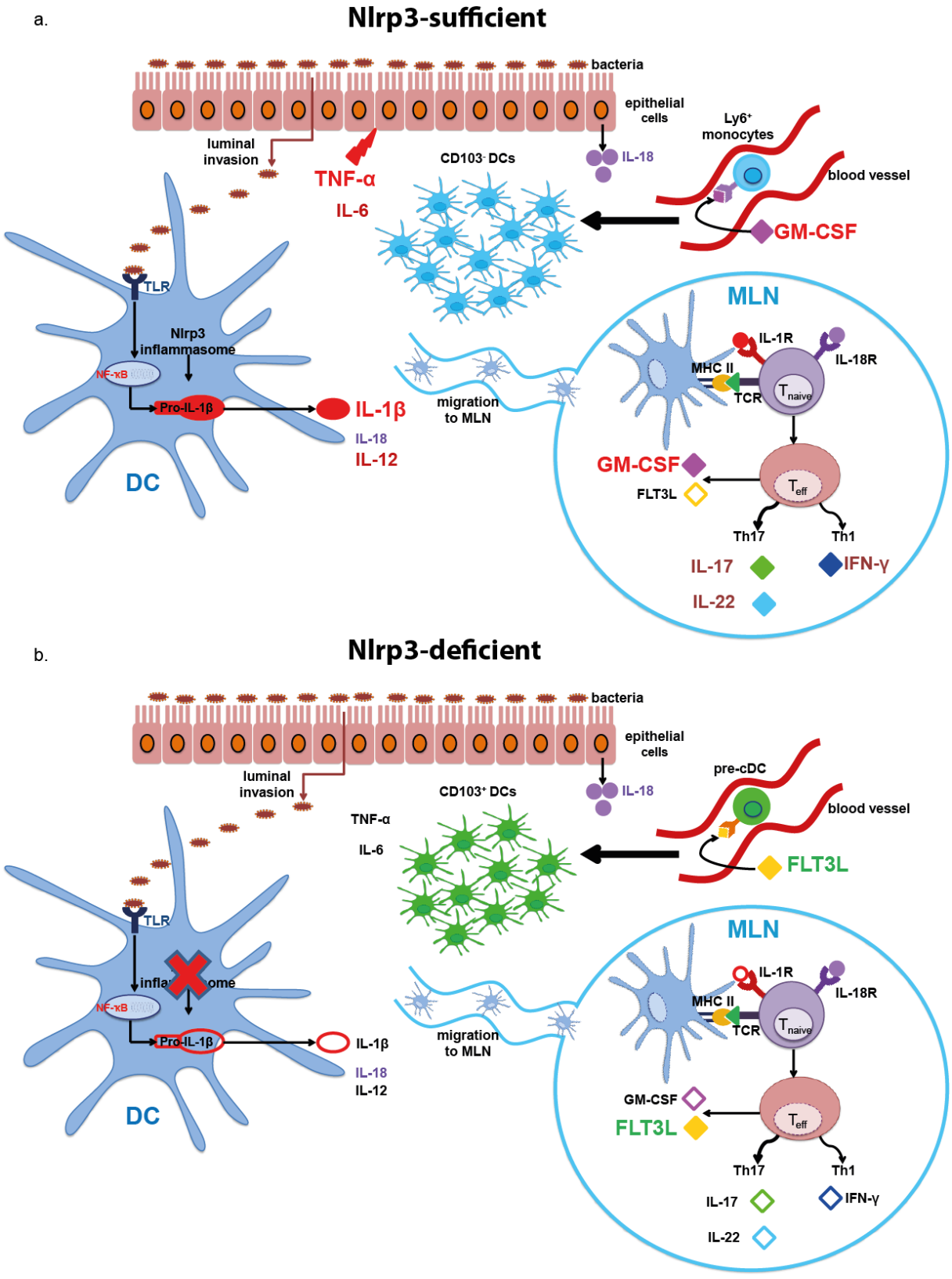

Figure 8-1: Proposed immunological mechanism in the induction of adoptive T cell transfer colitis in Nlrp3-sufficient and –deficient mice.

(a) TLR-dependent luminal trigger on DCs induce transcription of pro-IL-1β via NF-κB signalling. Pro-IL-1β is then activated to IL-1β by Nlrp3 inflammasome. Active IL-1β is a potent inducer of proinflammatory cytokines, such as TNF-α, IL-12 and IL-6. Activated DCs then migrate to the MLN where they present antigen to naïve $CD4^+$ T cells. The combination of antigen recognition via TCR and

binding of IL-1β via IL-1R induces differentiation of Th1 and Th17 effector T cells, secreting increased levels of IFN-γ, IL-17 and IL-22, respectively. IL-1β-activated effector T cells predominantly produce GM-CSF which favours the recruitment of $CD103^-$ DCs into LP and MLN. Importantly, the secretion of IL-18 appears to be predominantly Nlrp3-independent. Its signalling in T cells is critical for the limitation of intestinal inflammation. (b) Conversely, luminal microbial invasion in a Nlrp3-deficient organism results in significantly reduced levels of IL-1β, resulting in reduced levels of proinflammatory cytokines like TNF-α, IL-12 and IL-6. Reduced IL-1β results in T cells, which have a reduced secretion of T cell-associated proinflammatory cytokines, like IFN-γ, IL-17 and IL-22. Additionally, these T cells upregulate FLT3L secretion, resulting in a cytokine milieu favouring $CD103^+$ DC differentiation.

9 Literature

Abraham, C. and J. H. Cho (2009). "Inflammatory bowel disease." N Engl J Med **361**(21): 2066-2078.

Acosta-Rodriguez, E. V., G. Napolitani, et al. (2007). "Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells." Nat Immunol **8**(9): 942-949.

Agace, W. W. and E. K. Persson (2012). "How vitamin A metabolizing dendritic cells are generated in the gut mucosa." Trends Immunol **33**(1): 42-48.

Agostini, L., F. Martinon, et al. (2004). "NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder." Immunity **20**(3): 319-325.

Alex, P., N. C. Zachos, et al. (2009). "Distinct cytokine patterns identified from multiplex profiles of murine DSS and TNBS-induced colitis." Inflamm Bowel Dis **15**(3): 341-352.

Annacker, O., J. L. Coombes, et al. (2005). "Essential role for CD103 in the T cell-mediated regulation of experimental colitis." J Exp Med **202**(8): 1051-1061.

Antoni, L., S. Nuding, et al. (2014). "Intestinal barrier in inflammatory bowel disease." World J Gastroenterol **20**(5): 1165-1179.

Arai, Y., H. Takanashi, et al. (1998). "Involvement of interleukin-1 in the development of ulcerative colitis induced by dextran sulfate sodium in mice." Cytokine **10**(11): 890-896.

Ashkar, S., G. F. Weber, et al. (2000). "Eta-1 (osteopontin): an early component of type-1 (cell-mediated) immunity." Science **287**(5454): 860-864.

Atarashi, K., J. Nishimura, et al. (2008). "ATP drives lamina propria T(H)17 cell differentiation." Nature **455**(7214): 808-812.

