Function and diversity of myeloid cells during chronic inflammation in non-lymphoid organs

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Submitted in total fulfilment of the requirements of the joint degree of

Doctor of Philosophy (PhD)

of

The Medical Faculty

The Rheinische Friedrich-Wilhelms-Universität Bonn

and

The Department of Microbiology and Immunology

The University of Melbourne

Bonn/Melbourne, 2020

Performed and approved by the Medical Faculty of The Rheinische Friedrich-Wilhelms-Universität Bonn and The University of Melbourne

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Date of submission: 18th of September 2019
Date of the oral examination: 10th of January 2020

Institute of Experimental Immunology (University Hospital Bonn),

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Abbreviations

AAMs	Alternatively activated macrophages
AKI	Acute kidney injury
AP-1	Activator protein 1
APC	Antigen-presenting cells
AT	Adipose tissue
BAT	Brown adipose tissue
BATF	Basic leucine zipper transcription factor ATF-like
Blimp-1	B lymphocyte-induced maturation protein-1
BMDCs	Bone marrow derived dendritic cells
BMI	Body-mass index
bZIP	Basic leucine zipper
CAMs	Classically activated macrophages
cDCs	Conventional or classical
CKD	Chronic kidney disease
CLPs	Common lymphoid progenitor
CMPs	Common myeloid progenitors
CTL	Cytotoxic T lymphocyte
CTLA4	Cytotoxic T-lymphocyte antigen 4
DAMPs	Damage-associated molecular patterns
DBD	DNA-binding domain
DCs	Dendritic cells
DIO	Diet induced obesity
ERα	Estrogen receptor alpha
ERβ	Estrogen receptor beta
GTT	Glucose tolerance tests
GVHD	Graft-versus-host-disease
H&E	Hematoxylin and eosin
HFD	high fat diet
IDO	Indoleamine 2,3-dioxygenase
IFN	Interferon
IL	Interleukin
IL-1RAcP	IL-1R accessory protein
IL1RL1	Receptor ST2
ILC	Innate lymphocytes
ILC2	Group 2 innate lymphoid cells
IRF	Interferon-regulatory factor
ITT	Insulin tolerance test
KIM-1	Kidney injury marker-1

KLRG-1	Killer cell lectin-like receptor G -1
ko	Knockout
LAG3	Lymphocyte activation gene 3
MDSC	Myeloid derived suppressor cells
MHC-I	Major histocompatibility complex class I
MHC-II	Major histocompatibility complex class II
ND	Normal diet
PAMPs	Pathogen associated molecular pattern
pDCs	Plasmacytoid DCs
PMNs	Polymorphonuclear leukocytes
PRRs	Pattern recognition receptors
SC	Subcutaneous
SLOs	Secondary lymphoid organs
ST-2	Suppression of tumorgenicity 2
SVF	Stromal vascular fraction
Teff	Effector T cells
TF	Transcription factor
TFH	Follicular T helper cell
TGFβ	Transforming growth factor beta
TLRs	Toll-like receptors
TNF	Tumor necrosis factor
Treg	Regulatory T cells
UUO	Unilateral ureteral obstruction
VAT	Visceral fat
WAT	White adipose tissue

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Abstract

Different immune cells play a major role in maintaining tissue homeostasis, tissue repair but also in inflammation. Tissue integrity is mainly controlled by tissue regulatory T cells (T_{regs}), type 2 innate lymphoid cells (ILC2) and myeloid cells. Here, we are using a chronic kidney disease (CKD) model of adenine enriched diet induced crystal-nephropathy and high-fat diet induced obesity to investigate the contribution of T_{regs}, macrophages and dendritic cells during inflammation. Using a modified gating strategy to distinguish macrophages and conventional dendritic cells (cDCs) we detected that macrophages and cDCs infiltrate the visceral adipose tissue (VAT) during obesity. Here, sex-specific differences show that macrophages and cDCs accumulate significantly more in male VAT. RNA sequencing confirmed a higher pro-inflammatory gene signature in male VAT macrophages and cDCs. Those data indicate that myeloid cells might be a significant contributor to the sex-specific differences in the adipose tissue.

Interleukin (IL)-33 maintaining tissue homeostasis by promoting Tregs and ILC2s in the VAT. Investigating IL-33 and its receptor ST2 during kidney inflammation in the adenine enriched diet induced crystal-nephropathy we show that ST2 deficiency is negligible in a severe CKD onset, whereas using a mild disease onset we detect a disease exacerbation. Additionally, we show that the deficiency for IL-33 leads to a more severe disease progression accompanied by a high influx of several immune cells and lower frequency of effector T_{regs}.

Transcription factors such as IRF1, IRF4 and BATF are linked to regulate suppressive cell functions e.g. in T_{regs} and macrophages. Using transcription factor knockout mice, we determined that IRF1 deficiency leads to disease amelioration by reducing macrophage and monocyte infiltration. IRF4 and BATF deficiency lead to reduced numbers of T_{regs} in the kidney. $IRF4^{-/-}$ but not $BATF^{-/-}$

showed significant increased myeloid cell infiltration. Bone marrow chimera reconstituted with IRF4 deficient CD4 T cells show reduced numbers of T_{regs} in the inflamed kidney and correlated with the myeloid cell infiltration. Collectively, the findings point to a sex-specific contribution of macrophages and cDCs in tissue inflammation and indicate that the IL-33/ST2 axis regulates immunosuppressive cells tissue and disease progression specific.

Declaration

The work that is presented in this thesis was conducted at the University of Bonn in the laboratory of PD Dr. Isis Ludwig-Portugall and at the University of Melbourne in the laboratory of Professor Dr. Axel Kallies. This work was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Council) within GRK 2168 (Bo&MeRanG) and the Medical Faculty of the Rheinische Friedrich-Wilhelms-Universität Bonn. Sebastian Liene was supported by the Deutsche Forschungsgemeinschaft, the Medical Faculty of the Rheinische Friedrich-Wilhelms-Universität Bonn and the Melbourne International Research Scholarship.

This is to certify that,

(i) the thesis only comprises my original work towards the PhD except where indicated

(ii) due acknowledgement has been made in the text to all other material used

(iii) the thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Bonn, 18th September 2019

Sebastian Liene

Preface

My contribution to the experiments within each chapter was as follows:

Chapter 3: 75%

Chapter 4: 95%

Chapter 5: 100%

I acknowledge the important contributions of others to experiments presented herein:

Chapter 3: Dr. Ajith Vasanthakumar, David Chisanga, Santiago Valle Torres, Elise Gressier

Chapter 4: Prof. Dr. Peter Boor (RWTH Aachen), Biomedical Histology Facility (University of Melbourne)

Dedication

This dissertation is dedicated to my family

Acknowledgments

I would like to express my sincere gratitude to my doctoral supervisor PD Dr. Isis Ludwig-Portugall and Prof. Dr. Axel Kallies for the opportunity to complete my dissertation at the institute of Experimental Immunology in Bonn and the Peter Doherty Institute at the University of Melbourne. Thanks for your guidance, research directions, knowledge and motivation throughout my entire thesis.

Furthermore, I want to thank my thesis board members: the reviewer of my dissertation of unknown identity, and my committee chair A/Prof. Sammy Bedoui for his help and supportive conversations.

Additionally, I would like to thank my colleagues and friends from Bonn Romina, Teresa, Ermanila and Miriam for their immense support and positive attitude as well as all my colleagues at the institute for the fruitful discussions and help.

Moreover, I have to thank my colleagues and friends from Melbourne: First of all, Tom! Thanks a lot for your help and support especially for reading the stuff I bothered you with! Also, Daniel: thanks for reading and funny comments…!
Thanks to all members of the Kallies lab: Ajith, Renee, Teisha, Sarah, Jonas and Santiago! It was a great pleasure to work with you in such a supportive environment.

I would like to thank the Bonn and Melbourne Bo&MeRanG coordination offices for putting such a remarkable program together and their continuous support throughout the past years.

I would also like to thank my fellow Bo&MeRanG students for all the great trips, encouragement and friendship.

Finally, I would like to give my heartfelt thanks to my loving family for their consistent encouragement and support, without them I would have never been able to achieve what I have till now. I am grateful to my friends, who have provided moral and emotional support along the way!

List of publications

Sex-specific adipose tissue imprinting of regulatory T cells

Nature, in press

Ajithkumar Vasanthakumar, David Chisanga, Jonas Blume, Renee Gloury, Kara Britt, Darren Henstridge, Santiago Valle Torres, **Sebastian Liene**, Nicholas Collins, Enyuan Cao, Tom Sidwell, Chaoran Li, Raul German Spallanzani, Yifan Zhan, Yang Liao, Paul A. Beavis, Thomas Gebhardt, Natalie Trevaski, Stephen Nutt, Jeffrey D. Zajac, Rachel A. Davey, Mark A. Febbraio, Diane Mathis, Wei Shi and Axel Kallies

Chapter 1 - Literature Review

1.1 Introduction

Every day we are exposed to pathogens, such as viruses, bacteria or parasites, which are able to harm our body. To protect us from these threats the human body has developed different defense mechanisms: the first level of defense are anatomic barriers such as the skin, oral mucosa, respiratory epithelium and the intestine, which prevent pathogens from entering internal areas of the body. Yet, pathogens can overcome these first layers, for example through wounds. Here, the second layer of defense acts, the innate immune system and in particular the complement system (Chaplin 2005). The complement system enhances immune responses by triggering three main-immune processes: phagocytosis, inflammation and membrane attack (rupturing bacteria cell walls). Furthermore, the complement system can “mark” pathogens for the cellular immune system (Murphy and Weaver 2017). The immune system can be divided into two subsystems: the innate immunity and adaptive immunity. The innate immune system reacts to pathogens in an immediate response, which is non-specific. Further, the innate response can activate the adaptive immune system (Iwasaki and Medzhitov 2015). This branch of the immune system is able to adapt its response and improves its ability to detect a specific pathogen.

All immune cells belong to the group of white blood cells (leukocytes), which together with red blood cells (erythrocytes) and platelets (thrombocytes) are generated in a process called hematopoiesis (Maton 1993). This term describes the development of specialized cells from hematopoietic stem cells and occurs in the bone marrow. Stem cells will give rise to common progenitor cells of the two lineages myeloid and lymphoid. Common myeloid progenitors (CMPs) will further develop, amongst others, to monocytes, granulocytes, macrophages or dendritic cells. Common lymphoid progenitor (CLPs) cells will give rise to T and B cells as well as innate lymphocytes (ILC) (Birbrair and Frenette 2016).

With its acquirement to distinguish between its own tissue (“self”) and foreign cells (“non-self”) the cellular immune system is our defense against pathogens. However, in the process of immune cell maturation, not all potentially

harmful T or B cells that recognize self-antigens are removed from the immune cell repertoire. Depending on many factors this might lead to autoimmune diseases such as diabetes mellitus type1, inflammatory bowel disease, systemic lupus or multiple sclerosis.

Furthermore, immune responses can cause strong inflammation while eradicating pathogens. In an uncontrolled inflammatory environment these processes can lead to severe destruction of tissue and therefore have to be guided and stopped. Therefore, the immune system contains different regulatory cell populations, such as, alternatively activated or type 2 macrophages (M2), regulatory dendritic cells (DC) and myeloid derived suppressor cells (MDSC) as part of the myeloid arm of the immune system but also regulatory T cells (T_{reg}) and group 2 innate lymphoid cells (ILC2) from the lymphoid immune system. These cell types have the function of maintaining or returning the host system to homeostasis (Almeida and Belz 2016; Anon 2013; Hu and Pasare 2013; Lisovska and Shanazarov 2019; Senovilla et al. 2013).

1.2 The innate immune system

The innate immune system is comprised of different cells, which are able to sense components of pathogens that are evolutionary conserved, such as bacterial cell wall structures or specific surface molecules on viruses. Those specific patterns are consolidated in the term pathogen associated molecular pattern (PAMPs) (Maverakis et al. 2015; Silhavy, Kahne, and Walker 2010). The recognition of these patterns is facilitated by specific receptors called pattern recognition receptors (PRRs)(Kumar, Kawai, and Akira 2011). Similarly, innate immune cells are also able to detect molecules which derive from the host itself and have been released due to cell damage or cell stress and can be associated with infection. These so-called damage-associated molecular patterns (DAMPs) are together with PAMPs sensed by a specialized group of PRRs the toll-like receptors (TLRs)(Kumar et al. 2011). Tissue resident immune cells are strategically located as first layer of defense at barrier sites, which have a high

potential to be breached by microorganisms. Most of these immune cells are phagocytes, which are able to eliminate the intruders by “eating” them. To this class of immune cells belong monocytes, granulocytes macrophages and dendritic cells (Murphy and Weaver 2017).

1.2.1 Monocytes

Monocytes are involved in homeostasis and tissue repair, microbial resistance and tumor surveillance (Das et al. 2015; Mitchell, Roediger, and Weninger 2014; Olingy, Dinh, and Hedrick 2019). They derive from hematopoietic stem cells in the bone marrow. After bone marrow egress monocytes will typically circulate for one to three days inside the blood stream and eventually enter peripheral tissues, where they can further differentiate to macrophages (chapter 1.2.3) or dendritic cells (chapter 1.2.4) (Jakubzick et al. 2013). However, the spleen holds about half of the monocyte population as a reserve (Swirski et al. 2009). Blood monocytes consist of at least two (in human three) functionally distinct subset: CX3CR1^{med} CCR2^{+} CD62L^{+} Ly6C^{hi} (**Ly6C^{+}**) and CX3CR1^{hi}CCR2^{-}CD62L^{-}Ly6C^{lo} (**Ly6C^{-}**) cells (Mildner, Marinkovic, and Jung 2016). The infiltration of Ly6C^{+} monocytes during infectious and sterile inflammation is a hallmark of host defense against injuries. Depending on context and spatial manner of monocyte tissue entry, their plasticity allows them to contribute to either pro- or anti-inflammatory responses (Mildner, Yona, and Jung 2013). In a model of chronic obstructive lesion in the kidney (a model of unilateral ureteral obstruction (UUO)), Braga *et al*. demonstrated that Ly6C^{+} monocytes infiltrate the kidney in early disease and led to higher monocyte recruitment in later stages, this steered the release of pro-inflammatory cytokines and fibrosis formation (Braga et al. 2018). In the low-grade inflammatory environment of adipose tissue a recent study showed that at later stage the accumulation of blood derived monocytes is involved in maintaining tissue inflammation (Zheng et al. 2016).

1.2.2 Granulocytes

Granulocytes or polymorphonuclear leukocytes (PMNs) are leukocytes that are predominantly characterized by their appearance with granules in their cytoplasm. The group of PMNs is comprised of four different cell types: basophils, eosinophils, mast cells and neutrophils. The most abundant cell type of PMNs are neutrophils, which make up around 60% of leukocytes in the blood (Ermert et al. 2013). These phagocytes are one of the very first responders to tissue disturbances and play important roles in bacterial infections, several cancers and environmental exposure (Jacobs et al. 2010; De Larco, Wuertz, and Furcht 2004; Waugh and Wilson 2008). Neutrophils have a unique mechanism to kill extracellular pathogens using so-called neutrophil extracellular traps (NETs) (Brinkmann et al. 2004). NETs consists of DNA stretches and different anti-microbial proteins such as elastase, myeloperoxidase or cathepsin G, which trap and kill microbes independent of phagocytotic processes (Clark et al. 2007; Thomas et al. 2014).

In a model of kidney ischemia-reperfusion it has been shown that the accumulation of neutrophils foremost happened in the interstitium (Awad et al. 2009). Further reports showed that the degranulation of neutrophils accompanied with release of proteases, the release of cytokines, and the generation of reactive oxygen species can aggravate injury and damage the kidney (Jang and Rabb 2009; Li and Okusa 2006; Okusa et al. 2000). Neutrophils had also been connected to participate in high-fat diet induced obesity. The authors report that the neutrophil protease elastase plays a crucial role in insulin resistance and that the deletion of this enzyme leads to reduced tissue inflammation (Talukdar et al. 2012).

1.2.3 Macrophages

Macrophages belong to the phagocytes and are located in essentially all tissues. They roam the extra cellular matrix and have the potential to phagocytose anything that does not belong to the body ("non-self") (Correa-Costa et al. 2011). Every organ harbors tissue resident macrophages. They are non-migratory, proliferate *in situ* and provide essential factors for physiological functions and protect the tissue from inflammation (Gosselin et al. 2014; Muller et al. 2014; Okabe and Medzhitov 2014; Uderhardt et al. 2019). Recent research has shown that macrophages can develop from two different sources: during embryonic development progenitor cells enter the tissue from the yolk sac and provide one source for macrophage replenishment (Yona et al. 2013). Alternatively, they can develop from monocytes which have entered the tissue from the blood circulation (Yona et al. 2013). Depending on their tissue location macrophages have been given different names; for instance, liver resident macrophages are coined Kupffer cells or neural tissue macrophages are called microglial cells. Next to the function of general phagocytosis one of the many roles of macrophages is antigen presentation. After the phagocytosis of a microbe, digested parts of microbial proteins (peptides) can be present on the major histocompatibility complex II (MHC-II) molecules of macrophages and lead to the activation of helper T cells. This property places them as bridge between innate and adaptive immune system (Mantegazza et al. 2013).

Macrophages have been historically classified into two subgroups, classically activated "M1" macrophages or alternatively activated "M2" macrophages (Mills et al. 2000; Mosser and Edwards 2008). "M1"-polarized macrophages have pro-inflammatory characteristics and are bactericidal and have phagocytic functions. M2-polarized macrophages are thought to lead to decreased inflammation, wound healing and tissue repair (Figure 1-1) (Alisi et al. 2017; Mills 2012). *In vivo* M1 macrophages are induced by mediators like LPS, interferon (IFN)-y, tumor necrosis factor (TNF), PAMPs or DAMPs (Bianchi 2007; Zhang and Mosser 2008). This pro-inflammatory M1 phenotype leads to the production, of Interleukin (IL)-6 and TNF among other cytokines (Hesketh et al.

2017). Amici *et al*. described *in vivo* a robust expression of CD38 on pro-inflammatory human macrophages. This was additionally supported by *in vitro* studies on BM derived murine macrophages, making CD38 a useful marker to identify M1 macrophages (Amici et al. 2018; Jablonski et al. 2015). M2 macrophages can be generated *in vitro* from bone marrow derived macrophages stimulated with IL-4 or IL-13 (Weisser et al. 2013). *In vivo*, $CD206^{+}$ (mannose receptor) macrophages had been found to participate in peritoneal fibrosis. By analyzing their gene expression pattern it was found that these $CD206^{+}$ cells express similar mRNA level of "M2" mediators compared to *in vitro* differentiated M2 macrophages (Bellón et al. 2011).

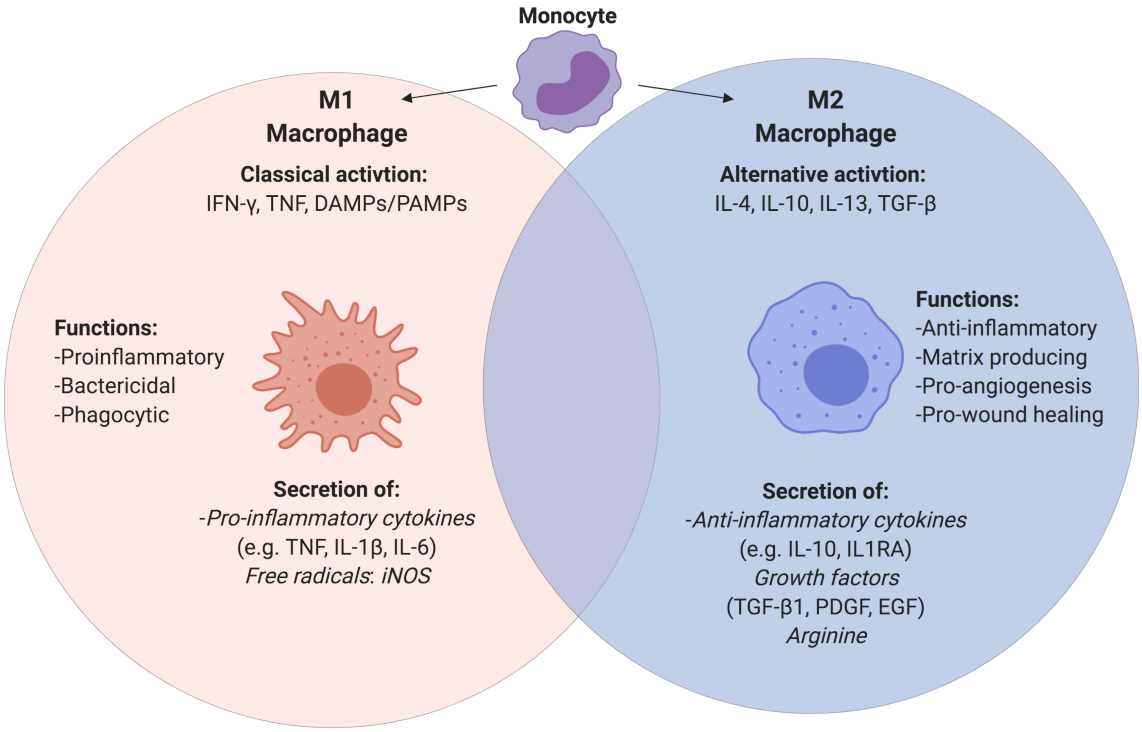


Figure 1-1 Polarization of macrophages.

Depending on the type of stimuli, monocytes can either differentiate into classically activated "M1" macrophages or alternatively activated "M2" macrophages. M1 macrophages are pro-inflammatory; M2 macrophages anti-inflammatory. Adapted from: Alisi et at. 2017

TheM1/M2 classification of macrophages is under debate among experts, but M1 or M2-classification delivers a simple construct. It is largely supported by *in vitro* data, with IFNγ + LPS stimulation to generate the M1 phenotype, or IL-4

+ IL-13 to generate M2 macrophages. However, *in vivo* macrophages are not only exposed to a limited and specific concentration of a few cytokines but encounter a high diversity of mediators and concentrations at any given time point (Gordon 2003; Ley 2017). Those manifold conditions give rise to macrophage phenotypes in-between the stages of M1 and M2, which results in highly complex macrophage compartments (Mosser and Edwards 2008). Due to the unresolved questions about these different phenotypes and for simplicity reasons, I will use in this thesis the M1 and M2 paradigm.

1.2.4 Dendritic cells

Dendritic cells (DCs) belong, next to macrophages and B cells, to the group of antigen-presenting cells (APC). DCs are located in tissues such as skin, lung, stomach or the intestine which are in contact with the external environment (Castell-Rodríguez et al. 2017). They scan their surroundings for pathogens via PRRs, similar to macrophages. Encountered microbes are phagocytized or endocytosed and digested, subsequently antigen can be presented. In contrast to neutrophils and macrophages, DCs show reduced degradation of ingested particles, which leads to more conserved presentation of antigens (Sallusto and Lanzavecchia 2002). Phagocytosis leads to DC activation, maturation and migration to the lymph node (Martín-Fontecha, Lanzavecchia, and Sallusto 2009). During maturation, DCs upregulate co-receptors like CD80, CD86 and CD40, which facilitate the activation of T cells (Chatzigeorgiou et al. 2009; Chen et al. 1994; van der Merwe et al. 1997). Inside the lymph node, mature DCs present processed proteins (antigens) of phagocytized pathogens on MHC molecules to helper or killer T cells. These T cells, that can recognize the specific antigens via their T cell receptors (TCRs), are activated and start different defense mechanisms of the adaptive immune system (Sallusto and Lanzavecchia 2002). DCs can present antigens to T helper cells (chapter 1.3.1) and cytotoxic T cells (chapter 1.3.3). Furthermore, they are able to perform cross-presentation, where exogenous antigen is presented via MHC class I to cytotoxic T cells to active them (den Haan, Arens, and van Zelm 2014).

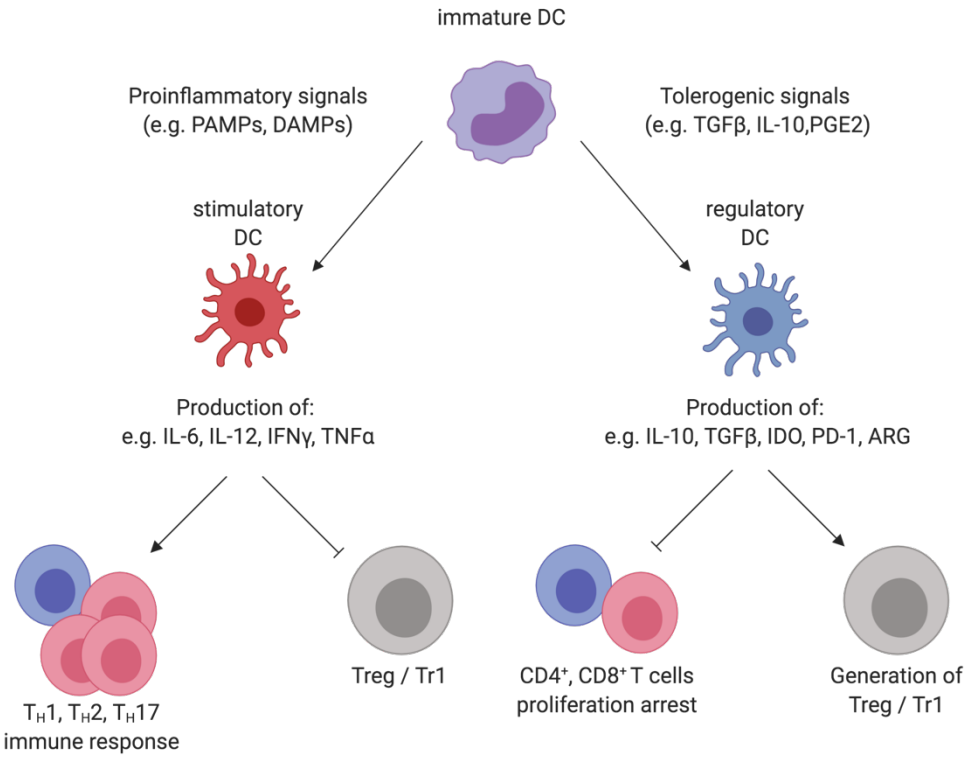


Figure 1-2 Dendritic cell properties are dependent on activation signals.

Immature DCs can develop into a stimulatory or a regulatory phenotype, depending on their activation and microenvironment. Stimulatory DCs will enforce a specific T helper cell immune response, whereas regulatory DCs will lead to accumulation of regulatory T cells. Figure adapted from Schmidt et al., 2012.

Dendritic cells are classified into different subtypes based on their phenotype and function: conventional (or classical) (cDCs) and plasmacytoid DCs (pDCs). Both arise from bone marrow progenitors, which egress as immature DCs and migrate via blood to peripheral lymphoid organs. However, unlike cDCs, pDCs leave the bone marrow not as immature DCs, but fully developed (Shortman and Naik 2007). Furthermore, they act less as APCs but release large amounts of type-1 interferon during viral encounter (Villadangos and Young 2008). Furthermore, it has been shown that cDCs can be separated into two functionally distinct subtypes: cDC1 and cDC2. “Lymphoid-resident DCs” reside in secondary lymphoid tissues (i.e. spleen and lymph nodes) whereas DCs from non-lymphoid tissues migrate to lymph nodes and are called “migratory DCs”. Splenic DCs express either CD8 (cDC1) or CD4 (cDC2). In non-lymphoid

organs DCs do not express those markers but upregulate the integrin CD103 (cDC1) or CD11b (cDC2). Different studies revealed that cDC1s are specialized in antigen cross-presentation and showing a polarization to T_H1 responses. cDC2s are specialized in antigen presentation to $CD4^+$ T cells, with a more T_H2/T_H17 dominant polarization (Merad et al. 2013; Schlitzer and Ginhoux 2014; Schlitzer, McGovern, and Ginhoux 2015).