Atarashi, K., T. Tanoue, et al. (2013). "Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota." Nature **500**(7461): 232-236.

Atif, S. M., S. Uematsu, et al. (2014). "CD103-CD11b+ dendritic cells regulate the sensitivity of CD4 T-cell responses to bacterial flagellin." Mucosal Immunol **7**(1): 68-77.

Atreya, R., J. Mudter, et al. (2000). "Blockade of interleukin 6 trans signaling suppresses T-cell resistance against apoptosis in chronic intestinal inflammation: evidence in crohn disease and experimental colitis in vivo." Nat Med **6**(5): 583-588.

Atzeni, F., A. Doria, et al. (2007). "Potential target of infliximab in autoimmune and inflammatory diseases." Autoimmun Rev **6**(8): 529-536.

Basu, R., R. D. Hatton, et al. (2013). "The Th17 family: flexibility follows function." Immunol Rev **252**(1): 89-103.

Basu, R., S. K. Whitley, et al. (2015). "IL-1 signaling modulates activation of STAT transcription factors to antagonize retinoic acid signaling and control the TH17 cell-iTreg cell balance." Nat Immunol **16**(3): 286-295.

Bauer, C., P. Duewell, et al. (2012). "Protective and aggravating effects of Nlrp3 inflammasome activation in IBD models: influence of genetic and environmental factors." Dig Dis **30 Suppl 1**: 82-90.

Bauer, C., P. Duewell, et al. (2010). "Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome." Gut **59**(9): 1192-1199.

Baumgart, D. C. and S. R. Carding (2007). "Inflammatory bowel disease: cause and immunobiology." Lancet **369**(9573): 1627-1640.

Baumgart, D. C. and W. J. Sandborn (2007). "Inflammatory bowel disease: clinical aspects and established and evolving therapies." Lancet **369**(9573): 1641-1657.

Ben-Sasson, S. Z., J. Hu-Li, et al. (2009). "IL-1 acts directly on CD4 T cells to enhance their antigen-driven expansion and differentiation." Proc Natl Acad Sci U S A **106**(17): 7119-7124.

Bernardo, D., S. Vallejo-Diez, et al. (2012). "IL-6 promotes immune responses in human ulcerative colitis and induces a skin-homing phenotype in the dendritic cells and Tcells they stimulate." Eur J Immunol **42**(5): 1337-1353.

Bethea, J. R., G. Y. Gillespie, et al. (1992). "Interleukin-1 beta induction of TNF-alpha gene expression: involvement of protein kinase C." J Cell Physiol **152**(2): 264-273.

Bettelli, E., Y. Carrier, et al. (2006). "Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells." Nature **441**(7090): 235-238.

Blyszczuk, P., S. Behnke, et al. (2013). "GM-CSF promotes inflammatory dendritic cell formation but does not contribute to disease progression in experimental autoimmune myocarditis." Biochim Biophys Acta **1833**(4): 934-944.

Bogunovic, M., F. Ginhoux, et al. (2009). "Origin of the lamina propria dendritic cell network." Immunity **31**(3): 513-525.

Brydges, S. D., J. L. Mueller, et al. (2009). "Inflammasome-mediated disease animal models reveal roles for innate but not adaptive immunity." Immunity **30**(6): 875-887.

Buonocore, S., P. P. Ahern, et al. (2010). "Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology." Nature **464**(7293): 1371-1375.

Buza, J., P. Benjamin, et al. (2008). "CD14+ cells are required for IL-12 response in bovine blood mononuclear cells activated with Toll-like receptor (TLR) 7 and TLR8 ligands." Vet Immunol Immunopathol **126**(3-4): 273-282.

Cahill, C. M. and J. T. Rogers (2008). "Interleukin (IL) 1beta induction of IL-6 is mediated by a novel phosphatidylinositol 3-kinase-dependent AKT/IkappaB kinase alpha pathway targeting activator protein-1." J Biol Chem **283**(38): 25900-25912.

Carter, J. D., J. Valeriano, et al. (2003). "Crohn disease worsened by anakinra administration." J Clin Rheumatol **9**(4): 276-277.

Casini-Raggi, V., L. Kam, et al. (1995). "Mucosal imbalance of IL-1 and IL-1 receptor antagonist in inflammatory bowel disease. A novel mechanism of chronic intestinal inflammation." J Immunol **154**(5): 2434-2440.

Cerovic, V., S. A. Houston, et al. (2013). "Intestinal CD103(-) dendritic cells migrate in lymph and prime effector T cells." Mucosal Immunol **6**(1): 104-113.

Chaudhry, A., D. Rudra, et al. (2009). "CD4+ regulatory T cells control TH17 responses in a Stat3-dependent manner." Science **326**(5955): 986-991.

Chen, G. Y. and G. Nunez (2011). "Inflammasomes in intestinal inflammation and cancer." Gastroenterology **141**(6): 1986-1999.

Chirdo, F. G., O. R. Millington, et al. (2005). "Immunomodulatory dendritic cells in intestinal lamina propria." Eur J Immunol **35**(6): 1831-1840.

Cho, J. H. (2008). "The genetics and immunopathogenesis of inflammatory bowel disease." Nat Rev Immunol **8**(6): 458-466.

Chung, Y., X. Yang, et al. (2006). "Expression and regulation of IL-22 in the IL-17-producing CD4+ T lymphocytes." Cell Res **16**(11): 902-907.

Coccia, M., O. J. Harrison, et al. (2012). "IL-1beta mediates chronic intestinal inflammation by promoting the accumulation of IL-17A secreting innate lymphoid cells and CD4(+) Th17 cells." J Exp Med **209**(9): 1595-1609.

Codarri, L., G. Gyulveszi, et al. (2011). "RORgammat drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation." Nat Immunol **12**(6): 560-567.

Cominelli, F., C. C. Nast, et al. (1990). "Interleukin 1 (IL-1) gene expression, synthesis, and effect of specific IL-1 receptor blockade in rabbit immune complex colitis." J Clin Invest **86**(3): 972-980.

Cominelli, F., C. C. Nast, et al. (1992). "Recombinant interleukin-1 receptor antagonist blocks the proinflammatory activity of endogenous interleukin-1 in rabbit immune colitis." Gastroenterology **103**(1): 65-71.

Coombes, J. L. and F. Powrie (2008). "Dendritic cells in intestinal immune regulation." Nat Rev Immunol **8**(6): 435-446.

Coombes, J. L., K. R. Siddiqui, et al. (2007). "A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism." J Exp Med **204**(8): 1757-1764.

Darrasse-Jeze, G., S. Deroubaix, et al. (2009). "Feedback control of regulatory T cell homeostasis by dendritic cells in vivo." J Exp Med **206**(9): 1853-1862.

De la Fuente, M., L. Franchi, et al. (2014). "Escherichia coli isolates from inflammatory bowel diseases patients survive in macrophages and activate NLRP3 inflammasome." Int J Med Microbiol **304**(3-4): 384-392.

Dinarello, C. A. (1996). "Biologic basis for interleukin-1 in disease." Blood **87**(6): 2095-2147.

Dinarello, C. A. (2009). "Immunological and inflammatory functions of the interleukin-1 family." Annu Rev Immunol **27**: 519-550.

Dinarello, C. A., D. Novick, et al. (2013). "Interleukin-18 and IL-18 binding protein." Front Immunol **4**: 289.

Dupaul-Chicoine, J., G. Yeretssian, et al. (2010). "Control of intestinal homeostasis, colitis, and colitis-associated colorectal cancer by the inflammatory caspases." Immunity **32**(3): 367-378.

El-Behi, M., B. Ciric, et al. (2011). "The encephalitogenicity of T(H)17 cells is dependent on IL-1- and IL-23-induced production of the cytokine GM-CSF." Nat Immunol **12**(6): 568-575.

Elinav, E., T. Strowig, et al. (2011). "NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis." Cell **145**(5): 745-757.

Elson, C. O., Y. Cong, et al. (2005). "Experimental models of inflammatory bowel disease reveal innate, adaptive, and regulatory mechanisms of host dialogue with the microbiota." Immunol Rev **206**: 260-276.

Farache, J., I. Koren, et al. (2013). "Luminal bacteria recruit CD103(+) dendritic cells into the intestinal epithelium to sample bacterial antigens for presentation." Immunity **38**(3): 581-595.

Farber, J. M. (1997). "Mig and IP-10: CXC chemokines that target lymphocytes." J Leukoc Biol **61**(3): 246-257.

Feng, T., H. Qin, et al. (2011). "Th17 cells induce colitis and promote Th1 cell responses through IL-17 induction of innate IL-12 and IL-23 production." J Immunol **186**(11): 6313-6318.

Franchi, L., N. Kamada, et al. (2012). "NLRC4-driven production of IL-1beta discriminates between pathogenic and commensal bacteria and promotes host intestinal defense." Nat Immunol **13**(5): 449-456.

Fujimoto, K., T. Karuppuchamy, et al. (2011). "A new subset of CD103+CD8alpha+ dendritic cells in the small intestine expresses TLR3, TLR7, and TLR9 and induces Th1 response and CTL activity." J Immunol **186**(11): 6287-6295.

Garrett, W. S., G. M. Lord, et al. (2007). "Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system." Cell **131**(1): 33-45.

Goto, Y., C. Panea, et al. (2014). "Segmented filamentous bacteria antigens presented by intestinal dendritic cells drive mucosal Th17 cell differentiation." Immunity **40**(4): 594-607.

Gregersen, P. K. and L. M. Olsson (2009). "Recent advances in the genetics of autoimmune disease." Annu Rev Immunol **27**: 363-391.

Hadis, U., B. Wahl, et al. (2011). "Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria." Immunity **34**(2): 237-246.

Hanauer, S. B., B. G. Feagan, et al. (2002). "Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial." Lancet **359**(9317): 1541-1549.

Harrison, O. J., N. Srinivasan, et al. (2015). "Epithelial-derived IL-18 regulates Th17 cell differentiation and Foxp3(+) Treg cell function in the intestine." Mucosal Immunol **8**(6): 1226-1236.

Hart, A. L., H. O. Al-Hassi, et al. (2005). "Characteristics of intestinal dendritic cells in inflammatory bowel diseases." Gastroenterology **129**(1): 50-65.

Hasegawa, M., N. Kamada, et al. (2012). "Protective role of commensals against Clostridium difficile infection via an IL-1beta-mediated positive-feedback loop." J Immunol **189**(6): 3085-3091.

Higashimori, A., T. Watanabe, et al. (2016). "Mechanisms of NLRP3 inflammasome activation and its role in NSAID-induced enteropathy." Mucosal Immunol **9**(3): 659-668.

Hironaka, I., T. Iwase, et al. (2013). "Glucose triggers ATP secretion from bacteria in a growth-phase-dependent manner." Appl Environ Microbiol **79**(7): 2328-2335.

Hirota, S. A., J. Ng, et al. (2011). "NLRP3 inflammasome plays a key role in the regulation of intestinal homeostasis." Inflamm Bowel Dis **17**(6): 1359-1372.

Horai, R., S. Saijo, et al. (2000). "Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice." J Exp Med **191**(2): 313-320.

Hu, X., T. Wang, et al. (2015). "Antibiotic-induced imbalances in gut microbiota aggravates cholesterol accumulation and liver injuries in rats fed a high-cholesterol diet." Appl Microbiol Biotechnol **99**(21): 9111-9122.

Huber, S., N. Gagliani, et al. (2012). "IL-22BP is regulated by the inflammasome and modulates tumorigenesis in the intestine." Nature **491**(7423): 259-263.

Hudcovic, T., R. Stepankova, et al. (2001). "The role of microflora in the development of intestinal inflammation: acute and chronic colitis induced by dextran sulfate in germ-free and conventionally reared immunocompetent and immunodeficient mice." Folia Microbiol (Praha) **46**(6): 565-572.

Ikejima, T., S. Okusawa, et al. (1990). "Interleukin-1 induces tumor necrosis factor (TNF) in human peripheral blood mononuclear cells in vitro and a circulating TNF-like activity in rabbits." J Infect Dis **162**(1): 215-223.

Iliev, I. D., I. Spadoni, et al. (2009). "Human intestinal epithelial cells promote the differentiation of tolerogenic dendritic cells." Gut **58**(11): 1481-1489.

Inoue, M., K. L. Williams, et al. (2012). "NLRP3 inflammasome induces chemotactic immune cell migration to the CNS in experimental autoimmune encephalomyelitis." Proc Natl Acad Sci U S A **109**(26): 10480-10485.

Ivanov, II, K. Atarashi, et al. (2009). "Induction of intestinal Th17 cells by segmented filamentous bacteria." Cell **139**(3): 485-498.

Ivanov, II, L. Frutos Rde, et al. (2008). "Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine." Cell Host Microbe **4**(4): 337-349.

Iwasaki, A. and B. L. Kelsall (2001). "Unique functions of CD11b+, CD8 alpha+, and double-negative Peyer's patch dendritic cells." J Immunol **166**(8): 4884-4890.

Iwase, T., H. Shinji, et al. (2010). "Isolation and identification of ATP-secreting bacteria from mice and humans." J Clin Microbiol **48**(5): 1949-1951.

Iwata, M., A. Hirakiyama, et al. (2004). "Retinoic acid imprints gut-homing specificity on T cells." Immunity **21**(4): 527-538.

Jaensson, E., H. Uronen-Hansson, et al. (2008). "Small intestinal CD103+ dendritic cells display unique functional properties that are conserved between mice and humans." J Exp Med **205**(9): 2139-2149.

Jager, S., E. F. Stange, et al. (2013). "Inflammatory bowel disease: an impaired barrier disease." Langenbecks Arch Surg **398**(1): 1-12.