Immature DCs that encounter inflammatory signals or TLR stimulation will get activated and "mature", which leads to a "stimulatory DC" type (Figure 1-2). On the other hand, DCs can get activated in a "regulatory" DC manner via tolerogenic signals. $CD103^+$ DCs in the gut were described to induce T cell tolerance due to their ability to induce $FoxP3^+$ T_{regs} (del Rio et al. 2010; Scott, Aumeunier, and Mowat 2011). Additionally, it has been reported that in a murine asthma model, fully mature DCs stimulated T_{regs} in an IL10-depended manner (Akbari, DeKruyff, and Umetsu 2001). Others showed that several soluble factors (IL-10, PGE_2 or TGFβ) play a role in the induction of tolerogenic DC_{regs} (Popov and Schultze 2008).

1.2.5 Myeloid derived suppressor cells

Myeloid derived suppressor cells (MDSCs) are a heterogenous group of cells with the ability to suppress the function of different cell types. MDSCs are present in low amounts in blood of healthy individuals, but increased in cancer, inflammation or infection (Almand et al. 2001; Ost et al. 2016). It has been shown that tumors are highly infiltrated by MDSCs, which is connected with therapy resistance (Gabrilovich, Ostrand-Rosenberg, and Bronte 2012). Due to their myeloid origin, murine MDSCs express high levels of CD11b and Ly6C and/or Ly6G. Depending on their Ly6C and Ly6G expression, they can further be subdivided in: monocytic MDSCs, which express high levels of Ly6C but low or no Ly6G; or granulocytic MDSCs with high levels of Ly6G and intermediate levels of Ly6C (Gabrilovich 2017). The existence of MDSCs has been under debate since they share the same markers with monocytes and granulocytes and have

a similar phenotype; however, they are functionally distinct (Bronte et al. 1999). Their potential to suppress $CD8^+$ T cell proliferation has first been characterized in tumors and they are now considered an important factor in tumor immunology. However, they also have emerged to play a crucial role in other immune regulation aspects in inflammation, chronic infections, and autoimmune diseases (Gabrilovich 2017). Their expansion has been reported in a mouse model for multiple sclerosis (EAE), where they enter the central nervous system during inflammation (Zhu et al. 2007). MDSCs have been also shown to play pro-inflammatory role in inflammatory bowel disease (Haile et al. 2008). MDSCs that are positive for the IFNγRβ receptor have been described during chronic kidney inflammation to exhibit exclusively suppressive activity (Höchst et al. n.d.).

1.3 The adaptive immune system

The pathogen recognition of the innate immune system is limited to the detection of molecular pattern by their PRRs. Evolution of the acquired immune system has overcome these limitations. Leukocytes belonging to this part of the immune system are called lymphocytes.

T and B cells develop in the bone marrow from common lymphoid progenitor cells (CLPs). However, as B cells develop in the bone marrow, CLPs also migrate via blood to the thymus, were they further develop into T(hymus) cells (Murphy and Weaver 2017). Even though deriving from the same progenitor, T and B cells mount very different immune responses: humoral immunity is a B cell mediated response, whereas T cells are responsible for the cell-mediated immune response (Miller 1975).

Humoral immunity involves macromolecules such as secreted antibodies, antimicrobial peptides or proteins of the complement system that can be found in the humors (body liquids) (Slifka et al. 1998). Antibodies produced by B cells are an important part of this response. After B cells mature in the bone marrow they enter the blood stream to migrate to secondary lymphoid organs (SLOs), such as

the spleen or lymph nodes (Harwood and Batista 2010). Inside the SLO B cells are activated when they encounter a cognate antigen, which is engulfed and digested (Yuseff et al. 2013). Parts of the antigen are now displayed on MHC-II molecules, which can be recognized by the matching mature T helper cell, normally a follicular T helper cell (T_{FH}) (Blum, Wearsch, and Cresswell 2013). Upon T cell stimulation, cytokines and co-stimulatory molecules such as CD40L are produced by the T_{FH} which help the B cell to further develop (McHeyzer-Williams et al. 2009). Activated B cells begin to proliferate and enter the germinal center. Here, they differentiate into short-lived plasmablasts, which provide immediate protection or early memory B cells (Nutt et al. 2015). The generation of long-lived affinity matured plasma cells and memory B cells occurs inside the germinal center. Here B cells proliferate, differentiate and mature (somatic hypermutation) into cells with high-affinity to the initial presented antigen (Shlomchik and Weisel 2012). Plasma cells produce large amounts of antibodies, which are transported via the blood stream or the lymphatic system to the site of infection. The antibodies will bind their targets and facilitate cell dependent immune responses, e.g. phagocytosis by macrophages or compliment activation (Thau and Mahajan 2019).

In contrast, cell-mediated immunity does not involve antibodies but the activation of cytotoxic and helper T-lymphocytes. The T cell family is comprised of different specialized cell types, depending on their surface protein expression, e.g. $CD4^+$ T helper cells (see 1.3.1) and $CD8^+$ cytotoxic T cells (see1.3.3). Naïve T cells get activated when their TCR encounters its cognate antigen presented on an APC (primary immune response). Activated T cells will expand and differentiate into short lived effector (which will eliminate the antigen) and long-lived memory T cells (details see chapter 1.3.1, 1.3.3) (Murphy and Weaver 2017).

Memory cells, such as memory B cells, memory CD4 and CD8 T cells, form the so-called immunological memory, one of the hallmarks of the adaptive immune system (Ratajczak et al. 2018). During a secondary antigen encounter (re-stimulation) memory cells will produce a stronger and faster immune response compared to the primary immune response (Sprent and Tough 1994).

After cognate antigen re-stimulation T cells will immediately expand to immense numbers of effector cells (Farber, Yudanin, and Restifo 2014; Kurosaki, Kometani, and Ise 2015).

This memory characteristic of lymphocytes is taken advantage of during vaccination, where patients are exposed to controlled antigens of specific pathogens. The immune system gets primed via these “artificial” antigens and is able to react, on re-encounter with the “real” pathogen, before it can damage the body (Plotkin, Orenstein, and Offit 2008).

1.3.1 CD4 T cells

$CD4^+$ T cells are a subgroup of T cells, which mount immune responses by boosting other immune cell responses via cytokine release. Hence, they are also called helper T cells. After thymic development, CD4 T cells will home similar to B cells into SLOs. Here, they encounter antigens presented by APCs on MHC-II molecules. The naïve T cell, which recognizes its specific antigen via the TCR will be activated by a two-signal activation process. Activation releases IL-2, a potent growth factor for T cells, which works in an autocrine and paracrine manner and subsequently leads to proliferation and clonal expansion of the T cell (Bonilla and Oettgen 2010). Depending on the prevailing microenvironment, T helper cells can differentiate into T_H1 (IFN-γ driven), T_H2 (IL-4 driven), T_H9 (TGFβ, IL-4), T_H17 (IL-17) or T_{FH} (IL-21) cells, which differ in their characteristics (Bonilla and Oettgen 2010; Wan and Flavell 2009). T_H1 cells have been connected to intracellular pathogens and autoimmunity. Their release of IFN-γ, lymphotoxin-α (LTa) and IL-2 are important for macrophage activation and their increase in phagocytosis. IL-2 is also critical for CD8 T cell stimulation (Mosmann and Coffman 1989; Paul and Seder 1994). Production of IL-4, IL-5, IL-10, IL-13 and amphiregulin are linked to T_H2 responses and host defense against extracellular parasites and helminths and play important roles in asthma and other allergic diseases (Wan and Flavell 2009). T_H17 cells produce pro-inflammatory cytokines and play an important role against extracellular bacteria and fungi (Korn et al. 2007). Like mentioned above, naïve T cells will develop in distinct non-

overlapping T helper cells lineages which are defined by the expression of specific master transcription factors, such as T-bet (T_H1 cells), GATA3 (T_H2 cells) RORγT (T_H17 cells) or Bcl6 (T_{FH} cells). However, the concept of one transcription factor one cell fate is under debate since some transcription factors are simultaneously expressed in the same lineage (Fang and Zhu 2017).

1.3.2 Regulatory T cells

Regulatory T cells (T_{regs}) belong to the adaptive immune system, more precisely they derive from $CD4^+$ T cells. They are modulators of the immune system, preserve immunotolerance and suppress autoimmune diseases (Li and Rudensky 2016). These immunosuppressive effects are established via different factors. Cytokines play an important role in the communication between cells. T_{regs} use inhibitory cytokines such as IL-10, IL-35 or transforming growth factor beta (TGF-β) to suppress the induction and proliferation of effector T cells (T_{eff}) (Vignali, Collison, and Workman 2008). In addition, it has been shown that T_{regs} are able to induce the production of IL-10 in other cell types (Figure 1-3, a) (Kearley et al. 2005). Another mechanism of suppression is the induction of apoptosis in T_{eff}, which is mediated via Granzyme B and can be a perforin dependent or independent process (Figure 1-3, b) (Gondek et al. 2005; Zhao et al. 2006). T_{regs} also influence T cell proliferation/maturation indirectly, by modulating DCs, which are necessary for the activation of T_{effs}. Different mechanisms have been reported to be important in this process: Lymphocyte activation gene 3 (LAG3), which is a CD4 homologue binding to MHC-II and induces an inhibitory signaling pathway that modulates DC maturation; Cytotoxic T-lymphocyte antigen 4 (CTLA4), that controls access to co-stimulatory signals by competition between T_{reg} (CTLA4) and T_{eff} (CD28) ; and the induction of indoleamine 2,3-dioxygenase (IDO) in DCs, which leads to the production of pro-apoptotic metabolites (Miyara and Sakaguchi 2007; Walker and Sansom 2015). Another mechanism is metabolic disruption, depicted in d) and e) of Figure 1-3. Finally, due to the high expression of the IL-2 receptor (CD25) T_{regs} reduces the

availability of IL-2 in the microenvironment and therefore “starve” T_{eff} cells which need IL-2 to survive (Figure 1-3, e)(Miyara and Sakaguchi 2007). Additionally, the expression of the coenzymes CD39 and CD73 leads to the generation of pericellular adenosine, which has the capability to suppress T_{eff} function through the adenosine receptor 2A (Figure 1-3,d) (Deaglio et al. 2007).

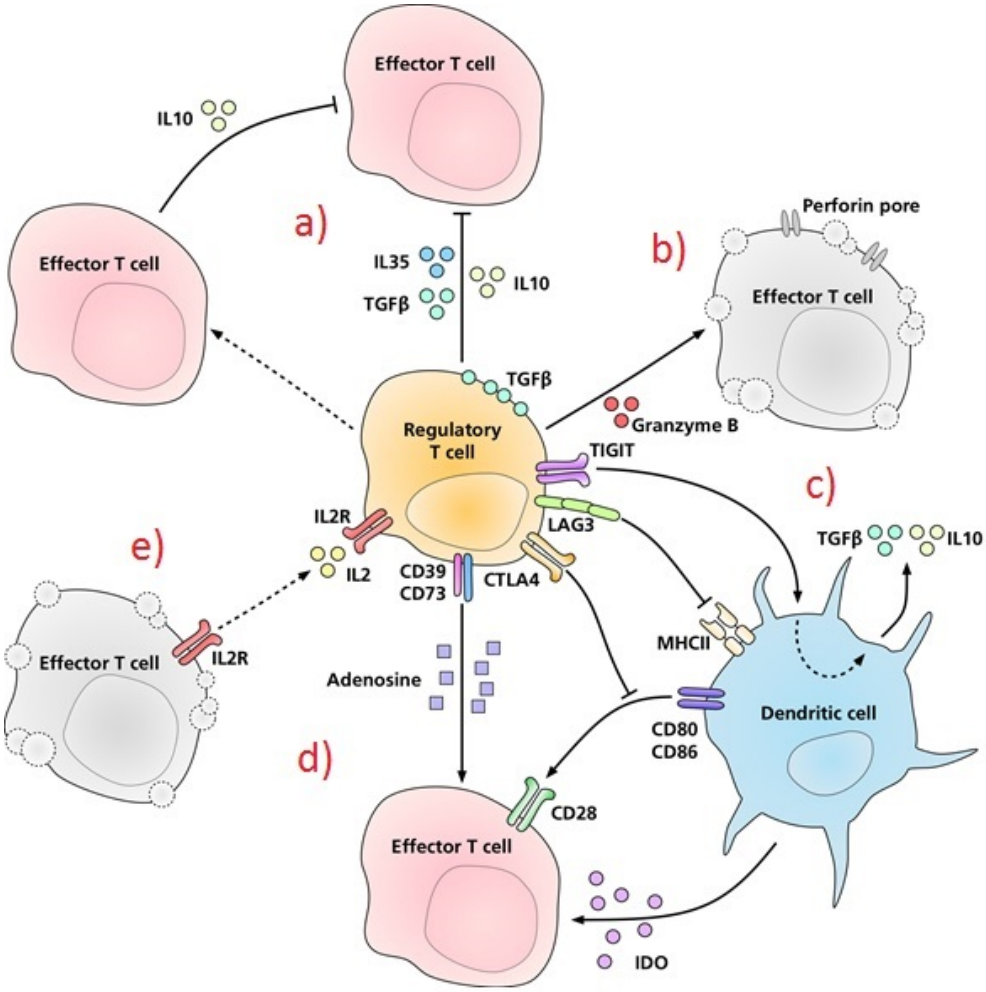


Figure 1-3 Basic regulatory T cell action mechanisms.

(a) Effector T cell inhibition via cytokines, including IL-10, IL-35 or TGFβ. (b). Induction of apoptosis in effector T cells via granzyme B. (c-d) Different mechanism of interaction between Tregs, DCs and Teff influence T cell activation. (e) Competition of local IL-2 leads to reduced proliferative capacity of Teffs. Figure adapted from (Gwilz 2017)

T_{regs} also suppress the innate immune response, for instance the accumulation of macrophages and therefore reduce kidney injury in an

adriamycin-induced chronic kidney disease model (Mahajan et al. 2006). It also has been shown that T_{regs} inhibit the production of ROS and cytokines by neutrophils (Lewkowicz et al. 2006).

However, T_{regs} are comprised of a distinct pool of heterogenous subsets. Generally, they are classified in central T_{regs} and effector T_{regs}. Central T_{regs} are located in lymphoid tissues and show a naïve phenotype with expression of CD62L and CCR7 and are dependent on IL-2 (Smigiel et al. 2014). On the contrary, effector T_{regs} are primarily found in non-lymphoid tissues and show an activated phenotype with downregulated expression of CD62L and CCR7 (Smigiel et al. 2014). Further, effector T_{regs} show expression of IL-10, CTLA-4 and TIGIT, which are functionally important (Cretney, Kallies, and Nutt 2013). Mature effector T_{regs} produce high level of IL-10 and are positive for the transcription factor B-lymphocyte-induced maturation protein 1 (Blimp-1)(Cretney et al. 2011). Even though CD4 T cell precursor can give rise to T_{regs} in the periphery, most T_{regs} are thymus derived (Teh, Vasanthakumar, and Kallies 2015). Similar to conventional T cells, also T_{regs} undergo a differentiation which is depend on microenvironmental stimuli (Burzyn, Benoist, and Mathis 2013; Cretney et al. 2013). Several studies showed that T_{regs} show a distinct phenotype in tissues, such as gut, skin, lung or the visceral adipose tissue (Panduro, Benoist, and Mathis 2016). Especially, VAT tissue T_{regs} have been shown to expand locally in an IL-33 dependent manner (Panduro et al. 2016; Vasanthakumar et al. 2015).

1.3.3 CD8 T cells

T cells positive for the TCR co-receptor CD8 (also cytotoxic T lymphocyte, CTL) are important immune cells that are able to kill cancer cells, virus infected cells, or cells that signal “damaged” on their MHC class I (MHC-I) molecule. All nucleated cells show MHC-I expression (Kulski et al. 2002). On this molecules cells display peptides processed from their cytosol and any “non-self” peptide recognized from CTLs will trigger an immune response against this antigen/cell (Heath and Carbone 2001). However, like CD4 T cells, CTLs require activation and therefore the interaction with an APC, which are mainly DCs. Furthermore,

for an effective CTL activation and the formation of CD8 memory CD4 T cell help is necessary (Hoyer et al. 2014; Janssen et al. 2003; Shedlock and Shen 2003). The activated CTL will undergo clonal expansion in an IL-2 dependent manner. After clonal expansion mature CTLs will roam the body and search for antigen-specific cells. CTLs are using perforin, granulysin or granzymes which are cytotoxins, to trigger programmed cell death in target cells (Murphy and Weaver 2017).

1.3.4 ILC cells

ILCs are comprised of three types: ILC1, ILC2 and ILC3. They develop from common lymphoid progenitors and belong to the lymphoid cells, but do not express B or T cell receptor, neither do they express markers of myeloid or DC origin (Spits and Cupedo 2012). Their classification is based on transcription factor expression (ILC1s express Tbet, ILC2s express GATA3 and ILC3s express RORγt) and their production of cytokines (Spits et al. 2013) similar to the T helper cell populations (Figure 1-4, Eberl et al., 2015). NK cells ($Tbet^+$ and $Eomes^+$) have been grouped due to their developmental similarity to the type 1 ILC group. Due to their cytotoxic effects they mirror $CD8^+$ T cells (Figure 1-4, Eberl et al., 2015).

In addition, ILCs have varying physiological functions, which are complementary to T helper cells. They play important roles in regulating tissue homeostasis and inflammation (Almeida and Belz 2016). ILC2s are particularly important for tissue homeostasis. They can be activated by Th2 polarizing cytokines and produce Th2 cytokines, which allows them to respond very early e.g. during helminth infections. ILC2s are involved in tissue repair processes via amphiregulin secretion (Palm, Rosenstein, and Medzhitov 2012). They are highly abundant in gut, skin, liver, adipose and lung (Kim et al. 2013; Neill et al. 2010; Ochel, Tiegs, and Neumann 2019; Roediger et al. 2013) and are the main population of ILCs in the kidney in homeostasis (Riedel et al., 2017). But they play also important roles during adipose regulation and kidney disease (Brestoff et al. 2015; Riedel et al. 2017).

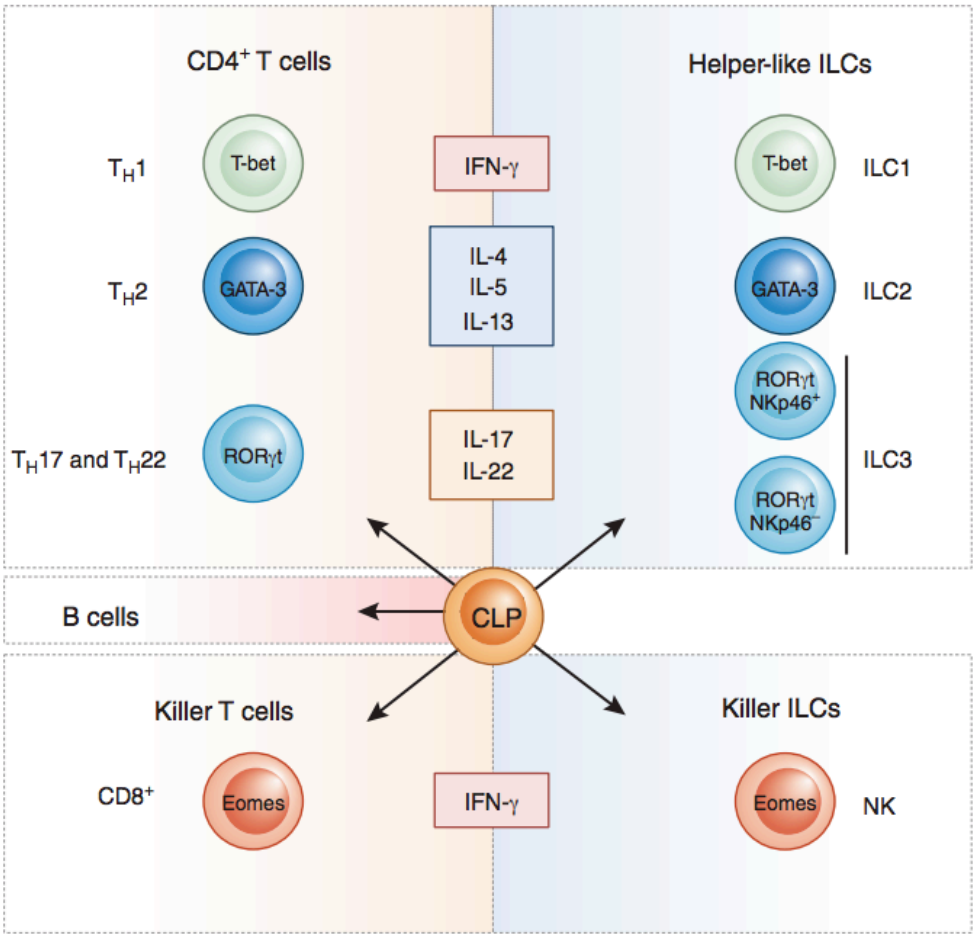


Figure 1-4 The diversity of ILCs mirrors the diversity of T cells.

Labels in cells indicate the main transcription factors that drive the function and differentiation of helper T cells, helper-like ILCs (ILC1, ILC2 and ILC3 cells), cytolytic T cells and cytolytic ILCs (NK cells). Adapted from: Eberl *et al.*, 2015

1.4 The adipose tissue

The adipose tissue (AT) is largely comprised of adipocytes and stromal vascular fraction (SVF) (Birbrair et al. 2013). Its main function is the storage of energy in form of lipids and insulation. The SVF consists mostly of vascular endothelial cells, fibroblasts and immune cells. However, recent studies showed that the AT produces huge amounts of hormones including estrogen, leptin and other

cytokines (Kershaw and Flier 2004). Leptin is foremost produced in the AT and is connected to weight gain or loss and directs fat storage (Campfield et al. 1995; Zhang et al. 1994). Leptin is regulated due to feeding and fasting but also by insulin or inflammatory cytokines, as well as glucocorticoids (Lee and Fried 2009; Pan, Guo, and Su 2014). Leptin acts directly on appetite inhibition, thermogenesis or fatty acid oxidation and furthermore studies suggest a role in maintaining glucose homeostasis (Cheung, Clifton, and Steiner 1997; Flier 2004; Morton and Schwartz 2011). Adiponectin is another important hormone, secreted from AT, which regulates glucose and fatty acid levels (Díez and Iglesias 2003). This hormone is released into the bloodstream and shows very high concentrations, with higher levels in females compared to males and lower concentrations in diabetic people opposing non-diabetic people (Coppola et al. 2009). Similar to leptin, adiponectin acts directly on the brain (Nedvídková et al. 2005). Different studies showed that it acts a suppressor in metabolic changes in diabetes, obesity and metabolic syndrome but also in non-alcoholic fatty liver disease (Díez and Iglesias 2003; Renaldi et al. 2009; Ukkola and Santaniemi 2002).

Two major kinds of AT are known: white adipose tissue (WAT) for energy storage, and brown adipose tissue (BAT) for heat generation. The WAT is divided in different subgroups depending on location. The main WAT depots are inside the abdominal cavity, referred to as visceral fat (VAT), which surrounds the internal organs. The VAT can at least be further divided into perirenal (surrounds the kidneys), perigonadal (surrounds the testes in males and ovaries in females) and mesenteric depot (surrounds the intestines). Beneath the skin is another depot the subcutaneous (SC) fat. Studies showed that depending on their location, different fat depots acquire different metabolic functions and therefore differ in their contribution to obesity (Macotela et al. 2009; Wueest, Schoenle, and Konrad 2012).

1.4.1 Obesity

Around the globe, obesity has become a pandemic and a challenging health problem (Roberto et al. 2015). Several studies show that a high body-mass index (BMI, body weight divided by square of height) is a high risk factor for cardiovascular disease, diabetes, chronic kidney disease and several cancers (Kovesdy et al. 2017; Lauby-Secretan et al. 2016; Singh et al. 2013; The Emerging Risk Factors Collaboration 2011). A recent study showed that in 2015 the prevalence of obesity had been 5% among children and approximately 12% in the adult population worldwide Interestingly, the study found more women to be obese, with a peak between the age 60 to 64. Analyzing those data, the study did not find sex differences before the age of 20 years. The report highlights that high BMI contributed to 7% of all deaths in 2015. Under those causes of death cardiovascular disease was the most common, followed by diabetes. Chronic kidney disease, cancers and musculoskeletal disorders contributed with each less than 10% (Collaborators 2017). Taken together, this report showed that obesity is a major health concern and that sexual dimorphism plays an important role.

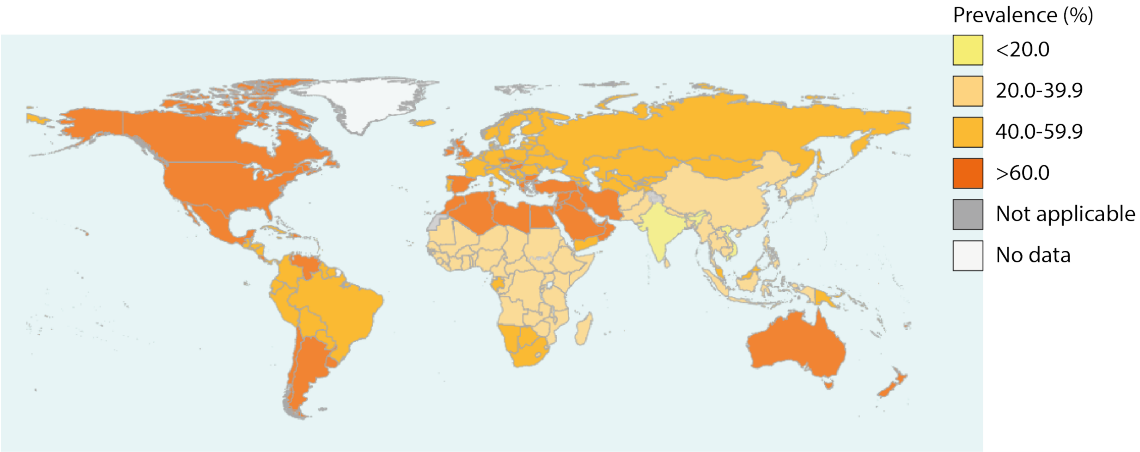


Figure 1-5 Prevalence of overweight (BMI >25kg/m2) adults (age 18+).

Source: World Health Organization 2017: Global Health Observatory

1.4.2 Obesity and the immune system

In addition to adipokines, the AT also secretes cytokines (1.4.1). Cytokines can be directly secreted by adipocytes but also immune cells contribute to the cytokine microenvironment (Lu et al. 2019). The chronic low-grade inflammation in the adipose tissue is associated with pro-inflammatory cytokines, which further steer the development of type 2 diabetes during obesity. Studies connect foremost the innate immune system to cytokines in the AT, such as tumor necrosis factor alpha (TNFα), IL-6 or IL-1 (Hotamisligil 2006; Pickup and Crook 1998), where macrophages (classically activated “M1” and alternatively activated “M2” macrophages) play an important role. During obesity macrophages increase in total numbers accompanied with an phenotype switch from M1 to M2 state (Olefsky and Glass 2010). M1 macrophages are the main source for TNFα in the AT, even though a minor amount is produced from adipocytes (Weisberg et al. 2003) and was the first cytokine connected with inflammation and obesity and the development of metabolic syndrome (Hotamisligil, Shargill, and Spiegelman 1993). TNFα exerts many effects, amongst others the inhibition of lipogenesis and stimulation of lipolysis, a decrease in PPARγ and GLUT4 transporter (Ranjit et al. 2011; Ruan and Lodish 2003). In obesity, TNFα levels are increased and it has been shown to directly inhibit insulin signaling (Moller 2000; Stephens, Lee, and Pilch 1997). Studies in rodents showed that blocking of TNFα reduced insulin resistance (Hadad et al. 2013; Hotamisligil et al. 1993).