Jang, M. H., N. Sougawa, et al. (2006). "CCR7 is critically important for migration of dendritic cells in intestinal lamina propria to mesenteric lymph nodes." J Immunol **176**(2): 803-810.

Johansson-Lindbom, B. and W. W. Agace (2007). "Generation of gut-homing T cells and their localization to the small intestinal mucosa." Immunol Rev **215**: 226-242.

Johansson-Lindbom, B., M. Svensson, et al. (2005). "Functional specialization of gut CD103+ dendritic cells in the regulation of tissue-selective T cell homing." J Exp Med **202**(8): 1063-1073.

Johansson, M. E., M. Phillipson, et al. (2008). "The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria." Proc Natl Acad Sci U S A **105**(39): 15064-15069.

Jones, S. C., J. E. Crabtree, et al. (1994). "Mucosal interleukin-6 secretion in ulcerative colitis. Effects of anti-inflammatory drugs and T-cell stimulation." Scand J Gastroenterol **29**(8): 722-728.

Kanai, T., M. Watanabe, et al. (2001). "Macrophage-derived IL-18-mediated intestinal inflammation in the murine model of Crohn's disease." Gastroenterology **121**(4): 875-888.

Killeen, M. E., L. Ferris, et al. (2013). "Signaling through purinergic receptors for ATP induces human cutaneous innate and adaptive Th17 responses: implications in the pathogenesis of psoriasis." J Immunol **190**(8): 4324-4336.

Kilshaw, P. J. and S. J. Murant (1990). "A new surface antigen on intraepithelial lymphocytes in the intestine." Eur J Immunol **20**(10): 2201-2207.

Kirkland, D., A. Benson, et al. (2012). "B cell-intrinsic MyD88 signaling prevents the lethal dissemination of commensal bacteria during colonic damage." Immunity **36**(2): 228-238.

Kojouharoff, G., W. Hans, et al. (1997). "Neutralization of tumour necrosis factor (TNF) but not of IL-1 reduces inflammation in chronic dextran sulphate sodium-induced colitis in mice." Clin Exp Immunol **107**(2): 353-358.

Kotenko, S. V., L. S. Izotova, et al. (2001). "Identification, cloning, and characterization of a novel soluble receptor that binds IL-22 and neutralizes its activity." J Immunol **166**(12): 7096-7103.

Kourepini, E., M. Aggelakopoulou, et al. (2014). "Osteopontin expression by CD103- dendritic cells drives intestinal inflammation." Proc Natl Acad Sci U S A **111**(9): E856-865.

Kroenke, M. A., T. J. Carlson, et al. (2008). "IL-12- and IL-23-modulated T cells induce distinct types of EAE based on histology, CNS chemokine profile, and response to cytokine inhibition." J Exp Med **205**(7): 1535-1541.

Kumar, N. P., K. Moideen, et al. (2016). "Coincident diabetes mellitus modulates Th1-, Th2-, and Th17-cell responses in latent tuberculosis in an IL-10- and TGF-beta-dependent manner." Eur J Immunol **46**(2): 390-399.

Laffont, S., K. R. Siddiqui, et al. (2010). "Intestinal inflammation abrogates the tolerogenic properties of MLN CD103+ dendritic cells." Eur J Immunol **40**(7): 1877-1883.

Lasiglie, D., E. Traggiai, et al. (2011). "Role of IL-1 beta in the development of human T(H)17 cells: lesson from NLPR3 mutated patients." PLoS One **6**(5): e20014.

Lee, J. K., S. H. Kim, et al. (2004). "Differences in signaling pathways by IL-1beta and IL-18." Proc Natl Acad Sci U S A **101**(23): 8815-8820.

Lehmann, J., J. Huehn, et al. (2002). "Expression of the integrin alpha Ebeta 7 identifies unique subsets of CD25+ as well as CD25- regulatory T cells." Proc Natl Acad Sci U S A **99**(20): 13031-13036.

Leppkes, M., C. Becker, et al. (2009). "RORgamma-expressing Th17 cells induce murine chronic intestinal inflammation via redundant effects of IL-17A and IL-17F." Gastroenterology **136**(1): 257-267.

Liang, S. C., X. Y. Tan, et al. (2006). "Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides." J Exp Med **203**(10): 2271-2279.

Ligumsky, M., P. L. Simon, et al. (1990). "Role of interleukin 1 in inflammatory bowel disease--enhanced production during active disease." Gut **31**(6): 686-689.

Lin, Z., J. P. Hegarty, et al. (2011). "Failure of anakinra treatment of pyoderma gangrenosum in an IBD patient and relevance to the PSTPIP1 gene." Inflamm Bowel Dis **17**(6): E41-42.

Littman, D. R. and A. Y. Rudensky (2010). "Th17 and regulatory T cells in mediating and restraining inflammation." Cell **140**(6): 845-858.

Liu, K., G. D. Victora, et al. (2009). "In vivo analysis of dendritic cell development and homeostasis." Science **324**(5925): 392-397.

Lopez, P., I. Gonzalez-Rodriguez, et al. (2011). "Immune response to Bifidobacterium bifidum strains support Treg/Th17 plasticity." PLoS One **6**(9): e24776.

Ludwiczek, O., E. Vannier, et al. (2004). "Imbalance between interleukin-1 agonists and antagonists: relationship to severity of inflammatory bowel disease." Clin Exp Immunol **138**(2): 323-329.

Mahida, Y. R., K. Wu, et al. (1989). "Enhanced production of interleukin 1-beta by mononuclear cells isolated from mucosa with active ulcerative colitis of Crohn's disease." Gut **30**(6): 835-838.

Mangan, P. R., L. E. Harrington, et al. (2006). "Transforming growth factor-beta induces development of the T(H)17 lineage." Nature **441**(7090): 231-234.

Mankertz, J. and J. D. Schulzke (2007). "Altered permeability in inflammatory bowel disease: pathophysiology and clinical implications." Curr Opin Gastroenterol **23**(4): 379-383.

Maraskovsky, E., K. Brasel, et al. (1996). "Dramatic increase in the numbers of functionally mature dendritic cells in Flt3 ligand-treated mice: multiple dendritic cell subpopulations identified." J Exp Med **184**(5): 1953-1962.

Mariathasan, S., D. S. Weiss, et al. (2006). "Cryopyrin activates the inflammasome in response to toxins and ATP." Nature **440**(7081): 228-232.

Martin, J. C., G. Beriou, et al. (2013). "Interleukin-22 binding protein (IL-22BP) is constitutively expressed by a subset of conventional dendritic cells and is strongly induced by retinoic acid." Mucosal Immunol.

Mascanfroni, I. D., A. Yeste, et al. (2013). "IL-27 acts on DCs to suppress the T cell response and autoimmunity by inducing expression of the immunoregulatory molecule CD39." Nat Immunol **14**(10): 1054-1063.

Matteoli, G., E. Mazzini, et al. (2010). "Gut CD103+ dendritic cells express indoleamine 2,3-dioxygenase which influences T regulatory/T effector cell balance and oral tolerance induction." Gut **59**(5): 595-604.

Mazzucchelli, L., C. Hauser, et al. (1996). "Differential in situ expression of the genes encoding the chemokines MCP-1 and RANTES in human inflammatory bowel disease." J Pathol **178**(2): 201-206.

McGeachy, M. J., K. S. Bak-Jensen, et al. (2007). "TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology." Nat Immunol **8**(12): 1390-1397.

Meng, G., F. Zhang, et al. (2009). "A mutation in the Nlrp3 gene causing inflammasome hyperactivation potentiates Th17 cell-dominant immune responses." Immunity **30**(6): 860-874.

Mowat, A. M. (2003). "Anatomical basis of tolerance and immunity to intestinal antigens." Nat Rev Immunol **3**(4): 331-341.

Munoz, M., C. Eidenschenk, et al. (2015). "Interleukin-22 induces interleukin-18 expression from epithelial cells during intestinal infection." Immunity **42**(2): 321-331.

Murugaiyan, G., A. Mittal, et al. (2008). "Increased osteopontin expression in dendritic cells amplifies IL-17 production by CD4+ T cells in experimental autoimmune encephalomyelitis and in multiple sclerosis." J Immunol **181**(11): 7480-7488.

Murugaiyan, G., A. Mittal, et al. (2010). "Identification of an IL-27/osteopontin axis in dendritic cells and its modulation by IFN-gamma limits IL-17-mediated autoimmune inflammation." Proc Natl Acad Sci U S A **107**(25): 11495-11500.

Muzaki, A. R., P. Tetlak, et al. (2016). "Intestinal CD103(+)CD11b(-) dendritic cells restrain colitis via IFN-gamma-induced anti-inflammatory response in epithelial cells." Mucosal Immunol **9**(2): 336-351.

Nakanishi, K., T. Yoshimoto, et al. (2001). "Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu." Cytokine Growth Factor Rev **12**(1): 53-72.

Neurath, M. F., S. Finotto, et al. (2001). "Regulation of T-cell apoptosis in inflammatory bowel disease: to die or not to die, that is the mucosal question." Trends Immunol **22**(1): 21-26.

Neville, L. F., G. Mathiak, et al. (1997). "The immunobiology of interferon-gamma inducible protein 10 kD (IP-10): a novel, pleiotropic member of the C-X-C chemokine superfamily." Cytokine Growth Factor Rev **8**(3): 207-219.

Ng, S. C., C. N. Bernstein, et al. (2013). "Geographical variability and environmental risk factors in inflammatory bowel disease." Gut **62**(4): 630-649.

Nowarski, R., R. Jackson, et al. (2015). "Epithelial IL-18 equilibrium controls barrier function in colitis." Cell **163**(6): 1444-1456.

O'Regan, A. (2003). "The role of osteopontin in lung disease." Cytokine Growth Factor Rev **14**(6): 479-488.

Ogawa, A., A. Andoh, et al. (2004). "Neutralization of interleukin-17 aggravates dextran sulfate sodium-induced colitis in mice." Clin Immunol **110**(1): 55-62.

Okamura, H., K. Nagata, et al. (1995). "A novel costimulatory factor for gamma interferon induction found in the livers of mice causes endotoxic shock." Infect Immun **63**(10): 3966-3972.

Okayasu, I., S. Hatakeyama, et al. (1990). "A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice." Gastroenterology **98**(3): 694-702.

Ouyang, W., S. Rutz, et al. (2011). "Regulation and functions of the IL-10 family of cytokines in inflammation and disease." Annu Rev Immunol **29**: 71-109.

Oz, H. S., J. Zhong, et al. (2012). "Osteopontin ablation attenuates progression of colitis in TNBS model." Dig Dis Sci **57**(6): 1554-1561.

Panea, C., A. M. Farkas, et al. (2015). "Intestinal monocyte-derived macrophages control commensal-specific Th17 responses." Cell Rep **12**(8): 1314-1324.

Pizarro, T. T., M. H. Michie, et al. (1999). "IL-18, a novel immunoregulatory cytokine, is up-regulated in Crohn's disease: expression and localization in intestinal mucosal cells." J Immunol **162**(11): 6829-6835.

Podolsky, D. K. (2002). "Inflammatory bowel disease." N Engl J Med **347**(6): 417-429.

Popivanova, B. K., F. I. Kostadinova, et al. (2009). "Blockade of a chemokine, CCL2, reduces chronic colitis-associated carcinogenesis in mice." Cancer Res **69**(19): 7884-7892.

Powrie, F. (1995). "T cells in inflammatory bowel disease: protective and pathogenic roles." Immunity **3**(2): 171-174.

Powrie, F. (2004). "Immune regulation in the intestine: a balancing act between effector and regulatory T cell responses." Ann N Y Acad Sci **1029**: 132-141.

Powrie, F., R. Correa-Oliveira, et al. (1994). "Regulatory interactions between CD45RBhigh and CD45RBlow CD4+ T cells are important for the balance between protective and pathogenic cell-mediated immunity." J Exp Med **179**(2): 589-600.

Powrie, F., M. W. Leach, et al. (1993). "Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice." Int Immunol **5**(11): 1461-1471.

Puren, A. J., G. Fantuzzi, et al. (1999). "Gene expression, synthesis, and secretion of interleukin 18 and interleukin 1beta are differentially regulated in human blood mononuclear cells and mouse spleen cells." Proc Natl Acad Sci U S A **96**(5): 2256-2261.

Qiao, Y., P. Wang, et al. (2012). "TLR-induced NF-kappaB activation regulates NLRP3 expression in murine macrophages." FEBS Lett **586**(7): 1022-1026.

Reinecker, H. C., E. Y. Loh, et al. (1995). "Monocyte-chemoattractant protein 1 gene expression in intestinal epithelial cells and inflammatory bowel disease mucosa." Gastroenterology **108**(1): 40-50.

Rescigno, M. (2009). "Before they were gut dendritic cells." Immunity **31**(3): 454-456.

Reynolds, G., J. R. Gibbon, et al. (2016). "Synovial CD4+ T-cell-derived GM-CSF supports the differentiation of an inflammatory dendritic cell population in rheumatoid arthritis." Ann Rheum Dis **75**(5): 899-907.

Rivollier, A., J. He, et al. (2012). "Inflammation switches the differentiation program of Ly6Chi monocytes from antiinflammatory macrophages to inflammatory dendritic cells in the colon." J Exp Med **209**(1): 139-155.

Round, J. L., S. M. Lee, et al. (2011). "The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota." Science **332**(6032): 974-977.

Saito, Y., C. S. Boddupalli, et al. (2013). "Dendritic cell homeostasis is maintained by nonhematopoietic and T-cell-produced Flt3-ligand in steady state and during immune responses." Eur J Immunol **43**(6): 1651-1658.

Saperstein, S., L. Chen, et al. (2009). "IL-1beta augments TNF-alpha-mediated inflammatory responses from lung epithelial cells." J Interferon Cytokine Res **29**(5): 273-284.