Interleukin 6, another cytokine produced by adipocytes, has been linked to various diseases such as atherosclerosis, Alzheimer Disease, systemic lupus erythematosus several cancer and most important to diabetes (Dubiński and Zdrojewicz 2007; Gadó et al. 2000; Kristiansen and Mandrup-Poulsen 2005; Smith et al. 2001; Swardfager et al. 2010; Tackey, Lipsky, and Illei 2004). An early study showed also a connection between IL-6 and obesity by influencing C-reactive protein concentrations (Bastard et al. 1999). Nevertheless, macrophages contribute to significant amounts of produced IL-6 and TNFα inside the AT and are thought to be a critical component in the development of VAT inflammation (Oh et al. 2012; Olefsky and Glass 2010; Weisberg et al. 2003). The adipose

tissue contains a variety of immune cells, such as γδ T cells, T_{regs}, CD4 and CD8 T cells, ILC2s, NK and NKT cells and several studies linked those cells to VAT homeostasis and inflammation (Dalmas et al. 2014; Feuerer, Herrero, et al. 2009; Kohlgruber et al. 2018; Lu et al. 2019; Lynch et al. 2012; Nishimura et al. 2009; Rana et al. 2019; Vasanthakumar et al. 2015). Adipose tissue inflammation and obesity are highly under investigation and the list of involved cell populations in this complex and diverse network of immune cells driving this disease is constantly expanded.

1.4.3 CD38

The surface molecule CD38 is not only expressed on macrophages (see 1.2.3) but had been first described as an activation marker on T lymphocytes and later to be expressed on a variety of immune cell types (Bhan et al. 1980; Malavasi et al. 1992). However, CD38 expression is not limited to immune cells but rather expressed in solid tissues, such as kidney, gut, brain and pancreas (Malavasi et al. 2008). CD38 plays a role in different biological processes and posses an ADP-ribosyl cyclase and cyclic ADP ribose (cADPR) hydrolase activity, in which NAD^+ is cleaved to generate ADPR or cADPR, which is a Ca^{2+} second messenger (Chini 2009; Graeff et al. 2009). Several reports showed a high expression of CD38 in the adipose of obese people (Chini 2009; Graeff et al. 2009). Further a study using CD38 knockout mice showed protection during HFD-induced obesity through increased NAD^+ levels and Sirt1 activity (Barbosa et al. 2007). Due to the important role of NAD^+ level in several age-related conditions, the upregulation of CD38 during aging had been linked to be a driver of NAD^+ decline (Camacho-Pereira et al. 2016; Gomes et al. 2013; Schultz and Sinclair 2016).

1.5 The kidney

The main function of the kidney is the purification of blood from metabolite end-products, the regulation of water excretion, as well as electrolyte and acid-base balance. The kidney filters the so-called primary urine, which gets concentrated in the renal tubules to secondary urine. The basic structure of the kidney are nephrons, which consist of glomeruli, the proximal tubule, the loop of Henle, and the distal tubule which is connected to the ureter (Figure 1-6). The kidney is divided into renal cortex and renal medulla. The loop of Henle is located in the medullary part; the rest of the nephron is located inside the cortex. The filter function of the kidney is maintained via glomeruli, which consist of capillary convolutes and the Bowman´s capsule. The tubule system withdraws the water and soluble nutrients from the primary urine after its filtration at the glomerulus (Eckert et al., Animal Physiology, 5th Edition). The tubular epithelium is metabolically very active and therefore very vulnerable to hypoxic and toxic injuries. Those injuries can lead within a few days to limitation of kidney function and even to complete organ failure (Kurts et al. 2013).

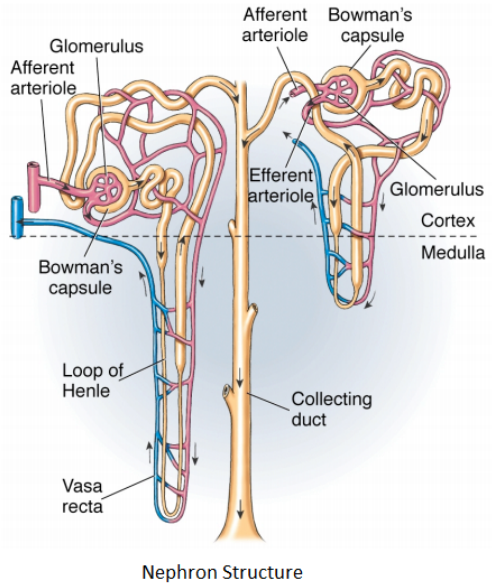


Figure 1-6 Structure of a nephron in the kidney.

(Eckert et al., Animal Physiology, 5th Edition)

1.5.1 Kidney injuries and fibrosis

Different inflammatory diseases can lead to kidney fibrosis. During type 1 diabetes changes in metabolism and blood circulation may occur and thereby damage the glomeruli in the kidney. In the progression of the disease this will evolve to albuminuria (Lim 2014). Another inflammatory disease which can damage the glomeruli is the autoimmune disease lupus nephritis. Here, autoantibodies against a range of antigens form immune-complexes, that lead to the destruction of the kidney (Jaryal and Vikrant 2017; Mortensen, Fenton, and Rekvig 2008).

Kidney damage manifests in many ways. Important part of the research focuses on crystal-induced inflammations in the kidney. Here, a variety of genetic and acquired metabolic disturbances, as well as drugs or toxins induce tissue damage. The kidney is especially prone to crystal formation because of its ability to concentrate the urine. Under non-inflammatory, steady-state conditions only a small number of immune cells, such as dendritic cells, macrophages and a few lymphocytes, reside in the kidney (Kurts et al. 2013). However, upon inflammation immune cells of the innate and adaptive immune system, such as monocytes, neutrophils and lymphocytes strongly infiltrate and accumulate in the kidney. Dependent on the kind of minerals, their concentration, and the signaling pathways triggered, crystals induce different kidney injuries. These damages are divided into three groups: renovascular damage (type 1), tubular damage (type 2), and urolithiasis (type 3) (Mulay and Anders 2017). Dehydration and super saturation will lead to crystal accumulation, which can lead to acute kidney injury (AKI). Other cases might lead to chronic kidney disease (CKD), which is normally induced by persistent mild super saturation. Kidney damage causes inflammation, which finally, if not resolved, leads to kidney fibrosis. Here, parenchymal tissue is replaced with extracellular matrix during wound healing processes, which leads to the loss of kidney function (Kurts et al. 2013; Meng, Nikolic-Paterson, and Lan 2014)

The acute or chronic exposure of patients to drugs, toxins or dietary elements, which have a tendency to crystallize in renal tubular cells may cause

AKI or CKD. Oxalate found in nuts, spinach or black tea, can lead to secondary hyperoxaluria and acute oxalate nephropathy (Mulay and Anders 2017). Similar effects have been described for adenine, which upon oral administration leads to the metabolite 2,8-DHA, which is insoluble and results over time in destructive changes in renal tubules via the formation of crystals in the tubulointerstitial compartment (Koeda et al. 1988) (Correa-Costa et al. 2011).

Using a mouse model of crystal nephropathy induced by adenine-enriched chow, we and others recently showed that NLRP3-dependent inflammasome activation causes severe kidney damage (Ludwig-Portugall et al. 2016). Here, oral administration of adenine leads to the insoluble metabolite 2,8-DHA, which results over time in the formation of crystals in the renal tubular cells and thereby causes tubular damage leading to sterile inflammation.

1.5.2 Innate immune cells in the kidney

Under inflammatory conditions several immune cell populations of the innate and adaptive immune system infiltrate and accumulate in the kidney. Kidney DCs were first described in the cortical interstitium (Hart and Fabre 1981). It has been shown that DCs are important in different disease models due to their ability to produce CXCL2, which drives the recruitment of other immune cells (Tittel et al. 2011). However, kidney resident DCs also harbor suppressive functions. In a crescentic glomerulonephritis (cGN) model, *Evers et al.* showed that $CD103^+$ DCs (cDC1) promoted T_{reg} accumulation in the kidney, which counteracted the “pro-inflammatory” cells (Evers et al. 2016). DC depletion at an early or late timepoint in a model of nephrotoxic nephritis showed that during kidney insult the DC phenotype might switch. Early depletion exacerbated disease, late depletion attenuated the disease, adding another level of complexity to kidney inflammation and the role of kidney DCs (Hochheiser et al. 2011).

Macrophages are seeded during embryonic development into the entire body and undergo *in situ* self-renewal throughout adulthood (Hoeffel et al. 2015; Schulz et al. 2012; Sheng, Ruedl, and Karjalainen 2015). However, the kidney harbors different types of fetal macrophages derived from yolk-sac, erythro-

myeloid progenitors (EMPs) or hematopoietic stem cells (HSCs) (Munro and Hughes 2017). However, during kidney inflammation monocytes recruited from the BM infiltrate the kidney and give rise to different macrophage types (Lin et al. 2009). In a model of ischemia/reperfusion injury (IRI) the depletion of macrophages with CD11b-DTR mice did not show kidney protection, whereas treatment with clodronate reduced kidney damage. The author concluded that clodronate did not deplete $CD206^{+}$ macrophages, which exert a protective function (Ferenbach et al. 2012).

1.6 Interleukin 33 and ST2

Interleukin (IL)-33 is a cytokine and member of the IL-1 superfamily, which is closely related to IL-18 (Schmitz et al. 2005). It mediates signal transduction through the *suppression of tumorgenicity 2* (ST-2) receptor (IL1RL1), which, upon IL-33 binding, forms a complex with IL-1R accessory protein (IL-1RAcP), a member of the IL-1R complex (Chackerian et al. 2007). This signaling leads to the recruitment of MyD88 and furthermore to the activation of different transcription factors such as NF-κB (Schmitz et al. 2005). In addition to its extracellular cytokine properties, IL-33 has been described to act intracellularly as a nuclear factor (Mirchandani, Salmond, and Liew 2012). IL-33 comprises a nuclear domain at its amino-terminus, which has been shown to associate with chromatin (Carriere et al. 2007). It has been shown that IL-33 is expressed by numerous cells, like DCs, macrophages, mast cells, fibroblasts and epithelial cells (Mirchandani et al. 2012).

The ST-2 receptor is a member of the Toll-like family and the binding of IL-33 leads to signaling through NF-κB (Schmitz et al. 2005). It can be found in two different isoforms: a soluble form (sST2) and a membrane-bound form (ST2 or ST2L). ST2 expression if found on a variety of immune cells, such as ILC2s, T_{regs}, macrophages, DCs, monocytes and mast cells (Liew, Girard, and Turnquist 2016; Mirchandani et al. 2012). The soluble form sST2 binds IL-33 and it is thought to

play an important role in IL-33 regulation due to its decoy receptor properties (Pascual-Figal and Januzzi 2015).

Recent reports indicate a dual function for IL-33. Originally, IL-33 has been described as an alarmin, first found to enhance expression of T helper (Th) 2 cytokines and to activate numerous immune cells (Schmitz et al. 2005). IL-33 expression has been found during tissue homeostasis in mainly non-hematopoietic cells, foremost epithelial and endothelial cells (Moussion, Ortega, and Girard 2008; Pichery et al. 2012). However, IL-33 levels are increased in inflammatory conditions (Kearley et al. 2015). IL-33 is a cytokine, which stimulates both myeloid and lymphoid cells and leads to their proliferation, survival and migration (Lott, Sumpter, and Turnquist 2015). Lymphoid cells with ST2 receptor expression includes populations such as T_H2 cells, T_{reg} cells and ILC2s (Liew et al. 2016). Due to the broad expression of IL-33 and its receptor ST2 on the surface of several cell types it has the potency to work in a pro-inflammatory or anti-inflammatory way. It has been associated with airway inflammation (Barlow et al. 2013), cardiac hypertrophy and fibrosis (Sanada et al. 2007). Additionally, IL-33 and ST2 were upregulated in a cis-platin model of acute-kidney injury, where the inhibition of IL-33 led to kidney damage alleviation (Akcay et al. 2011). However, IL-33 also takes part in the resolution of inflammatory processes and tissue damage repair, which is mediated trough T_{regs}, for example in graft-versus-host-disease (GVHD) (Matta et al. 2016). In a model of virus-induced inflammatory lung disease, it was shown that IL-33 drives tissue repair by induction of amphiregulin (Arpaia et al. 2015). One of the most extensive studied roles of IL-33 is the adipose tissue. Several studies reported the influence of IL-33 on ILC2s, tissue T_{regs} and M2 macrophages (Brestoff et al. 2015; Miller et al. 2010; Rana et al. 2019; Vasanthakumar et al. 2015).

1.7 The BATF and IRF transcription factor family

Transcription factors (TF) are crucial to most processes inside a cell. They regulate gene expression and therefore take part in the regulation of cell division, cell growth and cell death (Latchman 1997). Even though there is also a regulatory processes after transcription, the regulation of gene expression is mainly steered due to the fact which gene is actually transcribed (Latchman 1997). Those regulators are grouped together by their DNA-binding domain (DBD), a protein domain containing a structural motif that recognizes specific DNA areas. The TF binding to specific DNA areas regulates the activation or repression of specific genes (Stegmaier, Kel, and Wingender 2004).

The interferon-regulatory factor (IRF) transcription factor includes nine members and they all share the same helix-turn-helix motif at their N-terminal DBD (Yanai, Negishi, and Taniguchi 2012). Several studies showed that IRFs have defined roles in immune cell development and their function (Lohoff and Mak 2005; Taniguchi et al. 2001).

An early study showed that IRF4 deficiency leads to profound reduced antibody level and deficient mice were not able to generate cytotoxic responses. The authors found that IRF4 is essential to generate mature B and T cells (Mittrucker et al. 1997). Results of a later study showed that both IRF4 and IRF8 are necessary in B cell development by steering the pre-B-to-B transition by functioning as a molecular switch (Honda and Taniguchi 2006). T helper cells lacking IRF4 are unable to differentiate into T_H2 effector cells and show impaired IL-4 production, indicating a role for IRF4 in T_H2 responses (Rengarajan et al. 2002). Moreover, Zheng et al. demonstrated that IRF4 is additionally necessary for T_{reg} cells by direct interaction between IRF4 and Foxp3 (Zheng et al. 2009). A functional cooperation of the transcription factor BATF and IRF4 has been found which showed the importance of both factors to promote *Il10* transcription in T cells (Li et al. 2012). Additionally, IRF4 is responsible for B lymphocyte-induced maturation protein-1 (Blimp-1) expression and therefore plays a critical role in the establishment of functional aspects in regulatory T cells (Cretney et al. 2011).

Furthermore, it has been demonstrated that IRFs not only play a role in lymphocytes but also in myeloid cells. Especially, IRF4 had been shown to be required for a specific DC subset ($CD103^+$ $CD11b^+$) in the small intestine. IRF4 deficient mice showed reduced cell numbers of this DC subset and were unable to mount a strong T_H17 response (Persson et al. 2013).

Family members of the Basic leucine zipper transcription factor ATF-like (BATF) transcription factor are another important TF family. BATF is a subgroup of the basic leucine zipper (bZIP) TFs and is comprised of three members: *Batf*, *Batf2* and *Batf3* (Bateman et al. 2004). They all share an α-helical bZIP domain, containing a basic DNA-binding region. Early identified member of this group are the important regulators FOS and JUN, which constitute the heterodimeric TF "activator protein 1" (AP-1) 5. FOS and JUN contain additionally to their bZIP domain a distinct transactivation domain, which is a typical characteristic of bZIP family member (Murphy, Tussiwand, and Murphy 2013). However, functional distinct BATF and BATF3 lack other additional domains, whereas BATF2 has an additional domain with unknown function. It has been shown that on basis of their bZIP domain the BATF family forms heterodimer with JUN proteins; which includes TFs such as FOS, FOSB or ATF3 (Chinenov and Kerppola 2001; Vinson et al. 2002) These heterodimer can interact either with IRF4 or IRF8, which leads to different regulatory actions in immune cells (Chang, Zuo, and Stormo 2018).

BATF family member have intrinsic functions in DCs, B Cells and T cells (Murphy et al. 2013). It has been shown that this specific regulation is due to interactions with members of the IRF family (Murphy et al. 2013). In homeostatic conditions, high levels of BATF3 have been reported in cDCs, whereas BATF and BATF2 get upregulated during inflammatory conditions (Tussiwand et al. 2012). Vasanthakumar et al. demonstrated the dependency of VAT tissue T_{regs} on BATF and IRF4, highlighting their role in suppressive immune cell populations (Vasanthakumar et al. 2015).

It is necessary to distinguish between two different roles of TFs. All cells develop from stem cells and TFs play a crucial role as lineage-determining factors. However, the same TF can be involved in the differentiation of several cells not restricted to a single lineage. Once a cell has reached its terminal differentiation state, some TFs are dispensable, whereas some are continuously required to keep the cell identity with its specific characteristics. The latter TFs are coined “terminal selectors” (Hobert 2008; Holmberg and Perlmann 2012). In this regard, IRF8 has been described to be important in several immune cells, and inside the myeloid lineage especially during DC development. However, only in cDC1s it has the function of a terminal selector, this is reflected by high expression of IRF8, which is an additional characteristic of terminal selectors (Sichien *et al.*, 2016).

IRF1 is another member of the IRF family and has also been identified to play crucial roles in myeloid cell development. Myeloid cells of IRF1 knockout mice display immature macrophage and DC cells types, indicating developmental implications for IRF1 (Hoffman et al. 1991; Testa et al. 2004). Furthermore, polarization of macrophages to either M1 or M2 phenotype where connected to several member of the IRF family such as IRF1, IRF3, IRF4 and IRF8 (reviewd in Günthner et al., 2013).

1.8 Thesis aims

Interleukin-33 has been described as a multifaceted cytokine, which regulates many aspects from tissue homeostasis to inflammation. Adipose tissue T_{regs} (VAT-T_{regs}) have been shown to maintain tissue homeostasis by preventing obesity-associated inflammation, preserving insulin sensitivity and glucose tolerance (Cipolletta 2014; Feuerer, Herrero, et al. 2009). IL-33 signaling is necessary for the development and maintenance of VAT-T_{regs} and preserves their transcriptional profile. Furthermore, VAT-T_{regs} depend on the transcription factors IRF4 and BATF (Vasanthakumar et al. 2015). Moreover, unpublished data from the Kallies lab show a pronounced sexual dimorphism in the VAT, which is imprinted in VAT T_{reg} phenotype and transcriptional landscape and (Vasanthakumar *et al. under review*).

VAT inflammation is associated with the accumulation of pro-inflammatory (M1) macrophages, whereas anti-inflammatory (M2) macrophages can maintain tissue health (Castoldi et al. 2015). The transcription factors IRF1 and IRF4 had been associated with M1 and M2 macrophage polarization (Satoh et al. 2010; XIE et al. 2016). The pro-inflammatory VAT M1 macrophages had been identified using the cell surface marker F4/80 and CD11c. However, due to progress in the field it became apparent that F4/80 is not only a macrophage marker but can also be expressed by DCs (Guilliams et al. 2014). Here, we want to apply a new proposed gating strategy from an unsupervised high-dimensional analysis to differentiate macrophages and cDCs (Guilliams et al. 2016) to explore the diversity of myeloid cells inside the adipose and renal tissue. Furthermore, we will investigate if sex specific differences of macrophages or cDCs influence adipose tissue inflammation.

To address if the IL-33 VAT phenotype can be found in other non-lymphoid organs, we use a model of adenine-enriched diet induced crystal-nephropathy to induced chronic kidney disease. A protective role for exogenous IL-33 due to T_{reg} and ILC2 expansion had been described in other models (Riedel et al. 2017; Stremska et al. 2017). Here, we want to investigate how IL-33 influences the

progression of chronic kidney disease and if cells of the lymphoid but also from the myeloid lineage are affected. Furthermore, we aimed to understand if the transcription factors IRF1, IRF4 and BATF regulate suppressive immune cells during chronic kidney disease.

The objective of this dissertation is to address the following aims:

1. Examine sex-specific and diet-induced differences in the myeloid cell compartment of the visceral adipose tissue (Chapter 3)
2. Investigate the influence of ST2 and IL-33 in chronic kidney disease (Chapter 4)
3. Examine the role of transcription factors of the IRF family and their partners in chronic kidney disease (Chapter 5)

Chapter 2 - Materials and Methods

2.1 Materials

2.1.1 Mouse strains

Table 1 - Overview of mouse strains used in this study

Strain	Full strain name	Reference
B6	C57BL/6	
ST2ko	Il1rl1^{tm1Anjm}	(Townsend et al. 2000)
IL-33ko	Il33^{tm1Anjm}	(Wong et al. 2012)
IRF1ko	Irf1^{tm1Mak}	(Matsuyama et al. 1993)
IRF4ko	Irf4^{tm1Mak}	(Mittrucker et al. 1997)
IRF8ko	Irf8$^{tm1.2Hm}$	(Holtschke et al. 1996)
Irf4^{GFP}	Irf4^{GFP}	(Man et al. 2017)
Irf8^{RFP}	Irf8^{RFP}	Unpublished (Kallies lab)
IRF4$^{fl/fl}$	Irf4^{tm1Rdf}/J	(Klein et al. 2006)
CD4^{cre}	CD4^{cre}	(Sawada et al. 1994)
BATFko	B6.129S-Batf$^{tm1.1Kmm}$/J	(Schraml et al. 2009)

2.1.2 Equipment

Table 2 Overview of equipment used in this study

Equipment	Name and supplier
Centrifuge	Centrifuge 5810R, Eppendorf, Hamburg Germany NJ, USA
ELISA Plate reader Flow cytometer	Safire 2 (Tecan, Männedorf, Switzerland)
Flow cytometer	FACS LSR Fortessa^{TM} flow cytometry, BD Bioscience, USA
Freezer -20°C	Bosch, Munich
Freezer -80°C	Heraeus, Hanau

Ice machine	Icematic Scotsman®; Frimont Bettolinc, Pogliano, Italy
Incubator	HeraCell, HeraCell 240 (Heraeus, Hanau)
Microscope	Olympus CKX31 (Olympus, Düsseldorf) Smartflow
Mouse cages IVC	Tecniplast, Hohenpeißenberg, Germany
Neubauer chamber	Brand, Wertheim
pH- meter	Mettler Toledo
Pipettes (2 µl, 10 µl, 20 µl, 100 µl, 200 µl, 1000 µl)	Eppendorf, Hamburg
Vortex	Velp Scientifica, Italy
Water bath	Memmert, Germany
Workbench, sterile	HERAsafe; Heraeus, Braunschweig, Germany

2.1.3 Chemicals and reagents Reagent

Table 3 Overview of reagents used in this study

Reagent	**Supplier**
β-mercaptoethanol	Sigma-Aldrich, St. Louis, MO, USA, NJ, USA
Bovine Serum Albumin (BSA)	Roth, Karlsruhe, Germany
Brefeldin A (Golgi Plug) (1000x)	eBioscience, San Diego, CA, USA
Collagenase stock	200mg/ml Roche Diagnostic, Germany
Counting Beads (APC)	CountBrightTM Absolute Counting Beads, for flow cytometry (Life Technologies, Carlsbad, CA, USA)
Ethylene diamine tetracetic acid (EDTA)	Merck, Darmstadt, Germany
Ethanol, 70% (v/v)	Roth, Karlsruhe

Fetal Calf Serum (FCS)	Life Technologies, Carlsbad, CA, USA
Ionomycin	
rmIL-33 stock	10 µg/ml, Peprotech
Liquid nitrogen	Linde, Wiesbaden, Germany
Sodium hydrogen carbonate (NaHCO3)	Gibco, Thermo Fisher Scientific, Waltham, USA
Paraformaldehyde (PFA)	AppliChem, Darmstadt
Penicillin-Streptomycin-Glutamine (100x)	Thermo Fisher Scientific, Waltham, MA, US
Phosphate-buffered saline (PBS)	Thermo Fisher Scientific, Waltham, MA, US BD Bioscience
RPMI 1640 medium	Invitrogen, Darmstadt, Germany
Perm/Wash Buffer	Sigma-Aldrich, St. Louis, MO, USA
Tween80	Roth, Karlsruhe
RNase away spray	VWR, Darmstadt, Germany

2.1.4 Buffers, media and solutions

Table 4 Overview of buffers used in this study

Buffers	**Description**
Cell culture medium	RPMI 1640 (500 ml) FCS 10% (50 ml) 1% antibiotics (penicillin + streptomycin) (5 ml), 1% β-mercaptoethanol (5 ml)
ELISA Blocking and Dilution medium	500 ml PBS, 5 g Bovine Serum Albumin
Kidney digestion Medium	RPMI 1640, FCS 2%, 1% antibiotics (penicillin + streptomycin) Collagenase I and DNAse (both 1:100 from stock solution)
Red cells buffer (RCB)	15.58 g NH4Cl, 0.074 g EDTA (2Mm, 2.0 g NaHCO3 (10 mM, 2 L des. Water
Fixation/Permeabilization buffer (Foxp3 Kit)	1 Fix/Perm Concentrate: 3 Fix/Perm diluent

Permeabilization Buffer (Foxp3 Kit)	1	10x Stock solution Perm buffer : 9 ddH2o
Perm/Wash Buffer	1	Perm/Wash saponin buffer : 9 ddH2o

2.1.5 Antibodies

Table 5 Overview of antibodies used in this study

Antigen	**Fluorochrome conjugate**	**Clone**	**Stock conc.**	**Company**
CD11b	BV711	M1/70	0.2 mg/ml	Biolegend
CD11b	BV605	M1/70	0.2 mg/ml	Biolegend
CD11b	biotinylated	M1/70	0.5 mg/ml	Biolegend
CD11c	BV711	N418	0.2 mg/ml	Biolegend
CD11c	BV605	N418	0.2 mg/ml	Biolegend
CD11c	biotinylated	N418	0.5 mg/ml	Biolegend
CD19	PerCP/Cy5.5	1D3/CD19	0.2 mg/ml	Biolegend
CD19	biotinylated	MB19-1	0.5 mg/ml	Biolegend
CD206 (MMR)	PE/Cy7	C068C2	0.2 mg/ml	Biolegend
CD31	PE	390	0.2 mg/ml	Biolegend
CD38	PE/Dazzle 594	90	0.2 mg/ml	Biolegend
CD3ε	biotinylated	145-2C11	0.5 mg/ml	Biolegend
CD45	BUV395	Clone 104	0.2 mg/ml	BD Biosciences
CD5	biotinylated	53-7.3	0.5 mg/ml	Biolegend
CD64	APC	X54-5/7.1	0.2 mg/ml	Biolegend
CD73	PE/Cy7	eBioTY/11.8	0.2 mg/ml	eBioscience
CD8	BV650	53-6.7	0.2 mg/ml	Biolegend
eBioscience™ Fixable Viability Dye	eFluor 780		1:1000	ThermoFischer Scientific
eBioscience™ Fixable Viability Dye	eFluor 506		1:1000	ThermoFischer Scientific

F4/80	BV421	BM8	0.2 mg/ml	Biolegend
F4/80	BV605	BM8	0.2 mg/ml	Biolegend
Foxp3	FITC	FJK-16s	0.5 mg/ml	invitrogen
Foxp3	PE	FJK-16s	0.2 mg/ml	invitrogen
GATA3	BV421	L50-823	50 test	BD Bioscience
IFNyR2	PE	MOB-47	0.2 mg/ml	Biolegend
KLRG1	APC	2F1/KLRG1	0.2 mg/ml	Biolegend
KLRG1	BV711	2F1/KLRG1	0.1 mg/ml	Biolegend
Ly6C	BV421	HK1.4	0.2 mg/ml	Biolegend
Ly6G	PE/Cy7	1A8	0.2 mg/ml	Biolegend
Ly6G	biotinylated	RB6-8C5	0.5 mg/ml	Biolegend
MHC II	APC-Cy7	M5/114.15.2	0.2 mg/ml	Biolegend
PD-L1	BV605	10F39G2	0.2 mg/ml	Biolegend
Sca-1	BV711	D7	25 µg/ml	Biolegend
ST2 (IL-33R)	PerCPeFluor710	RMST2-33	0.2 mg/ml	Biolegend
ST2 (IL-33R)	PE	RMST2-33	0.2 mg/ml	Biolegend
Streptavidin-Conjugated fluorocrome	PerCp5.5		0.2 mg/ml	Biolegend
Streptavidin-Conjugated fluorocrome	APC		0.2 mg/ml	Biolegend
TCRβ	PerCP/Cy5.5	H57-597	0.2 mg/ml	Biolegend
TCRβ	biotinylated	H57-597	0.5 mg/ml	Biolegend
Ter-119	PerCP/Cy5.5	TER-119	0.2 mg/ml	Biolegend
Thy1.2 (CD90.2)	APC-Cy7	30-H12	0.2 mg/ml	Biolegend
XCR1	BV650	ZET	0.2 mg/ml	Biolegend
γδTCR	biotinylated	UC7-13D5	0.5 mg/ml	Biolegend

2.1.6 Kits

Table 6 Overview of kits used in this study

Kit	Catalogue no.	Supplier
Mouse IL-33 DuoSet ELISA	DY3626-05	R&D system
Sandwich KIM ELISA	DY1817	R&D system
Sandwich TIMP-1 ELISA	DY980	R&D system
RNA isolation (NucleoSpin RNA mini)	740955	Macherey Nagel (MN)
High capacity reverse transcription kit	10400745	eBioscience
Fixation/Permeabilization Kit (Foxp3 Kit)	00-5523-00	Applied Biosystems

2.1.7 Software

Table 7 Overview of software used in this study

Name	Company
FACS Diva V6.1.1	Becton, Dickinson and Company, Lakes, NJ, USA
Magellan™	Tecan
FlowJo 10.0.7	Tree star, Ashland, OR, USA
Prism 8 for Mac	GraphPad, La Jolla, CA, USA
Microsoft Office 2011	Microsoft, Redmond, VA, USA
Adobe Illustrator CS6	Adobe Systems, San Jose, CA, USA
Mendeley	Mendeley Ltd.
Biorender	Biorender, www.biorender.com

2.2 Methods

2.2.1 Experimental treatment of mice

2.2.1.1 Induction of crystal nephropathy

WT or transgenic male or female mice between 12- and 20-weeks of age were fed with either 0.15% or 0.2% adenine-enriched diet for 42 or 21 days respectively.