Satsangi, J., R. A. Wolstencroft, et al. (1987). "Interleukin 1 in Crohn's disease." Clin Exp Immunol **67**(3): 594-605.

Saunders, D., K. Lucas, et al. (1996). "Dendritic cell development in culture from thymic precursor cells in the absence of granulocyte/macrophage colony-stimulating factor." J Exp Med **184**(6): 2185-2196.

Schaubeck, M., T. Clavel, et al. (2016). "Dysbiotic gut microbiota causes transmissible Crohn's disease-like ileitis independent of failure in antimicrobial defence." Gut **65**(2): 225-237.

Schottelius, A. J. and H. Dinter (2006). "Cytokines, NF-kappaB, microenvironment, intestinal inflammation and cancer." Cancer Treat Res **130**: 67-87.

Schreiber, H. A., J. Loschko, et al. (2013). "Intestinal monocytes and macrophages are required for T cell polarization in response to Citrobacter rodentium." J Exp Med **210**(10): 2025-2039.

Schroder, K., R. Zhou, et al. (2010). "The NLRP3 inflammasome: a sensor for metabolic danger?" Science **327**(5963): 296-300.

Schulz, O., E. Jaensson, et al. (2009). "Intestinal CD103+, but not CX3CR1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions." J Exp Med **206**(13): 3101-3114.

Scott, C. L., C. C. Bain, et al. (2015). "CCR2(+)CD103(-) intestinal dendritic cells develop from DC-committed precursors and induce interleukin-17 production by T cells." Mucosal Immunol **8**(2): 327-339.

Seki, E., H. Tsutsui, et al. (2001). "Lipopolysaccharide-induced IL-18 secretion from murine Kupffer cells independently of myeloid differentiation factor 88 that is critically involved in induction of production of IL-12 and IL-1beta." J Immunol **166**(4): 2651-2657.

Semmrich, M., M. Plantinga, et al. (2012). "Directed antigen targeting in vivo identifies a role for CD103+ dendritic cells in both tolerogenic and immunogenic T-cell responses." Mucosal Immunol **5**(2): 150-160.

Seo, S. U., N. Kamada, et al. (2015). "distinct commensals iInduceinterleukin-1beta via NLRP3 inflammasome in inflammatory monocytes to promote Intestinal inflammation in response to injury." Immunity **42**(4): 744-755.

Shaw, M. H., N. Kamada, et al. (2012). "Microbiota-induced IL-1beta, but not IL-6, is critical for the development of steady-state TH17 cells in the intestine." J Exp Med **209**(2): 251-258.

Shinohara, M. L., M. Jansson, et al. (2005). "T-bet-dependent expression of osteopontin contributes to T cell polarization." Proc Natl Acad Sci U S A **102**(47): 17101-17106.

Shinohara, M. L., J. H. Kim, et al. (2008). "Engagement of the type I interferon receptor on dendritic cells inhibits T helper 17 cell development: role of intracellular osteopontin." Immunity **29**(1): 68-78.

Siddiqui, K. R., S. Laffont, et al. (2010). "E-cadherin marks a subset of inflammatory dendritic cells that promote T cell-mediated colitis." Immunity **32**(4): 557-567.

Siddiqui, K. R. and F. Powrie (2008). "CD103+ GALT DCs promote Foxp3+ regulatory T cells." Mucosal Immunol **1 Suppl 1**: S34-38.

Siegmund, B. (2002). "Interleukin-1beta converting enzyme (caspase-1) in intestinal inflammation." Biochem Pharmacol **64**(1): 1-8.

Siegmund, B. (2010). "Interleukin-18 in intestinal inflammation: friend and foe?" Immunity **32**(3): 300-302.

Siegmund, B., H. A. Lehr, et al. (2001). "IL-1 beta -converting enzyme (caspase-1) in intestinal inflammation." Proc Natl Acad Sci U S A **98**(23): 13249-13254.

Sims, J. E. and D. E. Smith (2010). "The IL-1 family: regulators of immunity." Nat Rev Immunol **10**(2): 89-102.

Sonnenberg, G. F., L. A. Fouser, et al. (2011). "Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22." Nat Immunol **12**(5): 383-390.

Sonnenberg, G. F., M. G. Nair, et al. (2010). "Pathological versus protective functions of IL-22 in airway inflammation are regulated by IL-17A." J Exp Med **207**(6): 1293-1305.

Spadoni, I., I. D. Iliev, et al. (2012). "Dendritic cells produce TSLP that limits the differentiation of Th17 cells, fosters Treg development, and protects against colitis." Mucosal Immunol **5**(2): 184-193.

Stagg, J. (2007). "Immune regulation by mesenchymal stem cells: two sides to the coin." Tissue Antigens **69**(1): 1-9.

Steinman, R. M. (2012). "Decisions about dendritic cells: past, present, and future." Annu Rev Immunol **30**: 1-22.

Steinman, R. M., D. Hawiger, et al. (2003). "Tolerogenic dendritic cells." Annu Rev Immunol **21**: 685-711.

Stepankova, R., F. Powrie, et al. (2007). "Segmented filamentous bacteria in a defined bacterial cocktail induce intestinal inflammation in SCID mice reconstituted with CD45RBhigh CD4+ T cells." Inflamm Bowel Dis **13**(10): 1202-1211.

Sugimoto, K., A. Ogawa, et al. (2008). "IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis." J Clin Invest **118**(2): 534-544.

Sujino, T., T. Kanai, et al. (2011). "Regulatory T cells suppress development of colitis, blocking differentiation of T-helper 17 into alternative T-helper 1 cells." Gastroenterology **141**(3): 1014-1023.

Sun, C. M., J. A. Hall, et al. (2007). "Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid." J Exp Med **204**(8): 1775-1785.

Sutton, C., C. Brereton, et al. (2006). "A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis." J Exp Med **203**(7): 1685-1691.

Sutton, C. E., S. J. Lalor, et al. (2009). "Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity." Immunity **31**(2): 331-341.

Svensson, M., B. Johansson-Lindbom, et al. (2008). "Retinoic acid receptor signaling levels and antigen dose regulate gut homing receptor expression on CD8+ T cells." Mucosal Immunol **1**(1): 38-48.

Taub, D. D., A. R. Lloyd, et al. (1993). "Recombinant human interferon-inducible protein 10 is a chemoattractant for human monocytes and T lymphocytes and promotes T cell adhesion to endothelial cells." J Exp Med **177**(6): 1809-1814.

Taub, D. D., D. L. Longo, et al. (1996). "Human interferon-inducible protein-10 induces mononuclear cell infiltration in mice and promotes the migration of human T lymphocytes into the peripheral tissues and human peripheral blood lymphocytes-SCID mice." Blood **87**(4): 1423-1431.

Teixido, J., C. M. Parker, et al. (1992). "Functional and structural analysis of VLA-4 integrin alpha 4 subunit cleavage." J Biol Chem **267**(3): 1786-1791.

Ten Hove, T., A. Corbaz, et al. (2001). "Blockade of endogenous IL-18 ameliorates TNBS-induced colitis by decreasing local TNF-alpha production in mice." Gastroenterology **121**(6): 1372-1379.

Thomassen, E., T. A. Bird, et al. (1998). "Binding of interleukin-18 to the interleukin-1 receptor homologous receptor IL-1Rrp1 leads to activation of signaling pathways similar to those used by interleukin-1." J Interferon Cytokine Res **18**(12): 1077-1088.

Tosato, G. and K. D. Jones (1990). "Interleukin-1 induces interleukin-6 production in peripheral blood monocytes." Blood **75**(6): 1305-1310.

Trinchieri, G. (1998). "Interleukin-12: a cytokine at the interface of inflammation and immunity." Adv Immunol **70**: 83-243.

Uaesoontrachoon, K., D. K. Wasgewatte Wijesinghe, et al. (2013). "Osteopontin deficiency delays inflammatory infiltration and the onset of muscle regeneration in a mouse model of muscle injury." Dis Model Mech **6**(1): 197-205.

Uematsu, S., K. Fujimoto, et al. (2008). "Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5." Nat Immunol **9**(7): 769-776.

Uss, E., A. T. Rowshani, et al. (2006). "CD103 is a marker for alloantigen-induced regulatory CD8+ T cells." J Immunol **177**(5): 2775-2783.

Van der Sluis, M., B. A. De Koning, et al. (2006). "Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection." Gastroenterology **131**(1): 117-129.

Van Limbergen, J., D. C. Wilson, et al. (2009). "The genetics of Crohn's disease." Annu Rev Genomics Hum Genet **10**: 89-116.

Varol, C., A. Vallon-Eberhard, et al. (2009). "Intestinal lamina propria dendritic cell subsets have different origin and functions." Immunity **31**(3): 502-512.

Vasconcellos, R., N. A. Carter, et al. (2011). "IL-12p35 subunit contributes to autoimmunity by limiting IL-27-driven regulatory responses." J Immunol **187**(6): 3402-3412.

Veldhoen, M., R. J. Hocking, et al. (2006). "TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells." Immunity **24**(2): 179-189.

Vermeire, S. and P. Rutgeerts (2005). "Current status of genetics research in inflammatory bowel disease." Genes Immun **6**(8): 637-645.

Villani, A. C., M. Lemire, et al. (2009). "Common variants in the NLRP3 region contribute to Crohn's disease susceptibility." Nat Genet **41**(1): 71-76.

Waskow, C., K. Liu, et al. (2008). "The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues." Nat Immunol **9**(6): 676-683.

Wei, C. C., T. W. Ho, et al. (2003). "Cloning and characterization of mouse IL-22 binding protein." Genes Immun **4**(3): 204-211.

Wilson, N. S., P. Duewell, et al. (2014). "Inflammasome-dependent and -independent IL-18 production mediates immunity to the ISCOMATRIX adjuvant." J Immunol **192**(7): 3259-3268.

Worbs, T., U. Bode, et al. (2006). "Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells." J Exp Med **203**(3): 519-527.

Xu, W., S. R. Presnell, et al. (2001). "A soluble class II cytokine receptor, IL-22RA2, is a naturally occurring IL-22 antagonist." Proc Natl Acad Sci U S A **98**(17): 9511-9516.

Yamaguchi, M., N. Matsuzaki, et al. (1990). "Interleukin 6 possibly induced by interleukin 1 beta in the pituitary gland stimulates the release of gonadotropins and prolactin." Acta Endocrinol (Copenh) **122**(2): 201-205.

Yokota, A., H. Takeuchi, et al. (2009). "GM-CSF and IL-4 synergistically trigger dendritic cells to acquire retinoic acid-producing capacity." Int Immunol **21**(4): 361-377.

Yuan, X., M. J. Dee, et al. (2015). "IL-2Rbeta-dependent signaling and CD103 functionally cooperate to maintain tolerance in the gut mucosa." J Immunol **194**(3): 1334-1346.

Zaki, M. H., K. L. Boyd, et al. (2010). "The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis." Immunity **32**(3): 379-391.

Zenewicz, L. A., G. D. Yancopoulos, et al. (2008). "Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease." Immunity **29**(6): 947-957.

Zhang, H. X., Z. T. Wang, et al. (2014). "NLRP3 gene is associated with ulcerative colitis (UC), but not Crohn's disease (CD), in Chinese Han population." Inflamm Res **63**(12): 979-985.

Zheng, Y., D. M. Danilenko, et al. (2007). "Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis." Nature **445**(7128): 648-651.

Zhong, J., E. R. Eckhardt, et al. (2006). "Osteopontin deficiency protects mice from Dextran sodium sulfate-induced colitis." Inflamm Bowel Dis **12**(8): 790-796.

Zielinski, C. E., F. Mele, et al. (2012). "Pathogen-induced human TH17 cells produce IFN-gamma or IL-10 and are regulated by IL-1beta." Nature **484**(7395): 514-518.

10 Appendices

10.1 Abbreviations

APC	Allophycocyanin
APC	Antigen presenting cell
ASC	Apoptosis-associated speck-like protein containing a CARD
BMDC	Bone marrow-derived dendritic cell
BP	Binding protein
CARD	Caspase-recruitment domain
CCL	Chemokine (C-C motif) ligand
CCR	C-C chemokine receptor
CD	Crohn's disease
CD	Cluster of differentiation
cDC	Conventional dendritic cell
cDNA	Copy deoxyribonucleic acid
CFSE	Carboxyfluorescein diacetat succinimidyl ester
CXCL	Chemokine (C-X-C motif) ligand
DAMP	Damage-associated molecular pattern
DAPI	4',6-diamidino-2-phenylindole
DC	Dendritic cell
DMEM	Dubelcco’s modified magle medium
DMSO	Dimethyl sulfoxide
DSS	Dextran sodium sulphate
DTT	Dithiotreitol
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescence activated cell sorter
FBS	Fetal bovine serum
FITC	Fluorescein isothiocyanate
FLT3L	FMS-like tyrosine kinase 3 ligand
FoxP3	Forkhead box P3
GM-CSF	Granulocyte macrophage-colony stimulating factor
hr	Hour
H&E	Hematoxylin and eosin
HPRT	Hypoxanthin-phosphoribosyl-transferase
HRP	Horse radish peroxidase
i.p.	Intraperitoneal
IBD	Inflammatory bowel disease
IDO	Indoleamine 2,3-dioxygenase
IEL	Intraepithelial lymphocytes
IFN	Interferon
IHC	Immunohistochemistry
IL	Interleukin
IP-10	Interferon gamma induced protein 10
LP	Lamina propria