Mice fitness, social behavior and signs of pain were monitored during the course of the experiment and scored in agreement with the Animal Ethic Application. In case a weigh loss of more than 15% was recorded, the animal was excluded from the experiment. Further, mice that showed a negative change in social behavior, in weight loss or in general fitness were sacrificed in accordance with the German regulation of animal experimentation.

All animal experiments were approved by the local government authorities (LANUV, NRW, Germany and The University of Melbourne Animal Ethics Committee) and were performed in adherence to the national and institutional guidelines for the care and use of laboratory animals.

2.2.1.2 High-fat diet

Male C57BL/6 mice were fed with a high-fat diet where 59% of the total energy is derived from lipids (59 kcal% fat, Specialty Feeds, SF03-002) for a minimum time of 20 weeks.

2.2.1.3 IL-33 treatment

For the *in vivo* stimulation of $ST2^+$ cell populations, we administrated intraperitoneally (i.p) per mouse 0.4 µg of recombinant mouse IL-33 diluted in 100 µl PBS.

These C57BL/6 male mice were between 12 and 20 weeks old and they received the treatment on day 3, day 5, day 7, day 9 and day 11 of the adenine-enriched diet. Mice were analyzed on day 14.

2.2.1.4 Generation of bone marrow chimera

Mixed bone marrow chimera were generated from lethally irradiated (2 × 550 R) wild-type Ly5.1^{+} mice and reconstituted with a mixture of congenically marked mutant and/or control bone marrow as indicated. After irradiation, 6-8 weeks time was given to allow mice to reconstitute. Mice were maintained and used in accordance with the guidelines of the Animal Ethics Committee (PDI/ University of Melbourne)

2.2.1.5 Glucose-tolerance tests

After mice had been fasted for 8h, 1.75 g per kilogram of body weight (g/kg), glucose was injected intraperitoneally, and blood samples were collected from the tail tip. The glucose concentration in the blood was measured using a handheld glucometer (Accu-Chek Performa, Roche).

2.2.1.6 Insulin measurement

Bio-Plex Pro mouse diabetes immunoassay kit (Bio Rad, 171F7001M) was used to measure serum concentrations of insulin according to manufacturer's protocol.

2.2.2 Isolation of cells from organs

2.2.2.1 Isolation from cells from the kidney and spleen

Prior to kidney removal, mice were perfused with 10 ml of ice-cold PBS. The kidney capsule was removed, and kidney and spleen were placed in 24 wells plate containing 2 ml digestion medium in each well (RPMI 1640 supplemented with 2%FCS, 1% Pen-Strep, 1% β-mercaptoethanol, DNase (0.1 mg/ml) and Collagenase (1 mg/ml)). In order to perform tissue digestion, the kidney was mechanically dissociated and incubated 20 min at 37°C. To optimize digestion of big kidney fractions, vigorously pipetting was performed after the first incubation time and orangs were incubated for an additional time of 20 min at 37°C. The cell suspension was filtered through a 100 μm nylon mesh and the single cell

suspension was treated with red blood cell lysis buffer, in order to eliminate erythrocytes from the cell suspension. A final washing step with FACS buffer or RPMI 1640 (2% FCS) medium was performed to remove lysate cells.
After digestion the spleen was resuspended in culture medium and the lysis of red blood cells was performed as described above. Subsequently, the spleen was resuspended in FACS buffer for further flow cytometric analysis.
To remove stromal cells, such as podocytes or tubular cells from the kidney cells suspension, samples were resuspended in 4 ml of medium and placed on ice for 4 minutes. After sedimentation of unwanted cells, the supernatant was collected and transferred to a new tube upon filtration through a 50 μm nylon mesh. After centrifugation (300 g / 5 min / 4°C), the pellet was re-suspended in cell culture medium or FACS buffer for further analysis.

2.2.2.2 Preparation of lymphocytes from the fat

The gonadal fat pad was collected from 30 to 35-week-old male or female mice and placed in PBS. After measurement of the organ weights, fat pad was finely minced in a petri dish and subsequently transferred to a 50 ml falcon and resuspended in digestion media (RPMI1640 containing 0.025% collagenase Type IV (Gibco) (2 ml collagenase per gram fat)). Next, the fat was incubated for 45 min at 37 °C placed on a vertical shaker to keep cells in suspension, and afterwards the suspension was diluted with PBS + 2% FCS in a 1:10 ratio.
A final centrifugation step was performed at 800 g for 15 min at 4 °C; here, the upper fraction was discarded, and the pellet was further purified in order to isolate leukocytes.

2.2.3 Biochemistry assay

2.2.3.1 ELISA

To evaluate possible physiological damage in the kidney, the Kidney Injury Marker (KIM-1) and tissue inhibitor of metalloproteinases 1 (TIMP1) were analyzed respectively in the urine and in the serum of treated and untreated mice.

Analysis of secreted IL-33 was performed in cell culture supernatant upon overnight incubation by sandwich ELISA (Enzyme Linked Immunosorbent Assay). Here, a 96 well plate (GBO Microplate) was coated with 50 µl capture antibody at a concentration recommended by the Kit manufacture (R&D system). After a 4°C overnight incubation, three washing steps were performed with wash buffer (200 µl/well PBS/ 0,1% Tween80) and a blocking step was performed by adding 200 µl/well PBS / 1% BSA. Plates were then incubated for one hour at room temperature.

Standard curve was prepared as recommended by the manufacture's protocol. In brief: to reach a working concentration of 4000 pg/ml, the stock solution (60 ng/ml) was diluted 1:15 in PBS / 1% BSA. The working concentration was further diluted by performing a 1:2 serial dilution on plate, in order to obtain a seven-point standard curve. Samples were diluted as followed: urine samples were diluted 1:5 in PBS/BSA (1%) and serum samples were diluted 1:10 in PBS/BSA (1%), supernatant for IL-33 measurements was plated undiluted. All samples and the standard curve were placed in doublets. After incubation for two hours at room temperature, three washing steps were performed with 200 µl wash buffer per well. Next, plates were incubated with detection antibody diluted in 1% PBS/BSA at a final concentration recommended by the manufacturer. Subsequently, the detection antibody was added to each well and incubated for two hours at room temperature, followed by three washing steps as described before. After three additional washing steps, plates were incubated for 45 minutes at room temperature with Streptavidin- Peroxidase (SA-POX) as described in the manufacture's protocol. After incubation, three more washing steps were performed.

The final phase of this assay is the development, where a solution prepared with 5 mg OPD and 5 µl H_2O_2 in 5 ml OPD-Buffer was added in a volume of 50 µl in each well.

The reaction was stopped by adding 50 µl H_2SO_4 (1M) in each well and the absorbance at 492 nm was measured at the ELISA plate reader (Tecan). Results were calculated as interpolated values of the linear regression of the standard

curve and the final concentration was calculated considering the specific dilution of the samples initially performed.

2.2.3.2 RNA isolation and qPCR

The left kidney of each mouse was longitudinally cut to obtain two identical kidney sections. One of these sections was additionally fractioned in smaller units, collected in a 1.5 ml tube and shock-freeze in liquid nitrogen. For long-term storage, samples were kept at -80°C.

RNA from whole tissue was extracted by using the RNA extraction kit purchased from Macherey-Nagel and performed as described in the manufacture's protocol. Next, RNA was reverse transcribed into cDNA using high-capacity reverse transcriptase kit (Applied Biosystems). cDNA was further diluted 1:5 in ddH_2O prior preparation for qPCR analysis.

Here, the SYBR Green kit (Applied Biosystems) was used following manufacture's protocol.

2.2.4 Histology

The left kidney of each mouse was longitudinally cut to obtain two identical kidney sections. One of these fractions was fixed by using 2 ml methacarn solution and stored overnight at 4°C. The next day, samples were placed in cold PBS and delivered to our collaboration partner Dr. Peter Boor at the Institute for Pathology at the University Hospital of Aachen.

Briefly, the fixed kidney sections were deparaffined with xylol, rehydrated with alcohol and stained 1 hour with anti-α-SMA or collagen III primary antibody. Samples were washed for 5 minutes with PBS, and then stained for 30 minutes with anti-IgG2A antibody. After an additional washing step samples were further incubated with ABC complex for 30 minutes. Samples were washed again and further incubated with 3,3′-Diaminobenzidine (DAB) solution in order to form a visible brown precipitate. For the quantitative analysis of the area positively stained, 16 to 22 photomicrographs covering almost the total cortical area were

taken for each mouse and the positive area for α-SMA or collagen III were quantified by using the open access image analysis software ImageJ.

2.2.4 Flow cytometry

2.2.4.1 Staining of surface molecules

Single cell suspensions of kidney, spleen or adipose tissue were resuspended in FACS buffer or RPMI1640 (2% FCS) and a volume of cell suspension specific for each organ was used to perform flow cytometry analysis: 1/5 Kidney, 1/20 spleen and 1/3 adipose tissue cell suspension. These fractions were transferred into a 96 well plate and centrifuged 300 x g / 5 minutes / 4°C and resuspended in a solution containing 50 µl PBS, viable dye, to discern living or dying cells (live/dead) and human serum, to prevent unwanted binding of antibodies with the FC receptor (FC-block). This solution was applied on samples and incubated for 20 min at 4°C. Next, cells were washed with FACS buffer and resuspended in a master mix containing fluorochrome labeled antibodies against cell surface molecules of interest. Cells were incubated with this antibody mix for 20 minutes at 4°C. Subsequently, cells were washed and resuspended in 200 µl of FACS buffer and analyzed at a LSRFortessa (BD) flow cytometer.

In case of ILC staining: lineage cocktail containing biotinylated antibodies against CD3ε, CD11b, CD5, CD19, TCRβ, γδ TCR and CD11c was applied on samples and incubated for 20 min at 4°C after the live/dead staining as described above. In order to detect biotinylated antibodies, an antibody against biotin streptavidin (SA) coupled to a fluorochrome (FITC or APC) was added to the cell suspension. After additional 20 minutes incubation, samples were washed and resuspended in PBS for LSRFortessa (BD) analysis as described before.

2.2.4.2 Staining of transcription factors

Intracellular staining for transcription factors were performed following manufacturer's protocol (eBioscience Foxp3 / Transcription Factor Staining Buffer Set). Briefly, after cell surface staining, cells were fixed and permeabilized (30 min / 4°C) by using permeabilization/fixation buffer. Next, cells were

incubated with 100 µl of the fluorochrome-labelled antibody mix for intracellular molecules diluted in permeabilization buffer for 30 minutes at 4°C. After incubation, cells were washed 2X with 100 µl permeabilization buffer, centrifuged at 300 x g, 5 minutes, 4°C and resuspended in 200 µl PBS for acquisition at the LSRFortessa (BD).

2.2.5 RNA-sequencing and analysis.

RNA was purified following manufacturer's protocol using a RNAeasy Plus Mini Kit (Qiagen). Two samples from a male and female mouse were pooled for each biological condition. Sequencing was performed on an Illumina NextSeq 500 generating 75 bp paired end reads. The mouse reference genome GRCm38/mm10 was used to align reads using the Subread aligner (version 1.6.2) (Liao, Smyth, and Shi 2013). NCBI RefSeq mouse genes were used to assign mapped reads and gene-wise counts were produced by using featureCounts (Liao, Smyth, and Shi 2014). If genes failed to achieve a CPM (counts per million mapped reads) value of 0.5 in at least 2 libraries, they were excluded from downstream analysis. The voom function of the limma package was used to quantile normalize and precision weighted read counts which had been converted to log2-CPM (Law et al. 2014; Ritchie et al. 2015). Differences in expression were assessed by using empirical Bayes moderated t-statistics, after genes were fitted to a linear model (McCarthy and Smyth 2009). Differentially expressed genes achieved a false discovery rate (FDR) less than 0.1 and a fold change greater than 1.2.

2.2.6 Statistical analysis

Results are expressed as mean ± SD. To determine statistical differences between two groups, a Student-t test with Mann-Whitney post-test was applied. To determine statistical differences between three or more groups, a One- or Two-Way ANOVA analysis of the variance with Tukey post-test was applied and always specified in each experiment. Analysis and graphs preparation were performed with Prism (GraphPad).

Chapter 3 - Contribution of the myeloid cell compartment in high-fat-diet induced obesity

3.1 Introduction

Obesity induces a chronic state of low-grade inflammation, so called chronic metabolic inflammation (metaflammation) (Christ and Latz 2019; Gregor and Hotamisligil 2011). Indeed, excess consumption of nutrients leads to metabolic changes which trigger inflammation. Adipocytes contribute to the inflammation by sustaining the insult and further mediating the “metabolic input and inflammatory output” (Hotamisligil 2006). Several studies in mouse and human showed that obesity leads to a dysregulation in inflammatory associated cytokines, i.e. TNFα, IL-6, IL-1β and CCL2 (Berg and Scherer 2005; Hotamisligil et al. 1995; de Luca and Olefsky 2008). Furthermore, obesity does not solely drive higher proinflammatory cytokine levels in the adipose tissue, but additionally in liver, pancreas, brain and muscle (Cai et al. 2005; Ehses et al. 2007; Saghizadeh et al. 1996; De Souza et al. 2005) .

Different populations of immune cells have been shown to infiltrate the obese adipose tissue and to contribute to the increased secretion of proinflammatory mediators (Ferrante 2013). However, the exact influence of different immune cells is not well defined yet, owed to the high complexity of the immunological network. Adding to this complexity are different functions of immune cells depending on their microenvironment. Pro-inflammatory macrophages, often referred to as “M1”, play a major role in adipose tissue inflammation (Chylikova et al. 2018; Fujisaka et al. 2009; Weisberg et al. 2003). Obesity leads to a shift from anti-inflammatory alternatively activated macrophages (AAMs, “M2” like) to the pro-inflammatory M1 phenotype (Lumeng et al. 2008). However, not only macrophages but also dendritic cells, natural killer T cells (NKT), NK cells, $\gamma\delta$ T cells and CD8 T cells and have been described to play important roles in AT inflammation (Cho et al. 2016; Chylikova et al. 2018; Ferrante 2013; Fujisaka et al. 2009; Nishimura et al. 2009). Especially immune cells belonging to type 2 responses, such as regulatory T cells, eosinophils, mast cells, M2 macrophages and ILC2s had been shown to regulate AT health and counteract inflammatory effects in obesity (Ferrante 2013; Feuerer, Hill, et al.

2009; Kumar et al. 2019; Odegaard and Chawla 2015; Vasanthakumar et al. 2015; Villarroya et al. 2018).

However, various studies have shown that one of the driving mechanisms of VAT inflammation is the accumulation of pro-inflammatory immune cells, e.g. macrophages, in human and mouse adipose tissue (Olefsky and Glass 2010; Weisberg et al. 2003). Macrophages are important regulators of the immune system and are positioned strategically throughout host tissues to sense danger signals via pathogen recognition receptors (PRRs), which may also include factors released due to tissue damage (Wong et al. 2012). Upon PRR activation macrophages take part in the initiation of a pro-inflammatory cascade, which may initiate tissue inflammation and pathology. However, macrophages can also exhibit regulatory functions and aid in tissue repair (Jeremy S Duffield et al. 2005).

To distinguish these two phenotypes of macrophages the concept of pro-inflammatory “M1” and anti-inflammatory “M2” macrophages has been established (Mills 2012; Mills et al. 2000). Even though, this concept is currently under debate it has been shown to provide in vivo the main macrophage polarization axis (Buscher et al. 2017; Ley 2017). Additional concepts discuss a continuum of macrophage polarization where M1 and M2 are extremes of polarization (Palma et al. 2018; Sica and Mantovani 2012). However, an in-depth characterization of cell surface marker expression on the different macrophages subsets has still to be addressed.

Adding to this complexity of macrophages is that further refinement of the characterization of the myeloid lineages, molecules previously used to identify DCs and macrophages (CD11c and F4/80, respectively) have been found to be expressed on either population (Gautier et al. 2012). Therefore, some studies in the adipose tissue, where CD11c has been associated with M1 macrophages (Fujisaka et al. 2009; Weisberg et al. 2003), did not consider that also DCs carry the marker CD11c and may upregulate the macrophage marker F4/80. Since it has been shown in other organs, such as the kidney or dermis, that DCs can be F4/80^{+}, it is necessary to have a closer look into the adipose tissue and the

different myeloid subsets (Bogunovic et al. 2006; Gottschalk and Kurts 2015; Rogers et al. 2014).

A recent study showed that the use of CD64 together with F4/80 is able to distinguish macrophages and DCs across tissues (Guilliams et al. 2016). The use and establishment of these marker might lead to uniform reports of macrophages and DCs.

Besides their characterization, the depletion of $CD11c^+$ cells during obesity showed rapid normalization of insulin sensitivity, directly linking macrophages and/or dendritic cells to play crucial roles in adipose tissue inflammation and metabolic disease (Patsouris et al. 2008).

An additional level of complexity stems from the observation of sex specific differences in the adipose tissue. In humans we know that the distribution of fat depots differs between male and females. Females tend to accumulate more subcutaneous fat, whereas males accumulate visceral fat depots, correlated to higher risk of cardiovascular disease (Palmer and Clegg 2015). Interestingly, in females adipose deposition changes after menopause and shifts to visceral adipose sites, similar to men, accompanied by a higher risk of metabolic disease (Gavin, Cooper, and Hickner 2013; Richelsen 1986). A growing body of evidence connects sex hormones, primarily estrogen, to adipose tissue inflammation and sex dimorphism (Davis et al. 2013). However, estrogen and its receptors estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) are expressed in female as well as male adipose tissue, but also androgen plays important roles in body fat deposition and health (Finkelstein et al. 2013; Lee, Kim, and Choi 2012). Intriguingly, androgen and estrogen receptors are expressed on immune cells and their function is of importance in many tissues and conditions, including cancer and lung disease (Becerra-Díaz et al. 2018; Kovats 2015; Thompson et al. 2017).

Taken together, it is well established that immune cells, especially macrophages and dendritic cells, play a critical role in adipose tissue homeostasis and disease. Furthermore, the Kallies lab found differences in stromal cell populations in the adipose tissue. This was accompanied by a pronounced sexual dimorphism in adipose tissue T_{regs} (Vasanthakumar *et al.* (*in press*). Therefore, we extended our investigations to macrophages and dendritic cells and if they contribute to the sexual dimorphism in the adipose tissue between males and females.

3.2 Results

3.2.1 High fat diet induced obesity leads to accumulation of different myeloid cell types in visceral adipose tissue

To examine the involvement of different myeloid cells in adipose tissue inflammation, a model of diet induced obesity (DIO) was applied. To this end, mice were fed for 16 weeks with high fat diet (HFD) in which 59% of the energy provided comes from lipids. Littermate control mice were fed over the same timeframe with regular mouse food (normal diet, ND) which provides 12% of its energy as fat (Figure 3-1, A). Over this period, HFD fed mice gained significantly more weight compared to ND fed controls (Figure 3-1, B). A closer examination of the weight of harvested visceral adipose tissue (VAT, Figure 3-1, C) identified significantly more VAT in HFD mice compared with ND controls, but no significant difference in splenic weight between groups (Figure 3-1, D). Furthermore, to detect alterations in the glucose metabolism following DIO, we performed insulin and glucose tolerance tests (ITT, GTT, details in 2.2.1, (Vinué and González-Navarro 2015)). Following intravenous injection of insulin, HFD-fed mice displayed significantly higher blood glucose levels than ND-fed controls (Figure 3-1, E). Similarly, following intra-peritoneal injection of glucose (GTT), mice from the HFD group were not able to remove glucose from the bloodstream as quickly as ND controls. (Figure 3-1, F). Those two parameters indicated that 16 weeks of high fat diet feeding is sufficient to induce insulin resistance.

Together, those results indicate successful establishment of DIO and metabolic impairments with the intervention described. This includes established markers of DIO including increased adipose tissue deposition and altered glucose homeostasis.

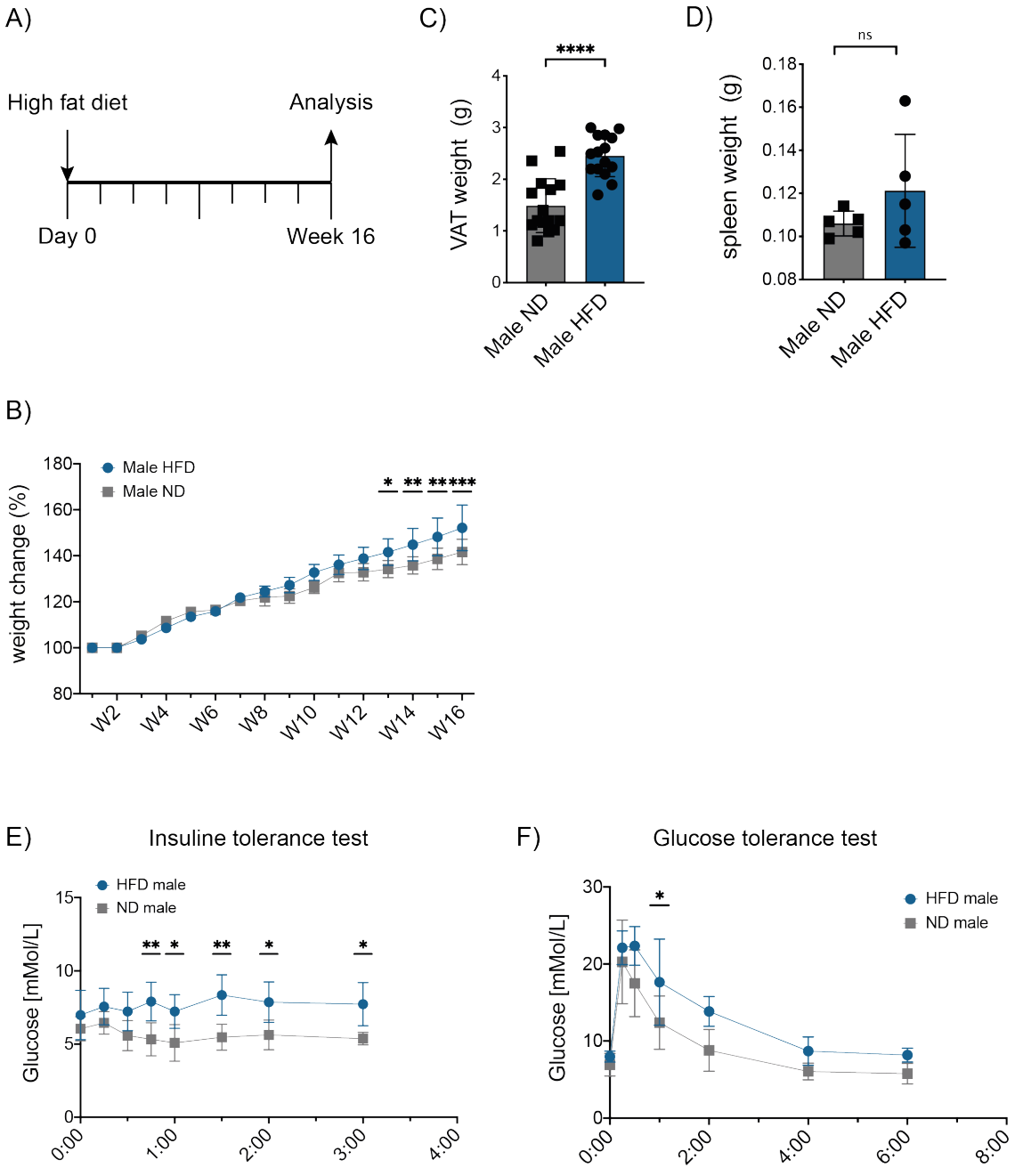


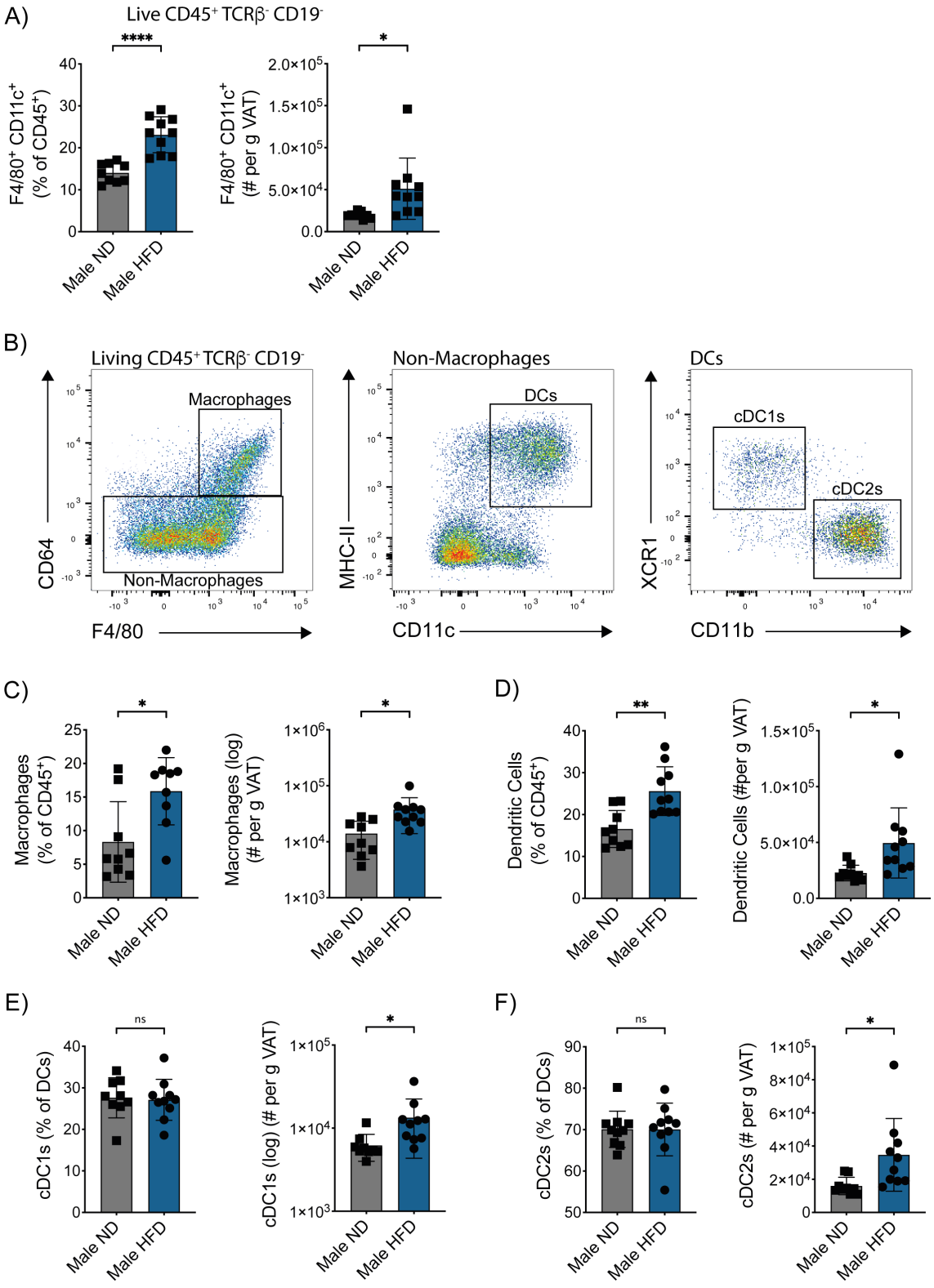
Figure 3-1 High fat diet feeding induces physiological changes in mice

(A) 6 weeks old male C57BL/6 mice were fed for 16 weeks with high fat diet (HFD) or control diet (ND). (B) Weight gain of mice. (C, D) VAT and spleen (one experiment) weight changes due to HFD. (E) Insulin and (F) glucose tolerance test of male mice at week 15. Data shown represents the mean ± SD (>3 mice per experiment, (B) Pooled data from at least two independent experiments, (B-F) Representative data from at least two independent experiments). Asterisks indicate statistically significant differences versus ND controls as assessed by (C-D) Student´s two-tailed T test or (B, E, F) one-way ANOVA (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

To study the role of the myeloid compartment in obese adipose tissue, we characterized macrophage and dendritic cell (DC) populations within the tissue using flow cytometry. In order to place our work in the context of the current literature, we first quantified $F4/80^+$ and $CD11c^+$ cells in the VAT (Figure 3-2, *A*). Consistent with previous reports, we identified a significant increase in both the number and frequency of $F4/80^+CD11c^+$ cells in the VAT of HFD mice compared to their control counterpart (ND) mice.

In order to more specifically characterize the VAT myeloid compartment of healthy and obese mice, we next applied the macrophage and DC gating strategy shown in Figure 3-2, *B*. We identified a significant increase in both macrophages and DCs in HFD treated mice, in frequencies and also in total cell numbers, (Figure 3-2, *B* and *C*). There was no change in the relative distribution of cDC1s and cDC2s (frequency of DCs), with both DC subtypes are increasing in a similar manner in total numbers (Figure 3-2, *D* and *E*).

Our analysis has corroborated and further refined previous reports, having identified a specific increase in both macrophages and DCs in DIO. Interestingly, neither class of cDC is predominantly increased in this disease model.


A)
Live CD45+ TCRβ- CD19-
****
*
F4/80+ CD11c+ (% of CD45+)
F4/80+ CD11c+ (# per g VAT)
Male ND
Male HFD
B)
Living CD45+ TCRβ- CD19-
Macrophages
Non-Macrophages
CD64
F4/80
Non-Macrophages
DCs
MHC-II
CD11c
DCs
cDC1s
cDC2s
XCR1
CD11b
C)
Macrophages (% of CD45+)
Macrophages (log) (# per g VAT)
D)
Dendritic Cells (% of CD45+)
Dendritic Cells (#per g VAT)
E)
cDC1s (% of DCs)
cDC1s (log) (# per g VAT)
ns
F)
cDC2s (% of DCs)
cDC2s (# per g VAT)

Having identified an increase in total macrophages in our DIO model, we asked if we can identify specific populations of VAT macrophages. We determined classically activated macrophages (CAMs, or 'M1 macrophages') by expression of CD38, and alternatively activated macrophages (AAMs, or 'M2 macrophages') by expression of CD206 (Mannose receptor) (Gordon 2003; Jablonski et al. 2015). To our surprise, macrophages isolated from the VAT did not fit the established strategy. We did not find classic CAM phenotype ($CD38^+$ $CD206^-$) macrophages, rather, $CD38^+$ cells in the VAT also expressed the mannose receptor CD206 (Figure 3-3, A). Interestingly, we observed a slight increase in the frequency of AAM-phenotype ($CD206^+CD38^-$) macrophages in the VAT of HFD mice, but no significant difference in the frequency of $CD38^+$ macrophages. This contrasts with earlier work which described that HFD feeding induces CAMs (Lumeng, Bodzin, and Saltiel 2007). However, in human adipose tissue a mixed expression of M1/M2 marker has been described (Wentworth et al. 2010). We further identified significantly higher levels of CD206 expression by ATMs from HFD mice compared with ND controls, independent of CD38 expression (Figure 3-3, D).

Here we have found the current strategy of identifying CAMs and AAMs to be insufficient to distinguish these populations in the visceral adipose tissue. Rather, we have identified a CD38 and CD206 double positive population in the adipose that undergoes substantial changes in DIO. Further we have observed enhanced expression of CD206 and expansion of the $CD206^+$ AAM population in DIO.

Figure 3-2. High fat diet induced accumulation of immune cells in visceral adipose tissue

(A-F) Male mice were fed either a high fat diet (HFD) or normal control diet for 16 weeks. All cells are pre-gated on live $CD45^+$ and TCR $beta^-$ $CD19^-$ cells. (A) Representative gating strategy used to identify macrophages ($CD64^+F4/80^+$) and DCs ($CD64^-F4/80^-MHC\text{-}II^+CD11c^+$). (B) Frequencies and numbers of $F4/80^+$ $CD11c^+$ cells in VAT. (C) Frequencies and numbers of macrophages and (D) DCs. (E) Frequencies and numbers of cDC1s (XCR1+ CD11b-) and (F) cDC2s ($XCR1^-$ $CD11b^+$). Data represent the mean ± SD from at least two independent experiments (n=3-5 mice per experiment). Asterisks indicate statistically significant differences versus ND controls as assessed by Student´s two-tailed T test (* $p<0.05$, ** $p<0.01$, **** $p<0.0001$).

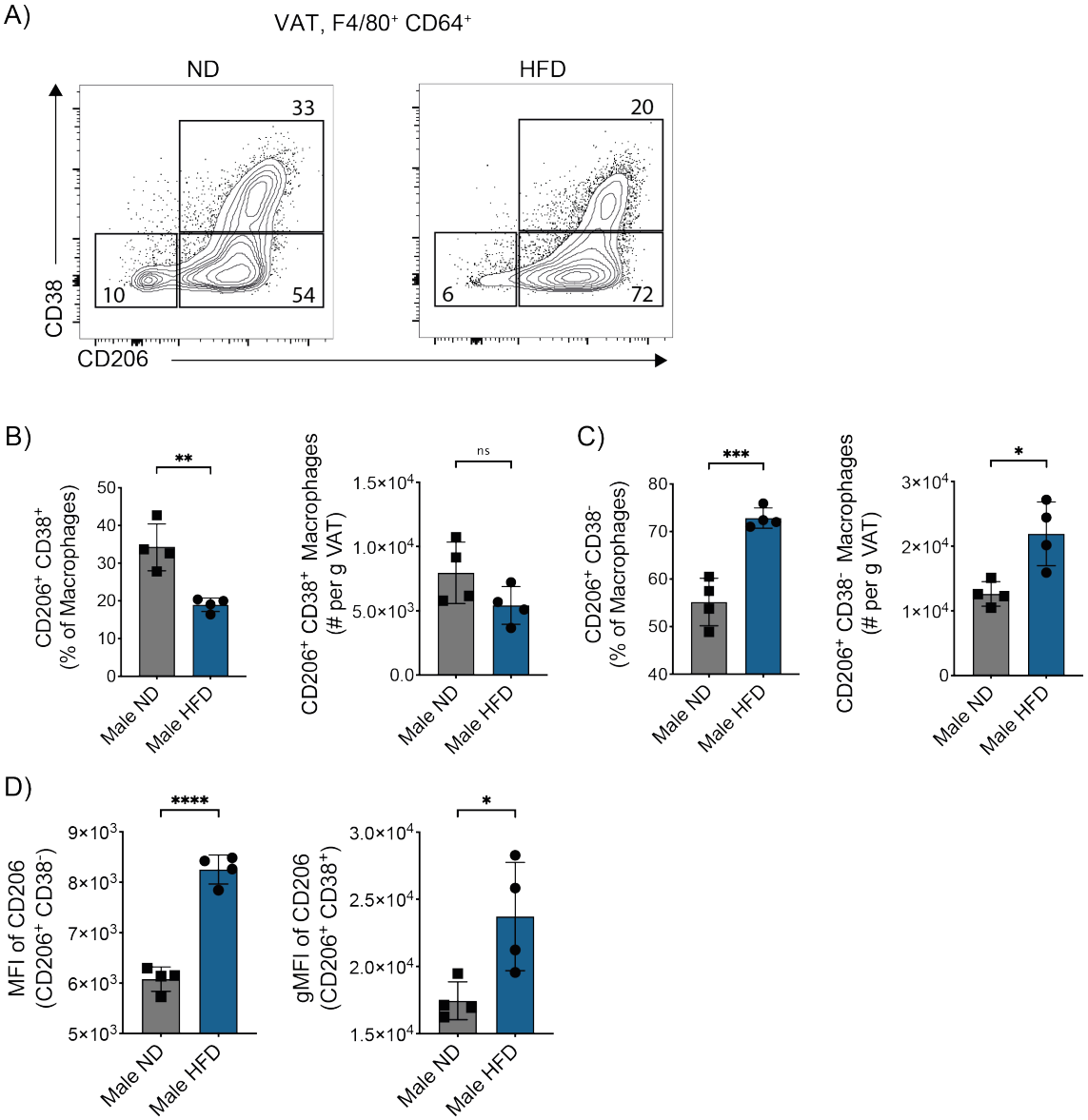


Figure 3-3 Visceral adipose tissue macrophages show phenotype shift during development of diet induced obesity.

(A) Representative FACS plots of "M1" ($CD38^+$) and "M2" ($CD206^+$) macrophages of VAT isolated from normal- and HFD fed mice. (B) Frequencies and numbers of $CD206^+$ $CD38^+$ VAT macrophages. (C) Frequencies and numbers of $CD206^+$ $CD38^-$ VAT macrophages. (D) Geometric MFI of CD206 expression on $CD206^+$ $CD38^-$ and $CD206^+$ $CD38^+$ cells. Data represent the mean ± SD (3-5 mice per group, representative data from at least two independent experiments). Asterisks indicate statistically significant differences versus ND controls as assessed by Student´s two-tailed T test (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$)

3.2.2 Sex specific differences in adipose tissue myeloid cells

We and others have identified specific differences in the regulatory T cell compartments of male and female mice (Vasanthakumar *et at., under review,* (Cipolletta 2014; Spallanzani et al. 2019). Further investigation showed that the VAT of male mice expressed higher levels of inflammatory mediators, such as MCP-1/CCL2 and TNFα, while female mice displayed superior metabolic function in terms of insulin and glucose uptake measured by ITT and GTT (Vasanthakumar *et at., under review)*. As macrophages have been described to play an important role in adipose tissue inflammation, we investigated whether the myeloid compartment displays sex specific differences, that may contribute to the higher inflammatory phenotype in the VAT of male mice.

First, we found significantly more perigonadal VAT in age-matched male compared with female mice (Figure 3-4, *A*). We further identified male mice to display poorer rates of glucose clearance compared with female mice, indicating fundamental metabolic differences between the sexes (Figure 3-4, *B*), which goes in line with previous reports (Macotela et al. 2009; Wintrob et al. 2014). Next, we used flow cytometry and applied the previous established gating strategy (Figure 3-2, *B*) to identify different immune cell populations. Compared with females, the VAT of male mice contained significantly more macrophages, both by frequency and number per gram (Figure 3-4, *C*). Further, we identified a significant increase in the frequency, but not number per gram, of dendritic cells in male vs female VAT. Further interrogating the DC phenotype, we could not identify significant differences in cDC1s or cDC2s between the two sexes, though we did observe a slight trend toward cDC1s and lower frequencies for cDC2s in female mice.

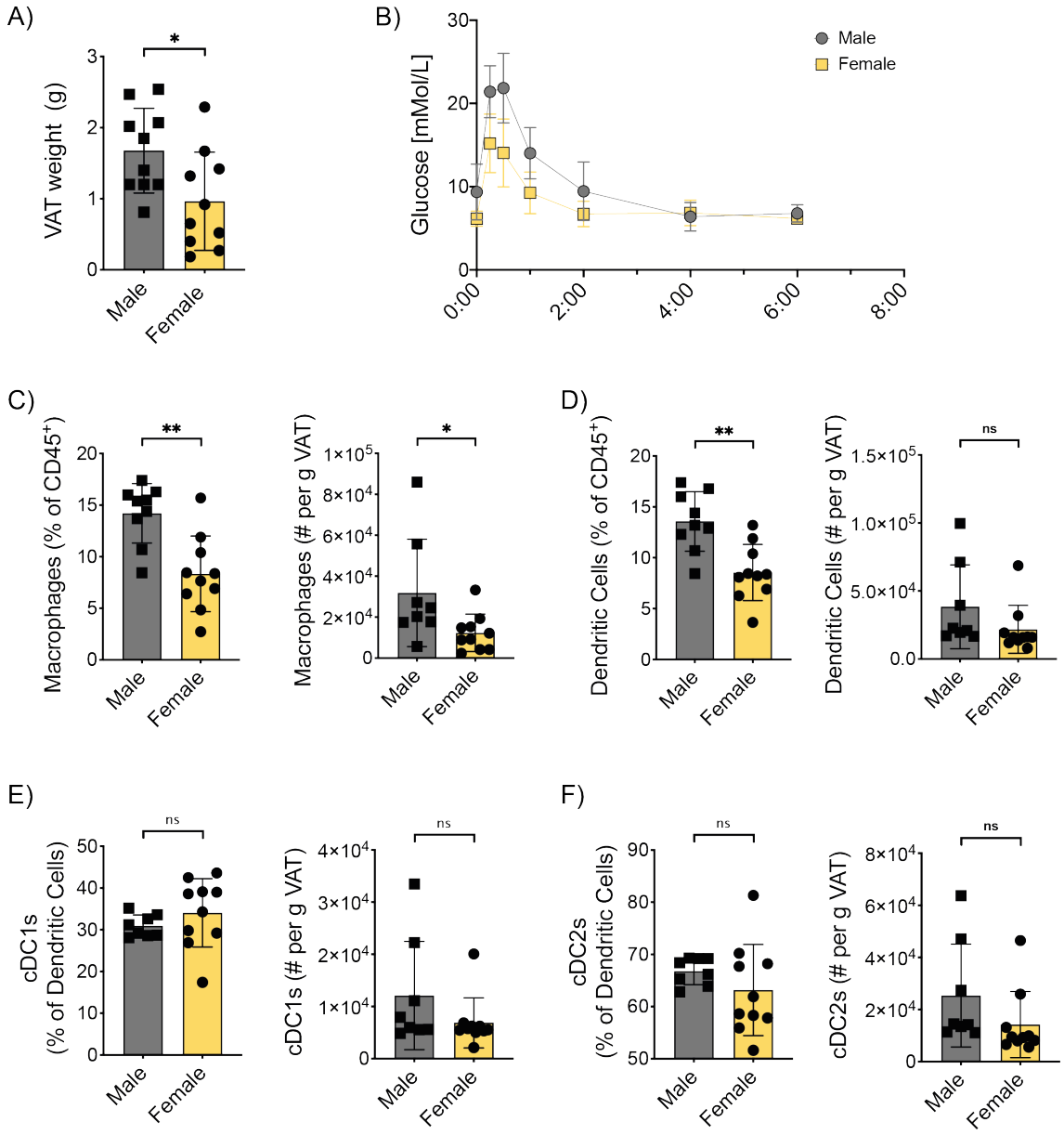


Figure 3-4 Male mice show elevated accumulation of macrophages in VAT.

(A) VAT weights of 24 weeks old male and female mice fed with normal diet. (B) Glucose tolerance test of male and female mice. (C) Frequency and total cell numbers of macrophages. (D) Frequency and total cell numbers of dendritic cells. (E) Frequency and total cell numbers of cDC1s. (F) Frequency and total cell numbers of cDC2s. Data shown represents the mean ± SD from at least two independent experiments (n=3-5 mice per experiment). Asterisks indicate statistically significant differences versus ND controls as assessed by Student´s two-tailed T test (* $p<0.05$, ** $p<0.01$).

These data show that male mice harbor more adipose tissue and perform worse in metabolic tests. Furthermore, male VAT contains higher frequencies of macrophages and dendritic cells compared to female VAT. Together, these observations identify sex-specific differences in the myeloid compartments of the VAT.

We next assessed whether the phenotype of the ATM population differed between sexes. Notably, both sexes displayed the CD38/CD206 distribution on macrophages as described above. In quantifying the three populations distinguishable by CD38 and CD206 expression, we identified a significant increase, both numerically and by frequency, of the $CD206^{+}CD38^{-}$ population in male compared with female VAT(Figure 3-5, C). We did not observe a significant difference in the frequency of the CD38/CD206 double positive population between the sexes. (Figure 3-5, B). A comparison of CD206 expression in those two populations revealed a slight, though nonsignificant, trend toward higher CD206 expression by both populations in male mice (Figure 3-5, D).

These data indicate that ATM cells from male VAT have an overrepresentation of the $CD206^{+}CD38^{-}$ population we described above. The accumulation of macrophages in AT has been associated with VAT inflammation and insulin resistance (see introduction). However, CD206 is frequently used to describe M2 macrophages, whereas AT inflammation is described to involve M1 macrophages. Therefore, our findings show an interesting new aspect of $CD206^{+}$ ATMs in HFD induced obesity. CD206/CD38 double positive macrophages are associated with the protective phenotype as seen both in females and lean mice.

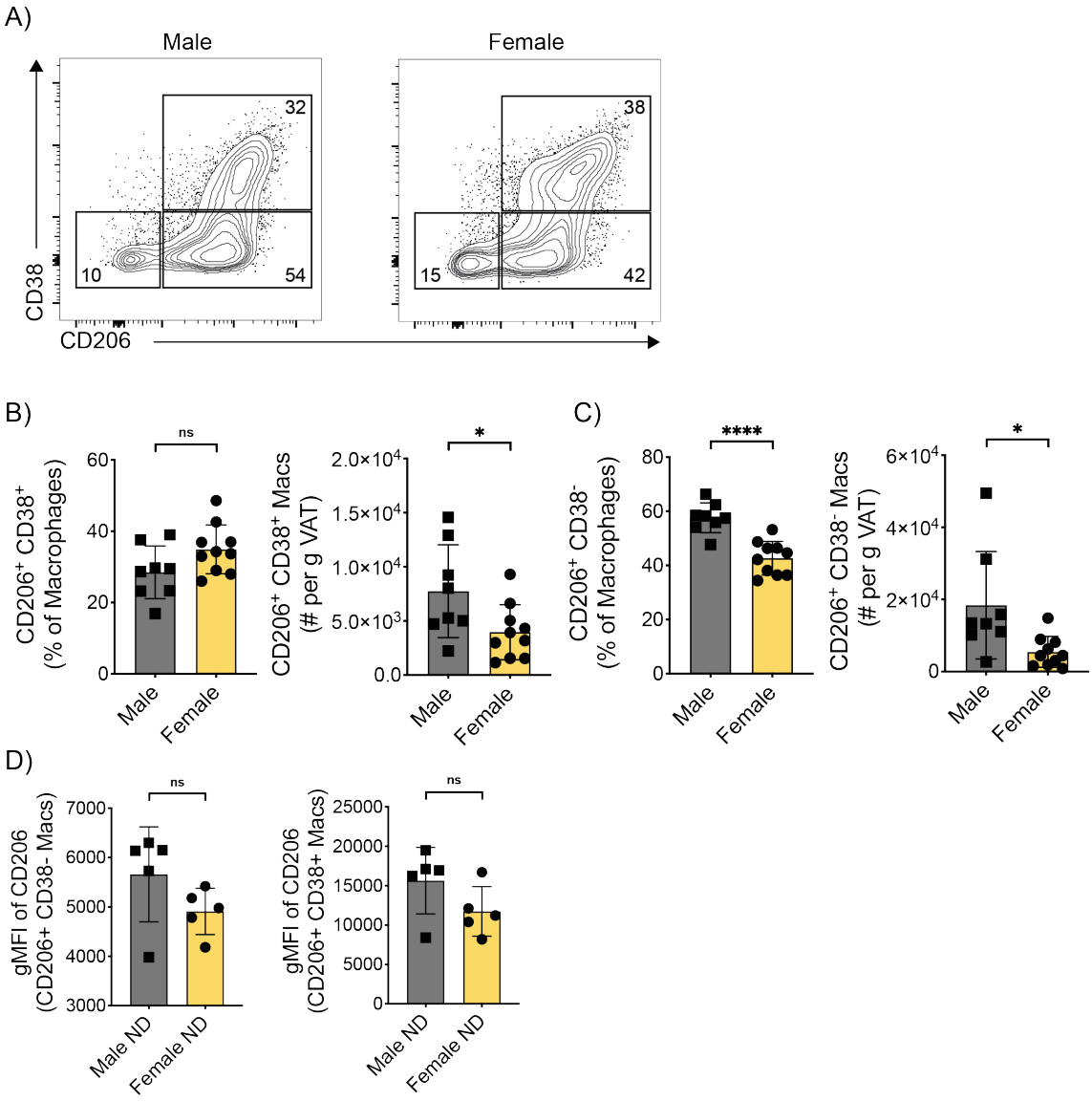


Figure 3-5 Male and female mice show significant differences in macrophage phenotype.

(A) Flow cytometry plot of male and female macrophages gated on CD38 and CD206. (B) Frequencies and numbers of $CD206^+$ and $CD38^+$ macrophages in male and female VAT. (C) Frequency and numbers of $CD206^+$ and $CD38^-$ macrophages in male and female VAT. (D) Geometrical mean fluorescence intensity (gMFI) of CD206 in the $CD206^+$ $CD38^-$ and $CD206^+$ $CD38^+$ macrophage populations. Data represent the mean ± SD from at least two independent experiments (n=3-5 mice per experiment). Asterisks indicate statistically significant differences versus ND controls as assessed by Student´s two-tailed T test (* $p<0.05$, **** $p<0.0001$).

3.2.3 RNA sequencing of male and female macrophages and dendritic cells reveals transcriptional differences

The results obtained so far indicate a clear correlation between the myeloid compartment and the degree of VAT inflammation. This, and the established role of myeloid cells to initiate and maintain inflammation, makes them prime candidates for mediating the effects we observed in the DIO model and the differences between male and female adipose tissue inflammation. Our results have shown that even under normal diet conditions male and female adipose tissue shows sex specific differences. Male mice showed decreased glucose tolerance compared to females, which was paralleled by an increased cell number of macrophages and a higher frequency of dendritic cells compared to female VAT.

In order to understand whether there may be specific differences in the transcriptional programs of male and female myeloid cells responsible for the difference between sexes, we performed transcriptional profiling of DCs and macrophages from male and female VAT. To this end, we used flow sorting to isolate macrophages and dendritic cells (sorting strategy followed gating in Figure 3-2, *B*) from the perigonadal adipose tissue of male and female mice. The male macrophages showed a more inflammatory gene signature with higher expression of *Il1b*, *Il6*, *Ccl2* and *Tnf*, and lower expression of *Ccl5,* depicted in the volcano plot (Figure 3-6*, A*). Similarly, proinflammatory *Il1b*, *Il6* and *Ccl2* were upregulated in male adipose tissue dendritic cells *(*Figure 3-6*, B*). All those genes are connected to inflammation and indicate that macrophages as well as dendritic cells of male adipose tissue display a more pronounced inflammatory signature. Additionally, we found a general difference between male and female macrophages and dendritic cells as shown in Figure 3-6*, C*. From this heatmap we can observe distinct transcriptional patterns dependent upon both the cell type and the sex of the donor mice. We found that DC associated genes like *Ccl22* or *Cxcl9* are upregulated in male and female DCs but not male or female macrophages.

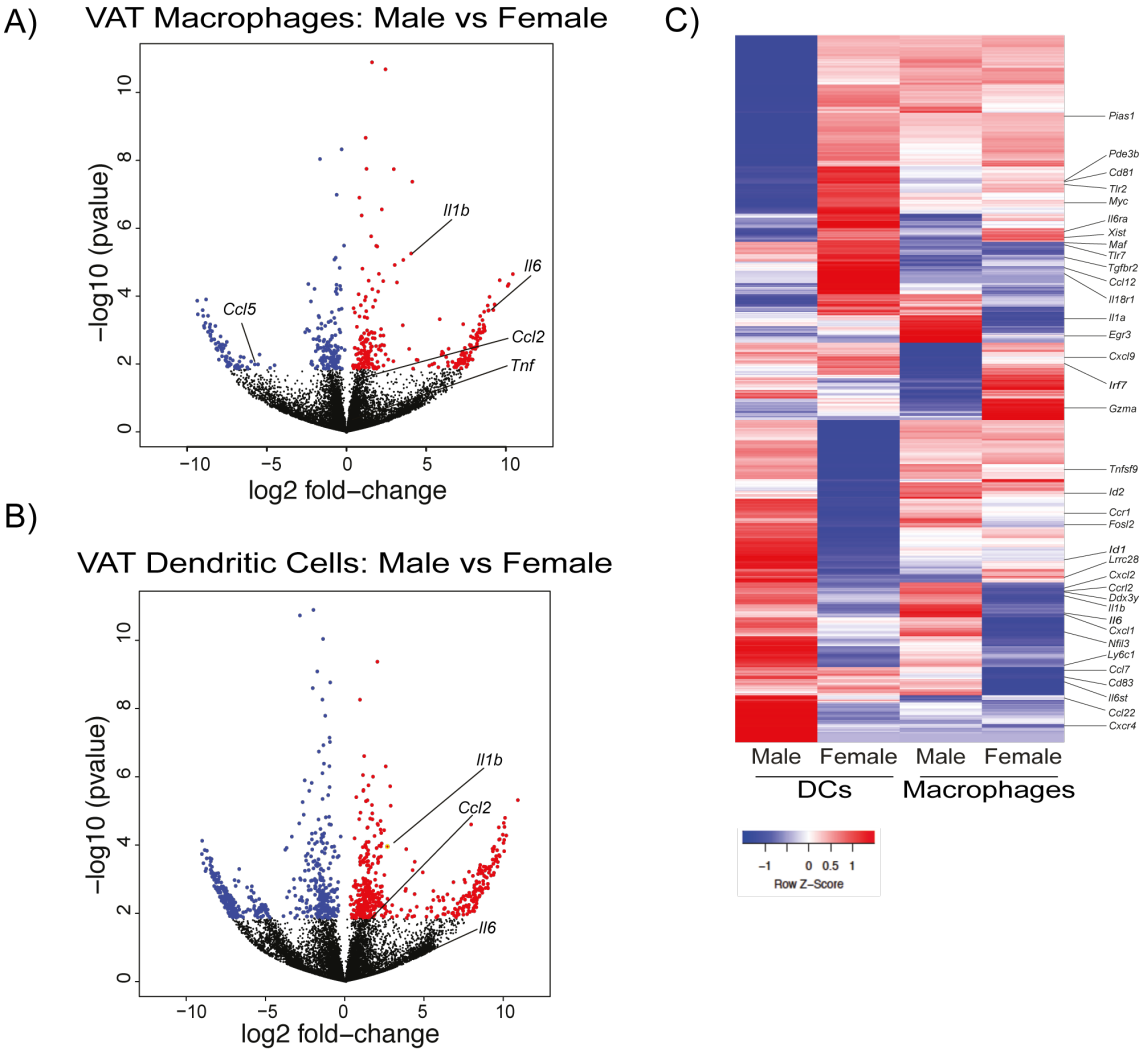


Figure 3-6 Male VAT macrophages and DCs show upregulation of pro-inflammatory genes.