LPS	Lipopolysaccheride
LRR	leucine-rich repeat
MFI	Median fluorescence intensity
Min	Minute
MHC	Major histocompatibility complex
MLN	Mesenterial lymph nodes
mRNA	Messenger RNA
NACHT	NAIP (neuronal apoptosis inhibitor protein), C2TA [class 2 transcription activator, of the MHC, HET-E (heterokaryon incompatibility) and TP1 (telomerase-asso
NLR	Nod-like receptor
NLRC4	NLR family CARD domain-containing protein 4
Nlrp	NACHT, LRR and PYD domains-containing protein
PAMP	Pathogen-associated molecular pattern
PCR	Polymerase chain reaction
PB	Pacific blue
PBS	Phosphate buffered saline
pDC	Plasmacytoid dendritic cell
PE	Phycoerythin
PerCP	Peridinin chlorophyll
PFA	Paraformaldehyde
PI	Propidium iodide
PYD	Pyrin domain
qRT-PCR	Quantitative real time PCR
Rag	Recombinant activating gene
RALDH	Retinal aldehyde dehydrogenase
RPMI	Roswell Park Memorial Institute
RNA	Ribonucleic acid
RT	Room temperature
sec	Second
s.c.	Subcutaneous
SDS	Sodium dodecyl sulphate
TBST	TRIS-buffered saline with Tween 20
TCR	T cell receptor
TGF-β	Transforming growth factor β
Th cells	T helper cells
TLR	Toll-like receptor
TMB	3, 3', 5, 5' –tetramethylbenzidine
TNBS	2,4,6-trinitrobenzene sulfonic acid
TNF-α	Tumour necrosis factor alpha
T_{reg}	Regulatory T cell
UC	Ulcerative colitis

10.2 List of figures

10.3 List of tables

11 Publications

11.1 Original publications

1. Proapoptotic and antiapoptotic proteins of the Bcl-2 family regulate sensitivity of pancreatic cancer cells toward gemcitabine and T-Cell-mediated cytotoxicity
   Bauer C, Hees C, Sterzik A, Bauernfeind F, **Mak'Anyengo R**, Duewell P, Lehr HA, Noessner E, Wank R, Trauzold A, Endres S, Dauer M, Schnurr M.
   J Immunother 2015 Apr; 38(3):116-26
2. Balance of Nlrp3-mediated IL-1β and IL-18 signaling regulates colitis induction in a T cell transfer model of inflammatory bowel disease
   Mak'Anyengo R, Duewell P, Denk G, Clavel T, Endres S, Schnurr M, Bauer C.
   In preparation

11.2 Abstracts

1. Aktivierung des Nlrp3-Inflammasoms induziert im Modell der CD45Rbhigh-Kolitis einen proinflammatorischen Phänotyp der adoptiv transferierten T-Zellen durch Vermittlung CD103+ DC
   R Mak'Anyengo, I Karl, P Duewell, H Lehr, S Endres, M Schnurr, C Bauer.
   Z Gastroenterol 2015; 53 - KG031
2. IL-18-Rezeptor-Signaling adoptiv transferierter T-Zellen reguliert die Induktion der CD4+CD45Rbhigh Transfer-Kolitis.
   R Mak'Anyengo, P Düwell, C Hörth, G Denk, HA Lehr, S Endres, M Schnurr, C Bauer.
   Z Gastroenterol 2016; 54 - KV018

11.3 Oral presentations

1. Poster "Role of the Nlrp3 inflammasome in Dextran sodium sulphate induced colitis"
   R Mak'Anyengo, P Düwell, HA Lehr, S Endres, C Bauer, M Schnurr.
   Inflammasomes in health and disease, June 2013, Boston, USA
2. Poster "Nlrp3 inflammasome in inflammatory bowel disease"
   R Mak'Anyengo, P Düwell, HA Lehr, S Endres, C Bauer, M Schnurr.
   Immunofest, September 2014, Munich, Germany
3. Poster "Role of the Nlrp3 inflammasome in murine models of inflammatory bowel disease"
   R Mak'Anyengo, P Düwell, HA Lehr, S Endres, C Bauer, M Schnurr.
   13th International Symposium on Dendritic Cells in fundamental and clinical immunology, September 2014, Tours, France

4. Talk “Nlrp3 takes centre stage in the balance of IL-1β and IL-18 during colitis”
   R Mak'Anyengo, P Düwell, HA Lehr, S Endres, C Bauer, M Schnurr.
   29. Jahrestagung der DACED (Deutsche Arbeitsgemeinschaf Chronisch-Entzündliche Darmerkrankung) , June 2016, Mainz, Germany

12 Acknowledgements

I would like to take this opportunity to express my sincere appreciation to those who have contributed to my thesis and have offered their support in one way or the other during the past four years.

First and foremost, I would like to express my sincere gratitude to my supervisor Prof. Max Schnurr for his constant support throughout this thesis. His motivation, boundless knowledge and encouragement escorted me throughout this journey.

I thank my second supervisor PD Christian Bauer who initiated this project by writing the grant, which made this project financially possible. I am very grateful for his supervision and his generous guidance throughout my thesis. Despite his very busy schedule, Dr. Bauer was always ready to offer his assistance.

I express my gratitude to Dr. Peter Düwell who offered an engaged and enthusiastic supervision during my thesis. His doors were always open for advice and discussions in both theoretical and practical problems.

I am very thankful to Prof. Stefan Endres for giving me the opportunity to do my thesis in the Division of Clinical Pharmacology. Prof. Endres has built a group of very enthusiastic researchers, making it really special to be part of the Division of Clinical Pharmacology.

I thank Prof. Hans-Anton Lehr, who evaluated all the colon tissue scores in this work.

My gratitude also goes to Prof. Gerald Denk, who provided us with IL-18R$^{-/-}$ and IL-1R$^{-/-}$ knock out mice.

I am grateful to the Else Kröner-Fresenius-Stiftung for financing my project and funding me for three years.

I thank CIPSM (Centre for Integrated Protein Science Munich) woman for two mini-grants which funded my two side projects.

I am grateful for being part of Graduiertenkolleg 1202 “Oligonucleotides in Cell Biology and Therapy” (research training group funded by German Research Foundation) and of i-Target” Immunotargeting of Cancer (International Doctorate Program funded by the Elite Network of the State of Bavaria).

My gratitude goes to Christine Hörth who was always ready to offer technical and practical support. Christine worked tirelessly with me and I am indebted for her great support.

I thank Nicole Lichter for her practical and technical support. Her very accurate way of working was in deed very helpful.

I thank all the present and past members of the Division of Clinical Pharmacology. Thank you for creating such a wonderful atmosphere.

I thank all members of the gastroenterology lab. I thank each and every one of you for creating such a friendly and warm atmosphere. I really enjoyed being part of your team.

Very special thanks to my husband Dirk and my family. Words cannot express how thankful I am for your support throughout this period.

13 Eidesstattliche Versicherung

Rachel Mak'Anyengo

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation mit dem Thema

Role of the Nlrp3 inflammasome in regulation of the tolerogenic function of CD103^{+} dendritic cells in CD4^{+}CD45Rb^{High} T cell transfer colitis and in steady state

selbstständig verfasst, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelleneinzeln nachgewiesen habe.

Ich erkläre des Weiteren, dass die hier vorgelegte Dissertation nicht in gleicher oder in ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht wurde.

München, 16.8.2017	Rachel Mak'Anyengo
Ort, Datum	Unterschrift Doktorandin