Visceral adipose tissue from male and female mice was collected and sorted for macrophages (TCRβ$^-$ CD19$^-$, CD64$^+$ F4/80$^+$) and DCs (TCRβ$^-$ CD19$^-$, CD64$^-$, MHC-II$^+$ CD11c$^+$). (A) Volcano plot indicating differentially expressed genes in male and female VAT macrophages. (B) Volcano plot indicating differentially expressed genes in male and female VAT DCs. (C) Heat-map of differentially expressed genes of male and female VAT DCs and macrophages, genes of interest are named on the right.

Myc, a gene found to be highly upregulated in macrophages and low in DCs, is another example represented in this data set, indicating that our gating strategy clearly distinguishes macrophages and dendritic cells.

These data are the first to specifically characterize the transcriptional profiles of male and female myeloid cells from the VAT. These datasets identify sex-dependent transcriptional programs, which are enriched for genes encoding inflammatory mediators. These results establish VAT-resident macrophages and dendritic cells as likely contributors to the maintenance and possible establishment of the inflammatory state of male perigonadal fat.

3.2.4 Sex hormone treatment did not influence cytokine production of bone marrow derived DCs

Our findings show that adipose tissue macrophages as well as DCs are molecularly distinct between males and females. Previous reports (summarized in Bianchi, 2019) are controversial about testosterone and its role as a pro- or anti-inflammatory mediator. Furthermore, a study showed that in ovariectomized mice, lacking the production of estrogen, the levels of pro-inflammatory cytokines increased, indicating an anti-inflammatory role for estrogen (Rogers et al. 2009).

To include the possibility of pro-inflammatory epigenetic imprinting, in bone marrow progenitors of high fat fed mice, in our investigations, we extracted bone marrow from HFD or ND mice, and male or female mice, and generated bone marrow derived dendritic cells (BMDCs): after isolating the bone marrow from male and female mice fed with control or HF diet, bone marrow cells where cultured with Flt3 ligand (150 ng/ml) for 8 days. Cells were then collected and seeded in 96 well plates and subsequently stimulated for 24 hours with media alone, estradiol or testosterone to investigate the effects of those sex hormones on the cytokine production of dendritic cells. After 24 hours cells were stimulated with LPS (50 ng/ml) for 24 hours, following which supernatant was collected and analyzed for expression of various cytokines (Summarized in Figure 3-7 A).

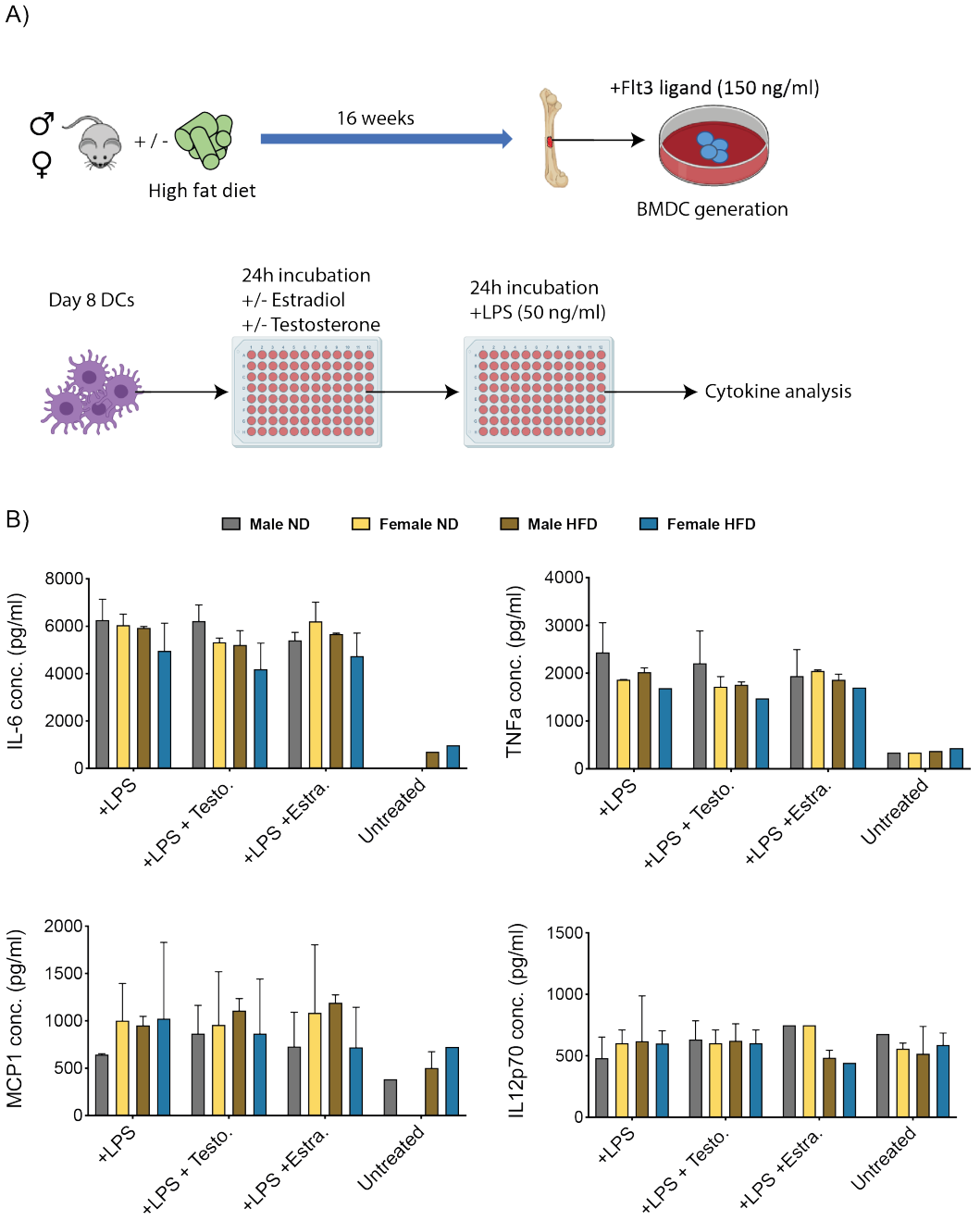


Figure 3-7 BMDCs from HFD treated mice show no significant differences in cytokine production.

(A) Experimental setup: Bone marrow was extracted from male and female mice treated with HFD or control diet for 16 weeks and subsequently cultured with Flt3 ligand (150 ng/ml) to generate BMDCs. At day 8 BMDCs were incubated with or without Estradiol (0.1 μM) or Testosterone (1 μM) for 24h and further incubated for an additional 24h with LPS (50 ng/ml). Supernatant was collected and further analyzed for different cytokines. (B) Concentrations (pg/ml) of IL-6, TNFα, MCP-1 (CCL2) and IL12p70 measured in supernatant. Data represent the mean ± SD from one experiment (n=2 mice).

We found no significant differences for IL-6, TNFα, MCP-1 or IL12p70 production. However, remarkably, DCs derived from HFD fed mice produced detectable amounts of IL-6 without being LPS stimulated (Figure 3-7, B, left upper corner). Additionally, only DCs from control diet treated females did not show any expression of MCP-1 in untreated conditions (Figure 3-7, B, left lower corner). Both findings might indicate that the ongoing inflammation in the adipose tissue alters the epigenetic landscape of BM progenitors. However, this experiment has only been done once and needs to be repeated and the number of animals has to be increased to make more evident observations.

Taken together, we could not demonstrate that BMDCs show significant differences in LPS induced cytokine production between male and female mice or high fat diet treated or untreated mice. However, untreated cells showed a tendency to a more pro-inflammatory phenotype.

The above presented experiments show that our high-fat diet induced obesity model leads to significant differences in physiological parameters, such as glucose uptake and insulin tolerance, but also to changes in immune cell composition. Furthermore, we found significant differences in male and female AT in homeostatic conditions. In both cases we observed a dysregulation of macrophages and DCs, suggesting their contribution to the physiological differences. These sex specific differences might be a consequence or cause of the more “pro-inflammatory” phenotype in male adipose. Testing if these differences are already imprinted into bone-marrow cells, we did not detect significant differences in LPS induced cytokine release in BMDCs generated from male or female BM under homeostatic or HFD treated mice. Additionally, the treatment of those BMDCs with sex hormones did not induce a change in their cytokine profile. These results might indicate a more complex network of immune cells and their microenvironment in the VAT.

3.3 Discussion

This chapter investigated the distribution of myeloid cells, namely macrophages and dendritic cells in the adipose tissue. We utilized a model of diet induced obesity, for which we fed C57BL/6 mice for 16 weeks with high fat diet. Analyzing male visceral adipose tissue revealed an increase in F4/80$^+$ CD11c$^+$ cells which had been previously reported (Lumeng et al. 2007). However, additionally to macrophages also dendritic cells express CD11c$^+$ and it has been shown that DCs are capable to upregulate the macrophage marker F4/80$^+$ (Austyn and Gordon 1981; Guilliams et al. 2014). Importantly, both cell types have specific characteristics and different roles in tissue homeostasis and inflammation. To define specific treatments for adipose tissue inflammation, and to fully understand the underlying reasons and disease progression, it is important to understand the multifaceted network between immune cells and their surroundings, such as immune cell - stromal cell interactions. Therefore, we have to better distinguish between macrophages and DCs. Here, we used a modified gating strategy proposed from unsupervised computational analysis of flow cytometry and mass cytometry data (Guilliams et al. 2016). Our results indicate that not only macrophages increased in the adipose tissue of mice in a DIO-model, but also dendritic cells. Furthermore, we found sex specific differences in the adipose tissue of male and female mice regarding macrophages in dendritic cells.

By feeding male mice a HFD we found a significant increase of body weight and the accumulation of visceral adipose tissue. Mice fed with HFD showed also significant changes in metabolic fitness regarding insulin and glucose uptake compared to normal diet fed mice, which is consistent with the literature (Palm and Medzhitov 2009; Reaven 2011; Xu et al. 2003). Analyzing the perigonadal adipose tissue we found increased numbers of F4/80$^+$ CD11c$^+$ cells in HFD fed mice. Those cells had been reported as pro-inflammatory macrophages and their increase is in line with older reports (Patsouris et al. 2008; Weisberg et al. 2003). However, aware that the expression of CD11c overlaps with conventional dendritic cells, we applied the above-mentioned gating strategy to distinguish

between macrophages and cDCs (Guilliams *et al.).* We found significantly increased frequencies and numbers of $CD64^+$ $F4/80^+$ adipose tissue macrophages. In addition, adipose tissue cDC were significantly increased, both in frequencies and numbers, which goes in line with reported results (Cho et al. 2016). Investigating the specific cDC subsets, we found no changes in cDC1 and cDC2 frequencies, both ATDC subtypes increased during VAT inflammation. Macdougall *et al.* showed in the adipose tissue that during homeostasis cDCs acquire a tolerogenic phenotype where cDC1s produce IL-10 and the expression of PPARγ in cDC2s suppresses the onset of inflammatory responses (Macdougall et al. 2018). However, in this study we did not analyze *in vivo* changes of adipose tissue DC metabolism or their cytokine profile.

It has been reported that with onset of adipose tissue inflammation macrophages undergo a phenotypic switch from the anti-inflammatory alternatively activated “M2” (AAM) phenotype to the pro-inflammatory classically activated macrophages (CAM) phenotype (Castoldi et al. 2015). The mannose receptor (CD206) is a well-known and accepted marker for AAMs (Cavallo et al. 2011; Martinez-Pomares 2012) Furthermore, it has been shown that CD38 is *in vitro* and in human SLE patients identifies pro-inflammatory “M1” macrophages (Amici et al. 2018; Jablonski et al. 2015). To distinguish between the macrophage phenotypes, we made use of both markers in our study. Interestingly, we found in mice fed with either control diet or HFD possessed a double positive (DP) population of CD38 and CD206 expressing macrophages. To our knowledge this population has not been reported before. However, in the onset of obesity we found a decrease in DP cells and an accumulation of CD206 single positive cells. Further, we found increased expression of CD206 on macrophages under inflammatory conditions. These results argue either against the model that VAT inflammation leads to a phenotypic switch of macrophages, or against the usefulness of CD206 as a marker to characterize “M2” macrophages. Even though CD38 had been described to be upregulated in human, as well as murine models during inflammation, its role is not yet fully understood. CD38 is a universal enzyme being involved in the modulation of many signal pathways, mainly due its role in intracellular Ca(2+) regulation (Malavasi et al. 2008). It has

been connected to diabetes by playing an important role in insulin responsiveness in pancreatic beta-cells (Johnson et al. 2006). Further, CD38-ko mice are resistant to HFD induced obesity, being part in a metabolic cascade for regulating energy expenditure (Barbosa et al. 2007). Therefore, the value of CD38 as CAM marker might be restricted to certain tissues and needs further investigation, especially in the adipose tissue.

It has become evident that sex dimorphism plays crucial roles in onset and progression of diseases such as, autoimmune diseases, several cancers but also obesity (Clocchiatti et al. 2016; Fitzgerald et al. 2018; Morrow 2015; Ngo, Steyn, and McCombe 2014). Having identified that macrophages and cDCs increase during HFD induced obesity, we were interested if these immune cells are also involved in sex differences observed in the visceral adipose tissue. Therefore, we investigated male and female perigonadal adipose tissue of age-matched mice. We found that male mice show significantly increased VAT weight and impaired glucose tolerance, which is consistent with reports studying sex dimorphism in a rat model of DIO (Estrany et al. 2013). Interestingly, we found significantly reduced frequencies and numbers of macrophages in the female VAT. We also found significantly decreased VAT cDC frequencies in female mice, however the difference in numbers were not significant. Further examination of cDC subsets did not show significant differences. Recapping the study of Macdougall et al. about IL-10 producing adipose tissue cDC1s during homeostatic conditions, higher frequencies of cDC1s might be an additional factor leading to differences between male and female VAT inflammation (Macdougall et al. 2018). However, we did not further investigate the specific phenotypes or cytokine profiles of the cDC subtypes.

Our characterization of the macrophage populations in the VAT of male mice fed with control or high fat diet showed an increase in $CD38^{-}$ $CD206^{+}$ macrophages and an elevated expression of CD206, the mannose receptor, in obese mice. Comparing male and female mice fed with normal diet, we found the same differences. The significantly lower cell count of macrophages and the shift to fewer $CD38^{-}$ $CD206^{+}$ macrophages in female mice suggests that this

population plays a role in adipose tissue inflammation. Using CD38 and CD206 could prove useful to distinguish adipose tissue macrophage populations; however, further work is needed to understand the development and function.

To further investigate the differences in male and female macrophages and cDCs, we performed RNAseq. We found that male macrophages and cDCs showed elevated gene expression of pro-inflammatory mediators, such as *Il1b*, *Il6* or *Ccl2*. Those genes are linked to VAT inflammation and insulin resistance (Coppack 2001; Hardy et al. 2011; Kang et al. 2016). Our data indicate that VAT macrophages and DCs differ between males and females not only numerically but also molecularly. This suggests that they play important roles in sex dependent differences in VAT inflammation.

Sex hormones control sexual dimorphism and sex-specific differences in immune responses (Taneja 2018). To determine if the observed differences in male and female cDCs could be induced directly via sex hormone signaling, we generated bone marrow derived DCs (BMDCs). Furthermore, we took into consideration that in recent years several studies showed indications for “trained immunity” (Mulder et al. 2019). A recent report from Christ *et al.* demonstrated that myeloid progenitor cells isolated from western diet treated mice showed an increased pro-inflammatory phenotype. This increased responsiveness to TLR stimuli was still evident after four weeks of control chow feeding (Christ et al. 2018). Therefore, we isolated BM from male and female mice from control chow or HFD treated mice. Furthermore, we exposed the BMDCs with the sex hormones estradiol or testosterone prior to LPS stimulation. Next, we measured their cytokine production. However, we did not detect significant differences between cells treated with estradiol or testosterone, nor differences between the sexes or HFD treated mice. An interesting finding is, that we obtained an IL-6 concentration from male and female mice treated with HFD but without LPS stimulation. Similar to MCP-1: here we found no signal in control diet fed female mice without LPS stimulation. However, these are preliminary results, further test have to validate these findings.

In this regard, we have to rule out that GM-CSF induced DCs might react different to treatment with sex hormones, since they already show a more monocytic DC-like phenotype, potentially resembling more closely DCs in the adipose tissue (Bhattacharya et al. 2015; Xu et al. 2007). Further, the use of an intermediate concentration of LPS and 24h stimulation might be able to override subtle differences between the BM generated cells. Due to difficulties during culturing of BM derived macrophages, we cannot yet comment if they show different phenotypes dependent on sex hormone stimulation or trained immunity. Lastly, this experiment has only been performed once. We have to further establish culture conditions and to test different concentrations of estradiol and testosterone but also LPS. Additionally, it might be of significant importance at what time point we stimulate the cells with the sex hormones. Here, we stimulated them after differentiation, however, sex hormones can already influence progenitor cells in the BM and affect their differentiation process, what we need to take into consideration as well (Carreras et al. 2008).

Taken together, we showed that both macrophages and cDCs accumulate in HFD treated perigonadal adipose tissue in male mice. Furthermore, we found increased frequencies of macrophages and cDCs in adipose tissue of control chow fed male mice, compared to female mice. Closer investigation of their genetic profile showed that male macrophages and cDCs show upregulated pro-inflammatory mediators. These differences indicate that both myeloid cell types could contribute to sex specific differences in male and female adipose tissue physiology. However, their specific contribution to VAT inflammation has to be addressed in further studies. Using the macrophage cell surface markers CD38 and CD206 showed that they distinguish at least three adipose tissue macrophage populations. We found a shift during obesity from double positive cells to CD206 single positive cells, which was also evident in differences between male and female AT macrophages. The usefulness of these marker needs to be further explored.

Chapter 4 - The IL-33/ST2 axis in crystal-induced nephropathy

4.1 Introduction

The alarmin IL-33 and its receptor ST2 play various roles in health and disease. A study of the host defense against *Trichuris muris,* a mouse nematode parasite, was one of the first to show a protective function of IL-33. Here, the exogenous administration of IL-33 prevented the parasite-driven T_H1 polarization, lead to IL-4, IL-9 and IL-13 cytokine induction and generated a protective T_H2 response (Humphreys et al. 2008). Further reports showed that mice deficient for IL-33 have a limited potential to clear worm infections, due to the role of T_H2 cytokines in worm expulsion (Hung et al. 2013; Yasuda et al. 2012). In a model of cerebral malaria strong T_H1 responses have been observed, which led to vascular leakage of blood vessels in the brain. Here, IL-33 treatment of mice infected with *Plasmodium berghei* ANKA resulted in protection from cerebral malaria due to the reduction of IFNγ, IL-12 and TNFα. The authors showed that IL-33 administration lead to the expansion of ILC2s, increased frequencies of M2 macrophages and T_{regs} and thus a suppression of pro-inflammatory damage. However, no significant impact on parasite burden was observed (Besnard et al. 2015). In inflammatory bowel disease (IBD) ST2-ko mice or the use of ST2 blocking antibody significantly reduced colitis in mice. Furthermore, IL-33 was shown to enhance intestinal inflammation via non-hematopoietic cells and therefore, the authors concluded that the IL-33/ST2 axis negatively influences colitis progression (Sedhom et al. 2013).

The adipose tissue shows high levels of IL-33 and ST2 expression, and high body mass index had been correlated with low serum level of IL-33 suggesting a protective role of IL-33 against obesity in humans (Hasan et al. 2014). In a high fat diet induced obesity model, ST2-ko mice showed increased body weight and adipose tissue accompanied by impairment of insulin and glucose regulation (N. Jovicic et al. 2015). Again, the T_H2 polarization and secretion of type 2 cytokines, polarization of M2 macrophages, accumulation of ILC2s and T_{regs} are thought to be mediated via a protective effect of IL-33 which is important for adipose tissue health (Kolodin et al. 2015; Miller et al. 2010; Vasanthakumar et al. 2015).

IL-33 mediates a dual role in kidney disease. In a model cis-platin model of acute kidney disease (AKI) Akcay *et al.* neutralized the function of IL-33 by using soluble ST2 (sST2). The authors found less CD4+ T cell infiltration, lower level of tubular necrosis, apoptosis and lower serum creatinine. Whereas, the administration of rIL-33 increased the before mentioned parameters, an effect not observed in CD4 knockout mice, suggesting a CD4 T cell/IL-33 axis involved in AKI tissue damage (Akcay et al. 2011).

In contrast, another group generated an IL-2 – IL-33 hybrid cytokine (IL233), which more efficiently enhanced T_{reg} and ILC2 mediated protection in AKI compared to a coinjection of IL-2 and IL-33 alone, and showed that the accumulation of T_{regs} and ILC2s is sufficient to ameliorated renal injury (Stremska et al. 2017). Similarly, Riedel et al reported that short-term injection of IL-33 in an adriamycin induced glomerulosclerosis model ameliorated disease by expanding ILC2s (Riedel et al. 2017).

Taken together, those studies showed a multifaceted function of IL-33. Depending, on disease model and organ IL-33 or ST2 might improve or exacerbate disease outcome. Mechanisms of protection are expansion of T_{regs}, ILC2 or the polarization of macrophages to alternative activated macrophages.

Also, in the kidney macrophages can possess dual properties. Their role as “pro-inflammatory” cells was shown in a cGN model, where the ablation of macrophages led to disease attenuation and tubular injury (Jeremy S. Duffield et al. 2005). However, they play also an important role in disease progression and the resolution of renal inflammation or fibrosis (Anders and Ryu 2011).

The aim of the following chapter was therefore to investigate what role the IL-33/ST2 axis plays in a crystal-induced nephropathy model and if IL-33 treatment might be applicate as a therapeutic agent to protect from renal damage.

4.2 Results

4.2.1 Il33 expression in the homeostatic and inflamed kidney

In order to assess the role of IL-33 during chronic inflammation we employed an established disease model of adenine-crystal-induced nephropathy (Correa-Costa et al. 2011; Ludwig-Portugall et al. 2016). In this model, mice are fed with 0.2% adenine-enriched food for 21 days and subsequently analyzed. First, we quantified the release of IL-33 due to disease induction. Here, we MACS separated $CD45^+$ hematopoietic from $CD45^-$ non-hematopoietic kidney cells from mice fed with either control diet or adenine-enriched (AF) diet and cultured them overnight. Subsequently, we analyzed the supernatant for the concentration of IL-33 protein and found increased concentrations in the $CD45^-$ cells populations due to kidney inflammation (Figure 4-1, A)

To further identify the IL-33 expressing compartment and how it may change with inflammation, we utilized a reporter mouse line, in which the coding sequence for the GFP variant citrine is inserted immediately after the *Il33* start codon ($Il33^{cit/+}$ mice (Hardman, Panova, and McKenzie 2013). First, the $CD45^-$ non-hematopoietic cell compartment was examined. Using multicolor flow cytometry to analyze single cell suspensions of total kidneys (gated on living $CD45^-$ $Ter119^-$ as depicted in Figure 4-1, B), we observed *Il33* to be predominantly expressed by $CD31^+$ endothelial cells (Figure 4-1, B, C). We detected *Il33* expression in animals on normal diet (control). Remarkably, around 50% of $CD31^+$ cells and less than 0.03% of $CD31^-$ cells expressed *Il33* in steady state. During kidney inflammation, the frequency of *Il33* expressing $CD31^+$ endothelial cells significantly increased, and we saw a non-significant increase among the $CD31^-$ cell compartment (Figure 4-1, D). However, we found that the total numbers of $CD31^+$ and $CD31^-$ cells expressing *Il33* did not significantly differ between animals on control or adenine-enriched diet.

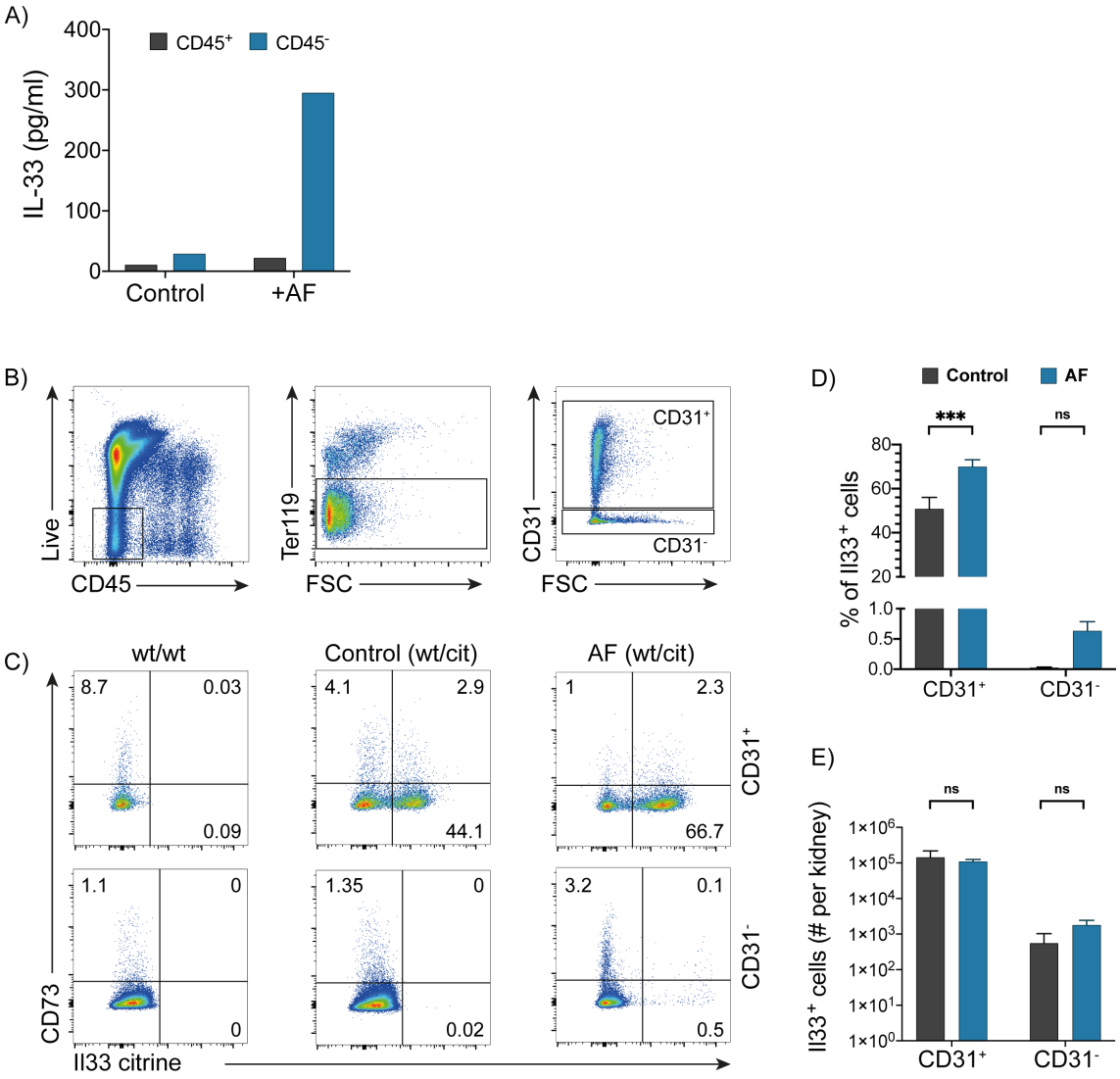


Figure 4-1 *Il33* expression increases in murine kidneys during crystal induced nephropathy

Mice are fed with normal diet (control) or 0.2% adenine-enriched diet (AF) for 21 days. (**A**) ELISA quantified concentration of IL-33 in supernatant of MACS ($CD45^{+/-}$) separated kidney cells after overnight incubation (**B**) Gating strategy for non-hematopoietic cells: live $CD45^-$ $Ter119^-$; $CD31^+$ vs $CD31^-$. (**C**) Gating of Il33 expression in $CD31^+$ (upper panel) and $CD31^-$ (lower panel) cells. Left plots show wild type control, middle plots *Il33* citrine reporter (wt/cit) on normal diet (control) and right plots *Il33* reporter on 0.2% adenine-enriched diet. (**D**) Frequency of *Il33* expressing cells among the $CD31^+$ and $CD31^-$ populations. (**E**) Total cell numbers per kidney of *Il33* expressing $CD31^+$ and $CD31^-$ cells (**D**). (A) n=1, 3 pooled mice per data point. (D, E) Data shown represents the mean ± SD (2-3 mice per group, representative for 2 independent experiments). Asterisks indicate statistically significant differences versus normal diet controls assessed by one-way ANOVA (*** $p<0.001$).

To more specifically characterize which cells are producing the IL-33 we gated *Il33* expression versus CD73. This is based on the unpublished observation of the Kallies lab according to which $CD73^+$ stromal cells are a major source of IL-33 in the adipose tissue. However, in the kidney only a small fraction of *Il33*$^+$ cells expressed additionally $CD73^+$ (Figure 4-1, C).

Our data indicate that *Il33* expression among kidney stromal cells is predominantly localized to $CD31^+$ endothelial cells. This population also displayed an increase in frequency of cells expressing the alarmin following the induction of sterile inflammation, suggesting a role of endothelia in *Il33* production in both homeostasis and disease.

We then investigated the *Il33* production of hematopoietic cells ($CD45^+$), which have also been reported to express this cytokine, in the kidney. We distinguished dendritic cells (DCs, live $CD45^+TCRbeta^-CD19^-CD64^-F4/80^-MHC\text{-}II^+CD11c^+$) in two distinct subsets: cDC1 ($XCR1^+$) and cDC2 ($XCR1^-$ $CD11b^+$) (gating strategy Figure 4-2, A). Further, we used XCR1 versus *Il33* citrine (Figure 4-2, B) to identify which DC subpopulation was producing IL-33. We observed minimal *Il33* expression among kidney DCs during homeostasis, but a significant increase in the percentage and total cell numbers of cDC2s but not cDC1s expressing *Il33* following crystal-induced kidney inflammation (Figure 4-2, C). Gating on macrophages (Macs, live $CD45^+TCRbeta^-CD19^-CD64^+F4/80^+$, Figure 4-2, A) we found that these cells also upregulated *Il33* expression during the course of inflammation. In homeostatic conditions we observed minimal *Il33* expression by macrophages, which increased to around 1% expression in inflammatory conditions (Figure 4-2, E). Similar to the DC compartment, *Il33* expression among macrophages was observed specifically by $CD11b^+$ cells (Figure 4-2, D). Finally, comparing the enumeration of *Il33* expressing cells in the hematopoietic (Figure 4-2 C, E) and non-hematopoietic cells (Figure 4-1, E), about one tenth as many DCs and macrophages express *Il33* in the kidney compared with stromal cells during crystal-induced nephropathy.

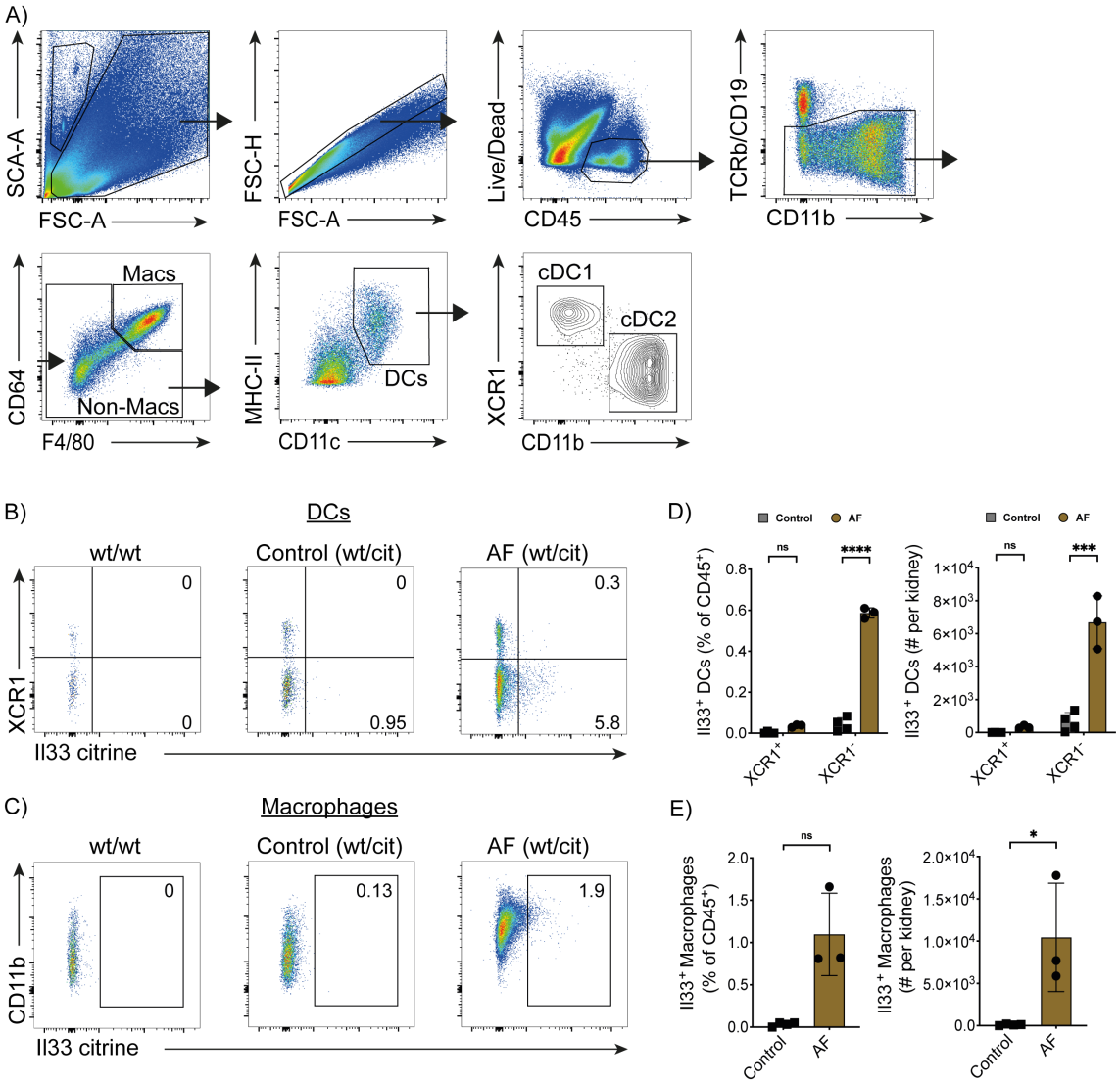


Figure 4-2 IL-33 expression of dendritic cells and macrophages in chronic kidney disease.

(**A**) Representative gating strategy for DCs and macrophages (FCS-A vs SSC-A to discriminate debris; FSC-A vs FSC-H gating out doublets; Live/dead vs CD45$^+$ to gate on hematopoietic cells; gating out T and B cells via TCRβ and CD19; CD64 / F4/80 double positive cells are considered macrophages (Macs). Non-macrophages (Non-Macs) are further gated on MHC-II vs CD11c: double positives are DCs. DCs are further subdivided by using XCR1$^+$ as cDC1s and CD11b$^+$ as cDC2s. (**B, C**) Left plots show wild type control, middle plots control chow fed *Il33* reporter (wt/gfp) and right plots 0.2% adenine food fed reporter cells at day 21. (**B**) Pre-gated on DCs and (**C**) macrophages. (**D, E**) Graphs show frequencies and total numbers of *Il33* expressing DC's (**D**) and macrophages (**E**). Data represent the mean ± SD (3-4 mice per group, representative for two independent experiments). Asterisks indicate statistically significant differences versus controls as assessed by (B) two-way ANOVA and (C) Student´s two-tailed T test (* p<0.05, **** p<0.0001).

Together these data identify populations of myeloid cells in the kidney that express *Il33* in response to sterile inflammation and constitute a notable proportion of the *Il33*$^{+}$ compartment within the organ.

Having observed DCs and macrophages to express *Il33* we asked if other myeloid cells could be identified to express *Il33* in the kidney. Therefore, we additionally applied the gating strategy depicted in Figure 4-3, A: cells pre-gated on TCRβ^{-} CD19^{-} (Figure 4-2, A) were further gated on CD11b^{+} and then divided into monocytes (Ly6C^{+} Ly6G^{-}) and PMNs (Ly6C^{int} Ly6G^{+}). We observed minimal expression of *Il33* in monocytes and PMNs during homeostasis (control, Figure 4-3, C and D). However, similar to macrophages and Dendritic cells we identified significantly elevated expression in inflammatory conditions (Figure 4-3, C and D). Interestingly, expression of PD-L1 (programed death-ligand 1, an inhibitor of T cell function) correlated with *Il33* expression among monocytes as well as PMNs (Figure 4-3, B).

Here, we identified that CD31^{+} endothelial cells represent the predominant *Il33* producing cells in the kidney. Additionally, we also found that immune cells of the myeloid cell lineage also contribute to the pool of *Il33*-producing cells. The relatively low number of those cells compared to the CD31^{+} endothelial cells may indicate a lesser role for myeloid-derived *Il33*, or perhaps a more targeted effect of *Il33* produced by these motile cell types.

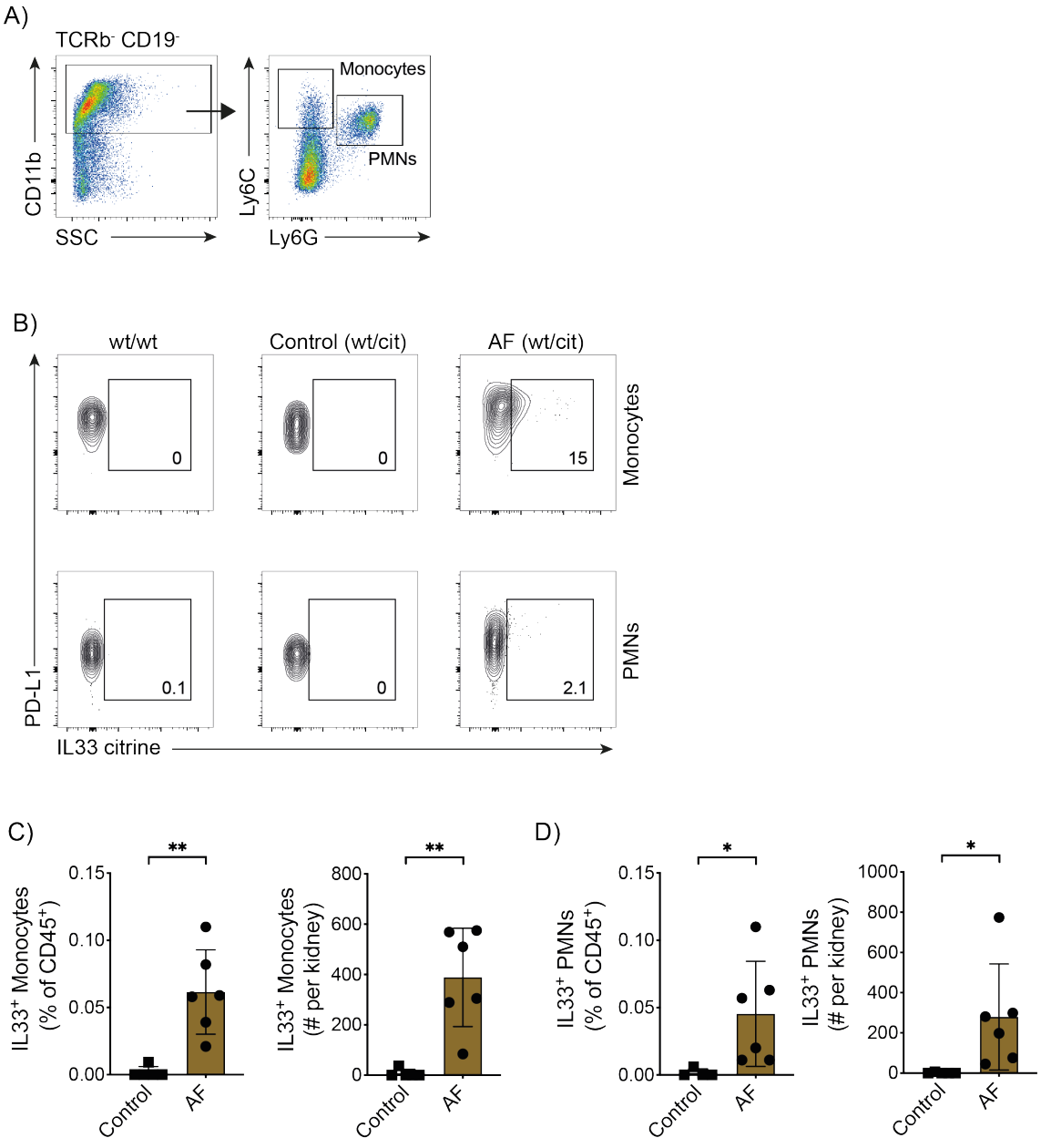


Figure 4-3 Monocytes and PMNs express IL-33 in chronic kidney disease.

(**A**) Representative gating strategy for monocytes and PMNs. Cells are pre-gated as TCRβ^{-}CD19^{-} (see Figure 1 A), CD11b^{+}, Ly6C^{+} Ly6G^{-} cells are considered monocytes, Ly6C^{int} Ly6G^{+} are considered PMNs. (**B**) Left plots show wild type control, middle plots control chow fed IL-33 reporter (wt/cit) and right plots 0.2% adenine food fed reporter cells at day 21. Upper panel is pre-gated on monocytes and lower panel on PMNs. (**C**) Graphs show percentages and total cell numbers of IL-33 expressing monocytes. (**D**) Graphs show percentages and total cell numbers of IL-33 expressing PMNs. Data shown represents the mean ± SD (n=4-5 mice per group, pooled data from two independent experiments).

4.2.2 ST2 receptor is expressed on myeloid cells

To determine whether hematopoietic cells might represent targets of the IL-33 produced during kidney inflammation we investigated the expression of the IL-33 receptor (ST2) on these cells using flow cytometry. First, we investigated monocytes and PMNs (Figure 4-4, A). Similar to *Il33*, we observed increased expression of ST2 during inflammation on all investigated cell types (Figure 4-4, A-G). We observed monocytes to not express ST2 in steady state (control) but upregulate it significantly in the inflammatory environment of adenine-enriched diet fed mice (AF) (Figure 4-4, A and B). A similar trend was evident among PMNs (Figure 4-4, C), although a small fraction (<1%) already expressed ST2 in non-inflammatory state (control).

We observed that a small of fraction macrophages and DCs already in steady state expressed ST2. Induction of crystal-induced nephropathy let to a significant increase in ST2 expression on macrophages, cDC1s and cDC2s. In total, macrophages displayed the greatest numerical increase in ST2 expressing cells (Figure 4-4, E).

These results show that inflammation leads to upregulation of the IL-33 receptor ST2 on monocytes, PMNs, macrophages and dendritic cells. Therefore, these cells may be able to react to the IL-33 released during the inflammatory process and might have an influence on the development of kidney fibrosis.

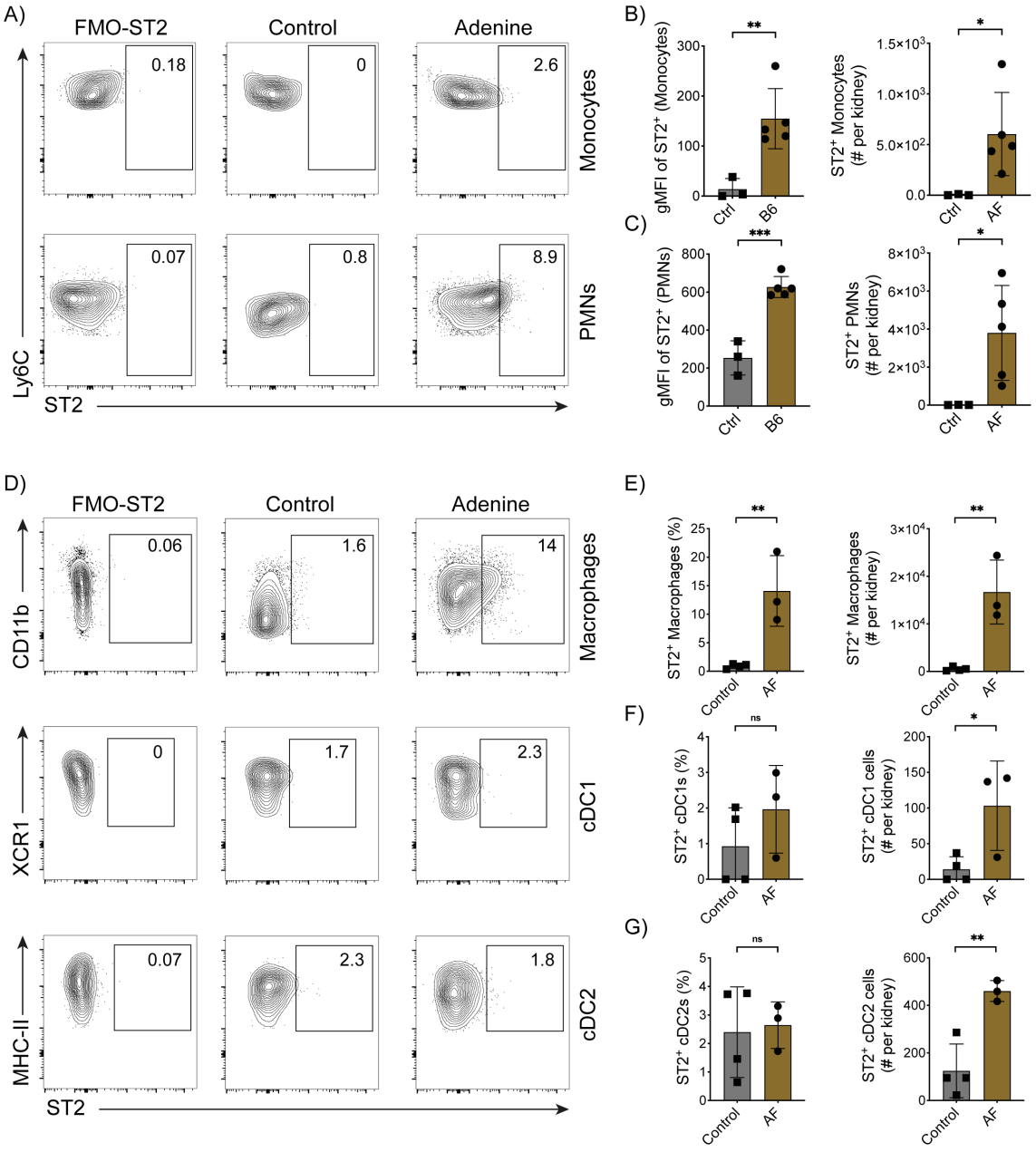


Figure 4-4 ST2 expression of myeloid cells during chronic kidney disease.

(**A+D**) Representative FACS plots showing ST2-FMO control (left), untreated control (middle) and 0.2% adenine food fed mice at day 21 (right). Pre-gating is depicted in Figure 1A and Figure 2A. (**A**) Representative FACS plots gated on Ly6C vs ST2, shown are monocytes (upper panel) and PMNs (lower panel). Graphs show frequencies and total numbers of ST2 expressing (**B**) monocytes and (**C)** PMNs. (**D**) Representative FACS plots for ST2 expression. Upper panel shows macrophages, middle panel shows cDC1 and lower panel cDC2. Graphs show frequencies and total numbers of ST2 expressing (**E**) macrophages (**F**) cDC1 and (**G)** cDC2. Data shown represent the mean ± SD (3-5 mice per group, representative for two independent experiments). Asterisks indicate statistically significant differences versus ND controls as assessed by Student´s two-tailed T test (* $p<0.05$, ** $p<0.01$, *** $p<0.001$)

4.2.3 The loss of the ST-2 receptor has no significant impact on severe crystal-induced nephropathy model.

Having identified considerable induction of both IL-33 and its receptor during sterile inflammation in the kidney, we sought to test the function of the IL-33/ST2 axis on disease. Since different publications show that IL-33 can work in a pro- or anti-inflammatory manner, we asked what the role of the IL-33/ST2 axis in crystal-induced nephropathy is. To answer this question we used ST2 receptor knockout mice (Townsend et al. 2000) and compared them to wild type mice using the same model of crystal-induced nephropathy as before (Figure 4-5, A). To analyze kidney damage different readouts were utilized. Kidney injury marker-1 (KIM-1, KIM) and tissue inhibitor of metalloproteinases 1 (TIMP1) are established readouts of renal fibrosis (for more details see introduction 1.5.1). Urine levels of KIM and serum levels of TIMP1 were strongly increased in mice on an adenine diet compared to control mice; however, we did not detect significant differences between wild type and ST2 knockout mice (Figure 4-5, A+B). Similarly, the pathohistological results for alpha-SMA and Collagen III, both indicators for kidney fibrosis, showed significant increases in mice on a adenine-enriched diet compared to control mice, but there were no differences between wild type and knockout mice (Figure 4-5 ,C+D). Additionally, we tested the urine and blood serum for creatinine levels, an additional established readout for kidney damage (for more details see introduction 1.5.1). No significant differences were found (Figure 4-5, E+F). The serum was further analyzed for blood urea nitrogen (BUN), an additionally indicator for loss of kidney function. This was the only marker that was significantly lower in ST2 knockout mice compared to wild type mice fed with adenine-enriched diet (Figure 4-5, G). Furthermore, we used RT-qPCR analysis to detect changes at mRNA level. We examined transcripts for several markers, such as αSMA, collagen I and IV, fibronectin, TGFβ, TNFα and vimentin, which are connected to kidney fibrosis. However, there were no significant differences between wild type and ST2 knockout mice (Figure 4-5, H).

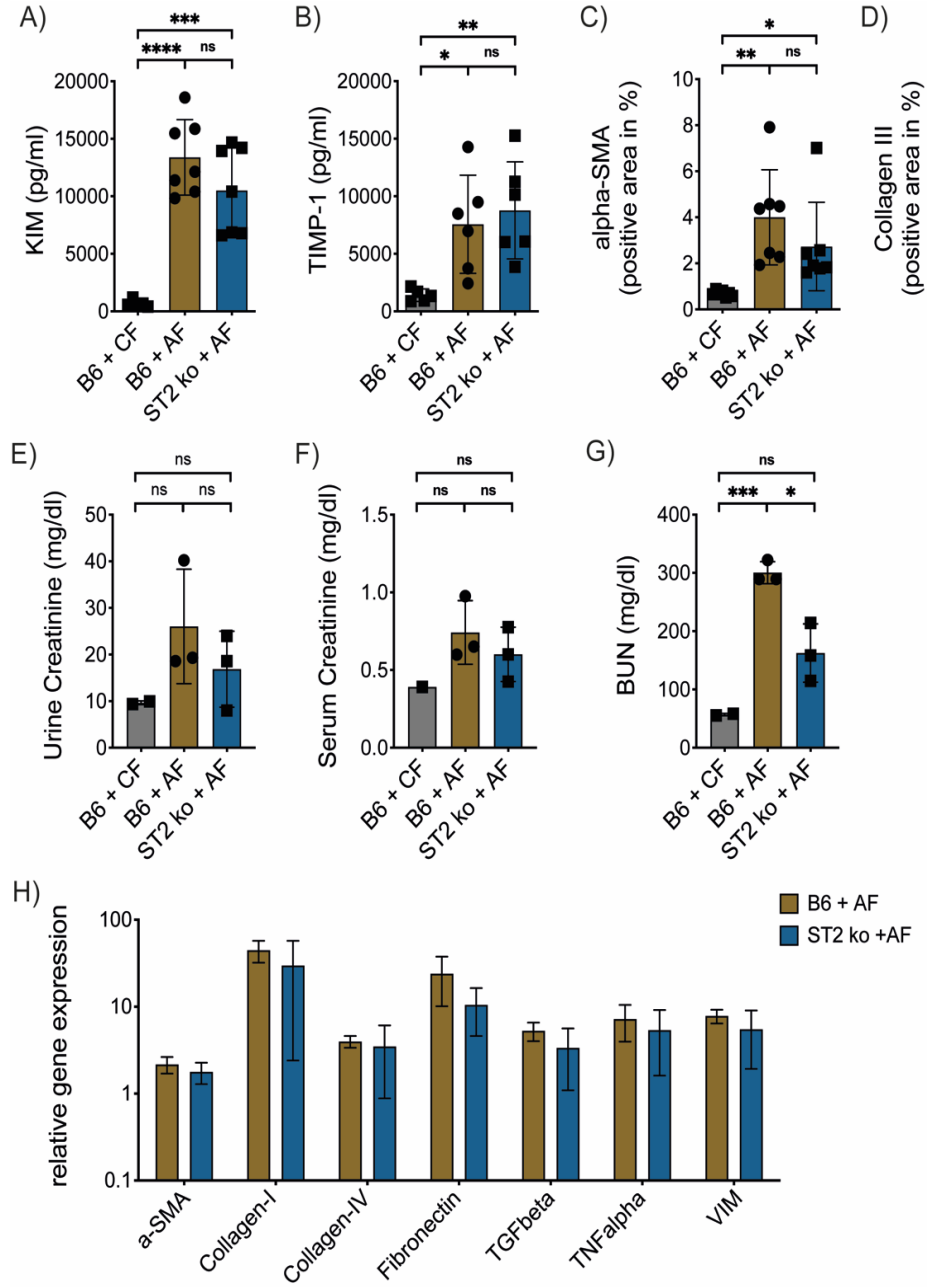


Figure 4-5 Influence of ST2 receptor loss on physiological kidney parameters during CKD.

Mice were fed with control diet (CF) or 0.2% adenine-enriched diet (AF) for 21 days. (**A**) KIM (urine) and (**B**) TIMP-1 (serum) measurements. (**C, D**) Pathohistological analysis of alpha-SMA and collagen III positive area of kidney sections. (**E**) Creatinine levels of urine. (**F**) Creatinine levels of serum. (**G**) Blood urea nitrogen (BUN) levels of serum. (**H**) Analysis of different kidney fibrosis related genes via qPCR of whole kidney lysate. Data represent the mean ± SD. (A-D) Data pooled from two independent experiments. (E-G) Data from one experiment. (H) Representative for two independent experiments. Asterisks indicate statistically significant differences versus ND controls as assessed by Student´s two-tailed T test (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

Taken together, our results indicate that deficiency in the IL-33 receptor ST2 has no significant impact on the development of kidney fibrosis in the severe adenine-induced crystal nephropathy. However, in most of the results we saw a general trend toward less damage among the ST2 knockout cohort, which might still indicate that IL-33 and ST2 play a role in kidney inflammation under some conditions.

4.2.4 Influence of the alarmin IL-33 and its receptor ST2 in a long term, mild onset chronic kidney disease model

In recent literature a time and dose dependent manner for the alarmin IL-33 is discussed. Furthermore, IL-33 appears to play a particularly important role in low-grade inflammation such as observed in the adipose tissue (see introduction of this chapter). To determine the role of IL-33 and its receptor ST2 in a mild onset chronic kidney disease model, we reduced the concentration of adenine in the mouse diet from 0.2% to 0.15%. The concentration reduction allowed us to keep the mice for 42 days on adenine-enriched diet and therefore mimics a more chronic disease progression with the possibility of healing of the kidney damage that was induced (Figure 4-6, A).

4.2.4.1 IL-33 expression in mild onset chronic kidney disease model

Using the new model setup by feeding the mice with 0.15% adenine-enriched chow for a mild onset chronic kidney disease, we first analyzed if and in which quantity the expression of IL-33 changes compared to the fast-onset model. To this end, we utilized flow cytometric analysis of *Il33* reporter (wt/gfp) mice. Notable, this *Il33* reporter line is a different line used for Figure 4-1, Figure 4-2 and Figure 4-3. *Il33*^{GFP} (Oboki *et al.*, 2010) mice have exon 2 of the *Il33* gene replaced with a *GFP* gene whereas a *Citrine* gene was directly inserted after the *Il33* start codon of *Il33*^{cit} mice (Hardman et al. 2013).

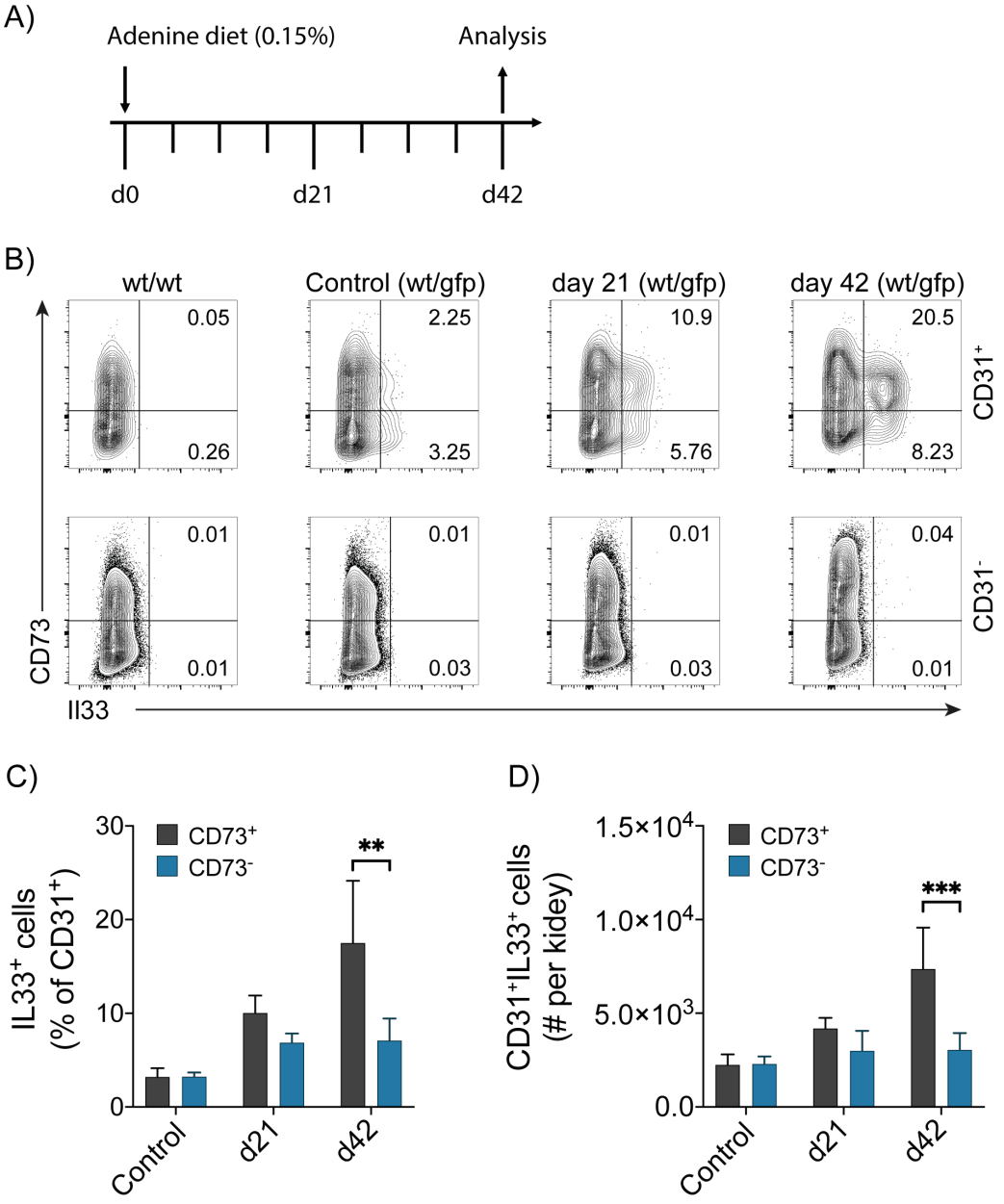


Figure 4-6 IL-33 expression of non-hematopoietic cells during long term chronic kidney disease.

(**A**) Model setup: mice were fed with control or 0.15% adenine-enriched diet for 21 days or 42 days. (**B**) Representative gating strategy pre-gated on live CD45$^-$, Ter119$^-$ cells (Figure 4-1, B). Upper panel represents CD31$^+$ fraction and lower panel CD31$^-$ fraction. Left panel shows wild type control (wt/wt), second panel IL-33 reporter (wt/gfp) with control diet, third and fourth panel *Il33* reporter with 0.15% adenine-enriched diet at day 21 and day 42. (**C**) Frequency and (**D**) total cell numbers of CD31$^+$ CD73$^+$ or CD73$^-$ cells. Data shown represents the mean ± SD (3-5 mice per group, representative for two independent experiments). Asterisks indicate statistically significant differences assessed by two-way ANOVA test (** p<0.01, *** p<0.001).

We applied the same gating strategy used before (Figure 4-1, B) and in Figure 4-6, B a representative FACS plot is depicted: $CD45^-$ $Ter119^-$ cells were further divided into $CD31^+$ endothelial cell and $CD31^-$ non-endothelial cell populations. $CD31^-$ cells neither in hemostasis nor during inflammation expressed any *Il33*; this occurred only in the $CD31^+$ cell population. Furthermore, we observed that during the time course of the inflammation the amount of $IL\text{-}33^+$ cells of the $CD73^-$ population stayed stable, whereas we see a continual increase from day 0 to day 42 in the $IL\text{-}33^+CD73^+$ fraction (Figure 4-6, C, D).

Our data indicate that *Il33* expression increase over time in a mild onset chronic kidney disease model. The predominantly *Il33* expressing populations are $CD31^+$ endothelial cells, where we found differences in $CD73^+$ and $CD73^-$ cell subsets. Also, in the severe onset model *Il33* shows an increased expression and therefore might play an important role for tissue damage or repair.

4.2.4.2 Mild onset model leads to influx of macrophages and DCs into kidney

Having identified elevated *Il33* expression in the mild-onset model, we further characterized this model. First, we analyzed the KIM-1 concentration in the urine of 0.15% adenine-enriched diet fed (AF) mice and found elevated concentrations compared to mice fed with normal chow (CF). This increase was not significant (Figure 4-7, *A*) and in addition, the serum concentration for Timp-1 did not show differences between both groups (Figure 4-7, *B*). On gene level, we observed non-significant elevated expression for αSMA, collagen I, fibronectin and vimentin in AF mice. Collagen III and TGFβ had been significantly elevated in AF mice and TNFα expression did not differ (Figure 4-7, *C*). These results indicated a weaker kidney inflammation and renal damage than described in the strong onset model. To further elucidate these findings, we next analyzed flow cytometry data for macrophages and DCs. Here, we found a not significant increase in macrophages due to adenine-diet feeding in AF mice (Figure 4-7, *D*). Conventional dendritic cells significantly increased in kidneys of AF mice (Figure 4-7, *E*).

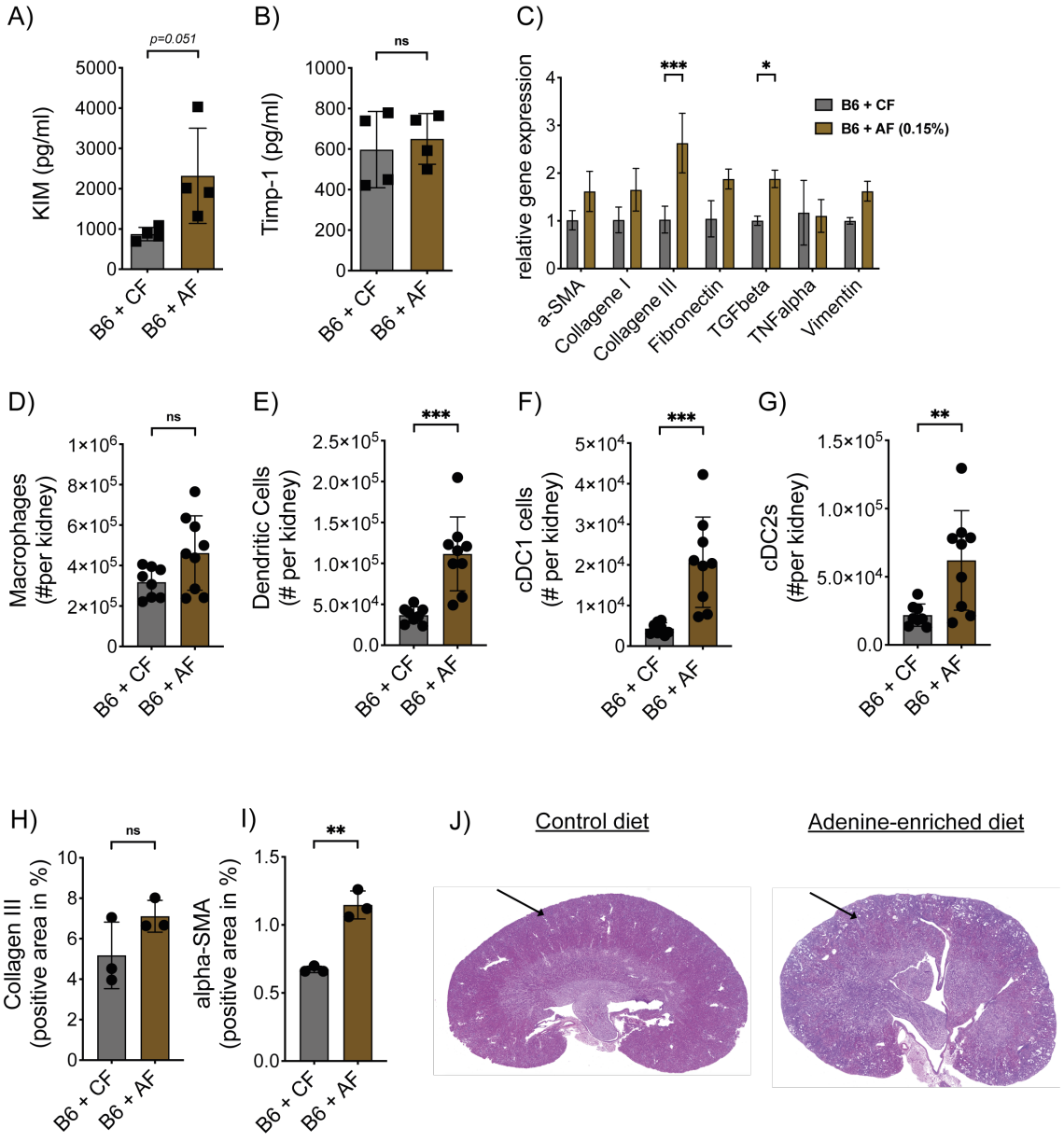


Figure 4-7 Mild onset model leads to myeloid infiltration and kidney damage

Mice were fed with control (CF) or 0.15% adenine-enriched diet (AF) for 42 days. (**A**) KIM-1 concentration. (**B**) TIMP-1 concentration. (**C**) RT-qPCR data for several fibrosis linked genes relative to B6 CF mice. Total cell numbers per kidney of (**D**) macrophages, (**E**), cDCs, (**F**) cDC1s and (**G**) cDC2s. Pathohistological data for alpha SMA (**H**) and Collagen III (**I**) positive stained area per kidney section. (**J**) Representative H&E stained kidney sections of CF mouse (left) and AF mouse (right). Data shown represent the mean ± SD (3-5 mice per group, (A-C) representative for two independent experiments, (D-G) pooled data from two independent experiments, (H, I) One experiment). Asterisks indicate statistically significant differences assessed by students T test (A, B, D-I) or one-way ANOVA (C) (* $p<0.05$, ** $p<0.01$, *** $p<0.001$)

Analyzing the two cDC compartments, we found that both cDC1s and cDC2s significantly accumulated in AF kidneys (Figure 4-7, *F, G*). Pathohistological analysis of kidney sections showed that collagen III is not significantly elevated in AF kidneys (Figure 4-7, H). However, treated kidneys showed significant increased positive areas for αSMA (Figure 4-7, H). The performed hematoxylin and eosin (H&E) staining for control and AF mice, showed a drastically change in cell infiltration (arrows) in the kidney (Figure 4-7, *H*).

Taken together, we found that in the mild onset model macrophages and DCs accumulate in the kidneys of treated mice. Even though KIM-1 and Timp-1 were not significantly elevated, we found significant increased gene expression of collagen III and TGFβ. Together with significantly increased staining for αSMA and the H&E staining, we found that feeding of 0.15% adenine-enriched diet leads to kidney damage. These results confirm the chronic character of this model.

4.2.4.3 Absence of IL-33 aggravates kidney damage in mild onset model

Having identified an increased cell number of *Il33* expressing non-hematopoietic cells in the mild-onset chronic disease model, we asked if the absence of the alarmin IL-33 or its receptor ST2 influences disease outcome.

First, we analyze the expression of molecules indicating kidney damage in urine (KIM-1) and blood serum (TIMP-1) via ELISA (Figure 4-8, *A*+*B*). KIM-1 concentration showed a significant increase in IL-33 knockout mice (IL-33ko) compared to wild type mice (B6). However, there was no significant difference in KIM-1 concentration between ST2 knockout mice (ST2ko) and B6 mice. The analysis for TIMP-1 showed that the absence of IL-33 led to significantly more TIMP-1 in the serum of the animals compared to B6 mice. Again, we saw no differences between B6 and ST2ko animals.

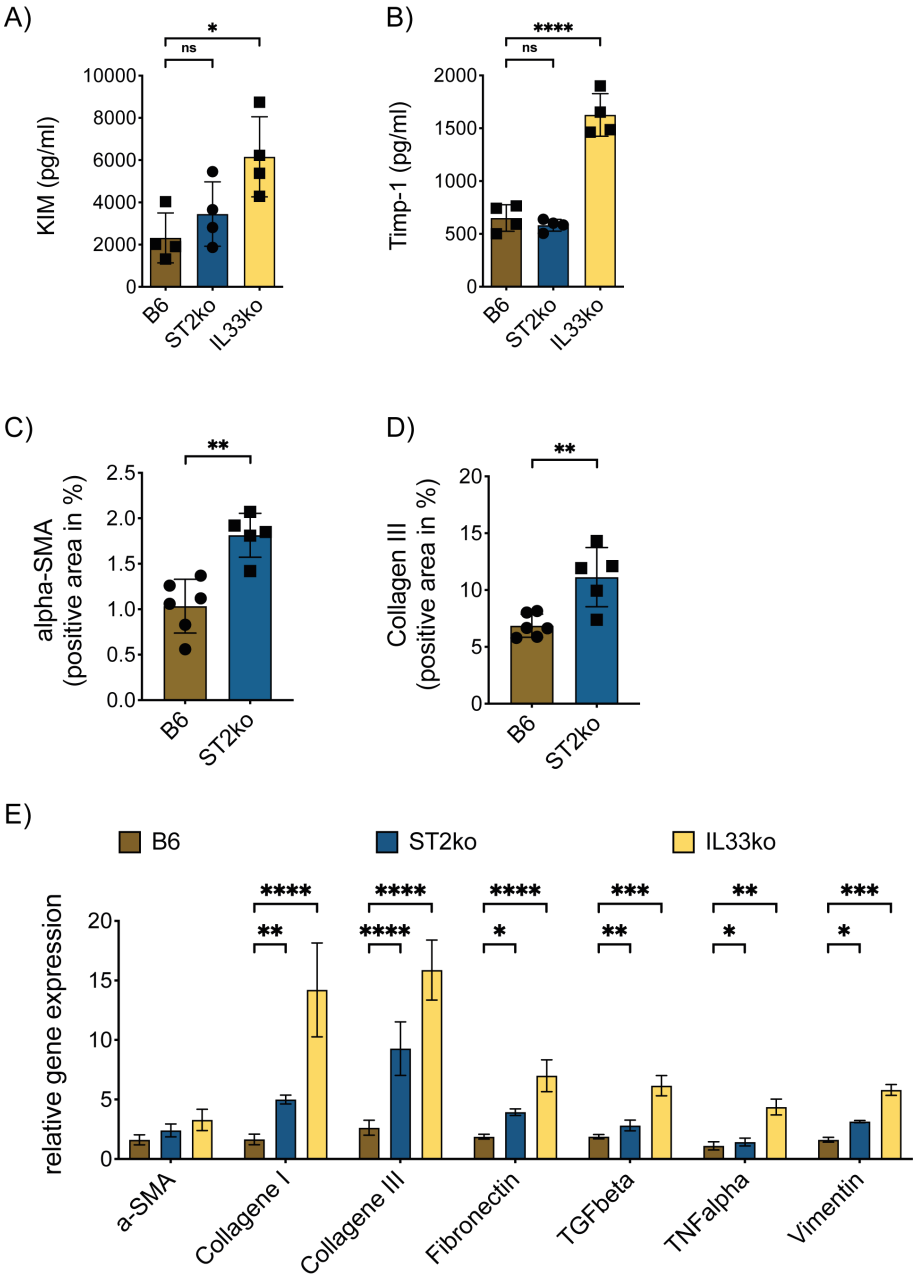


Figure 4-8 Influence of IL-33 or ST2 knockout in long term mild onset chronic kidney disease model.

Wild type (B6), ST2 knockout (ST2ko) and IL-33 knockout (IL-33ko) mice had been fed for 42 days with 0.15% adenine-enriched diet (AF). (**A**) KIM-1 concentration. (**B**) TIMP-1 concentration. (**C+D**) Pathohistological data for alpha SMA (**C**) and Collagen III (**D**) positive stained area per kidney section. (**E**) RT-qPCR data for several fibrosis linked genes relative to untreated control B6 mice. Data shown represent the mean ± SD. (A, B, E) 4 mice per group, representative for two independent experiments, (C) 5-6 mice per group, experiment performed once). Asterisks indicate statistically significant differences assessed by one-way ANOVA (A-E) or two-way ANOVA (**F**) (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$).

Pathohistological analysis of treated B6 and ST2ko mice revealed significant more positive area for αSMA and collagen III, indicating more tissue damage (Figure 4-8, *C, D*). Furthermore, differences in gene expression were analyzed with the help of RT-qPCR. We found that IL33ko mice and ST2koF showed a significant higher mRNA expression for collagen I and III, fibronectin, TGFβ, TNFα and vimentin but not for a-SMA, compared to wild type mice (Figure 4-8, *E*).

Together our data show increased damage in mice lacking either IL-33 or its receptor ST2 compared to treated wild type mice. This indicates that IL-33 and its receptor ST2 play a crucial role during the chronic crystal-induced kidney disease.

4.2.4.4 Absence of IL-33 leads to increased cell numbers of different myeloid cell types in mild-onset model

To identify a possible mechanism for those differences, we used flow cytometry to detect changes in the myeloid cell compartment, e.g. macrophages, dendritic cells, monocytes and PMNs.

Comparing the frequencies of macrophages and dendritic cells, we did not see any significant differences between wild type mice (B6), ST2 knockout (ST2ko) or IL-33 knockout (IL-33ko) mice (Figure 4-9, A). However, we found a significant increase between IL33ko and ST2ko and B6 mice in total cell numbers of macrophages (Figure 4-9, B). Interestingly, the macrophages B6 and ST2ko did not differ significantly. Also, total cell numbers of dendritic cells did not show significant differences between B6 and ST2ko mice. Dendritic cells of IL33ko mice were significantly elevated compared to B6 mice, but not compared to ST2ko mice.

Monocytes showed in frequency and total cell numbers a non-significant decrease comparing B6 mice to ST2ko.Monocytes of IL33ko mice had been significantly increased comparing them to ST2ko mice, but no significant difference to B6 mice. However, a tendency to increased monocyte numbers in IL33ko mice was observed. (Figure 4-9, *C*).

We found no significant differences in neither frequency nor total cell numbers of PMNs between the groups. (Figure 4-9, *D*).

Our observations showed that in the absence of IL-33, the number of macrophages, dendritic cells and monocytes was increased in the inflamed kidney. The additional infiltration of immune cells is an indicator for higher tissue damage observed in Figure 4-8. In the case of ST2ko mice, however, we were not able to observe a significantly higher influx of myeloid immune cells, which cannot explain the increased αSMA and collagen III scores in Figure 4-8. Lastly, we showed that the absence of the cytokine IL-33 had different effects on myeloid immune cells compared to the absence of its receptor ST2.

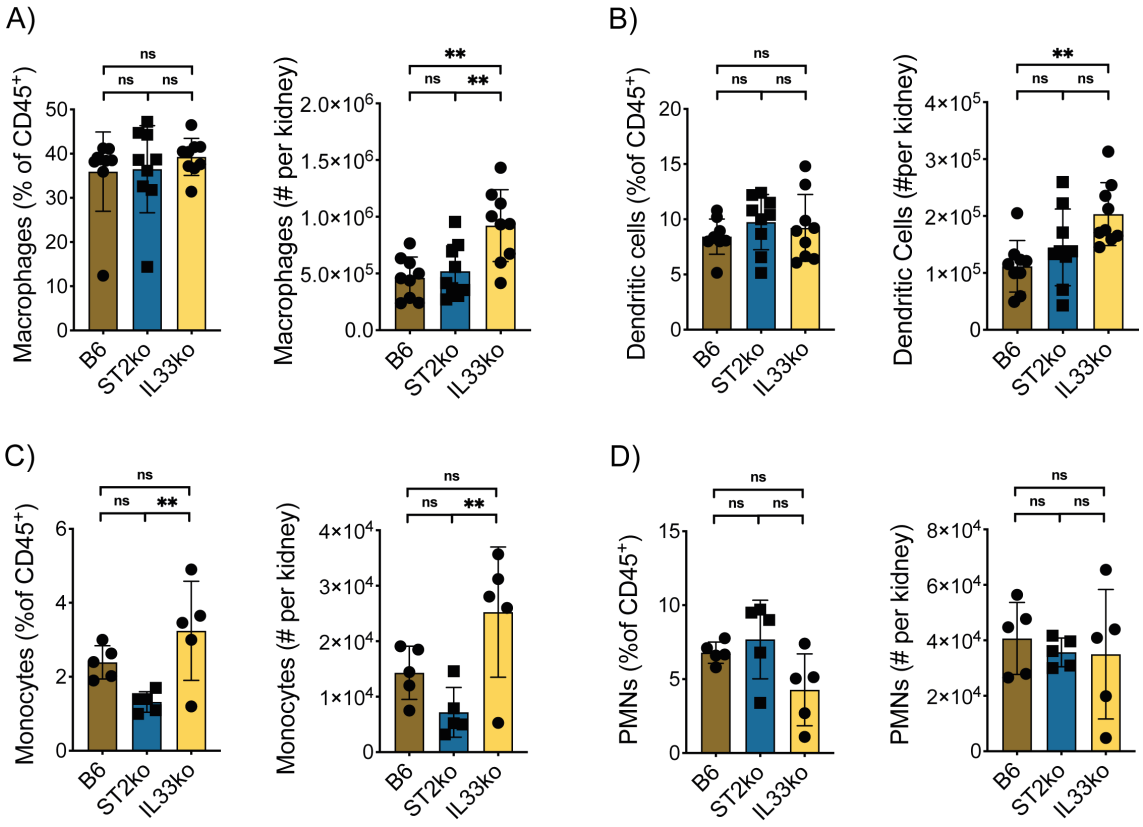


Figure 4-9 Myeloid cells during mild onset chronic kidney disease model.

Wild type (B6), ST2 knockout (ST2ko) and IL-33 knockout (IL-33ko) mice had been fed for 42 days with 0.15% adenine-enriched diet (AF). Frequencies and total cell numbers per kidney of (**A**) Macrophages, (**B**) dendritic cells, (**C**) Monocytes and (**D**) PMNs. Data shown represents the mean ± SD. (A, B) Data pooled from two independent experiments. (C, D) Data representative for two independent experiments. Asterisks indicate statistically significant differences assessed by one-way ANOVA (** $p<0.01$).

4.2.4.5 Regulatory T cells and ILC2s are increased in IL-33 knockout mice during mild onset model

Recent publications showed that ST2 and IL-33 play important roles for regulatory T cells and ILC2s in different tissues and disease models (Riedel et al. 2017; Schiering et al. 2014; Vasanthakumar et al. 2015). Therefore, we investigated in our mild-onset crystal-induced nephropathy model if those suppressive immune cells are affected due to the absence of IL-33 or ST2. Investigating CD4 T cells (Lineage$^+$ (CD3ε, CD11b, CD5, CD19, TCRβ, $\gamma\delta$ TCR, CD11c) CD4$^+$, gating strategy see Figure 4-10, *A*) we found no significant differences in frequency between the compared groups. However, the total numbers in IL33ko mice compared to B6 mice were significantly elevated (Figure 4-10, *B*). We found no significant differences of CD4 T cells between ST2ko and B6 mice.

Analyzing regulatory T cells (Lineage$^+$, CD4$^+$ Foxp3$^+$), we found no-differences in frequencies between the compared groups; however, total T_{reg} numbers per kidney were significantly increased in IL33ko mice. We did not observe significant differences in ST2ko compared to B6 mice (Figure 4-10, *C*). Interestingly, frequencies of KLRG1$^+$ effector T_{regs} were significantly increased in B6 mice, but neither in IL33ko nor ST2ko mice. However, total cell numbers of KLRG1$^+$ T_{regs} were not significantly different between B6 and ST2ko mice. Elevated, but not significantly, cell numbers were observed in IL33ko mice compared to the other groups (Figure 4-10, *D*). Furthermore, we found no significant differences in frequency or total cell numbers in CD8 T cells (Lineage$^+$CD8$^+$) between B6, ST2ko or IL33ko mice (Figure 4-10, *E*).

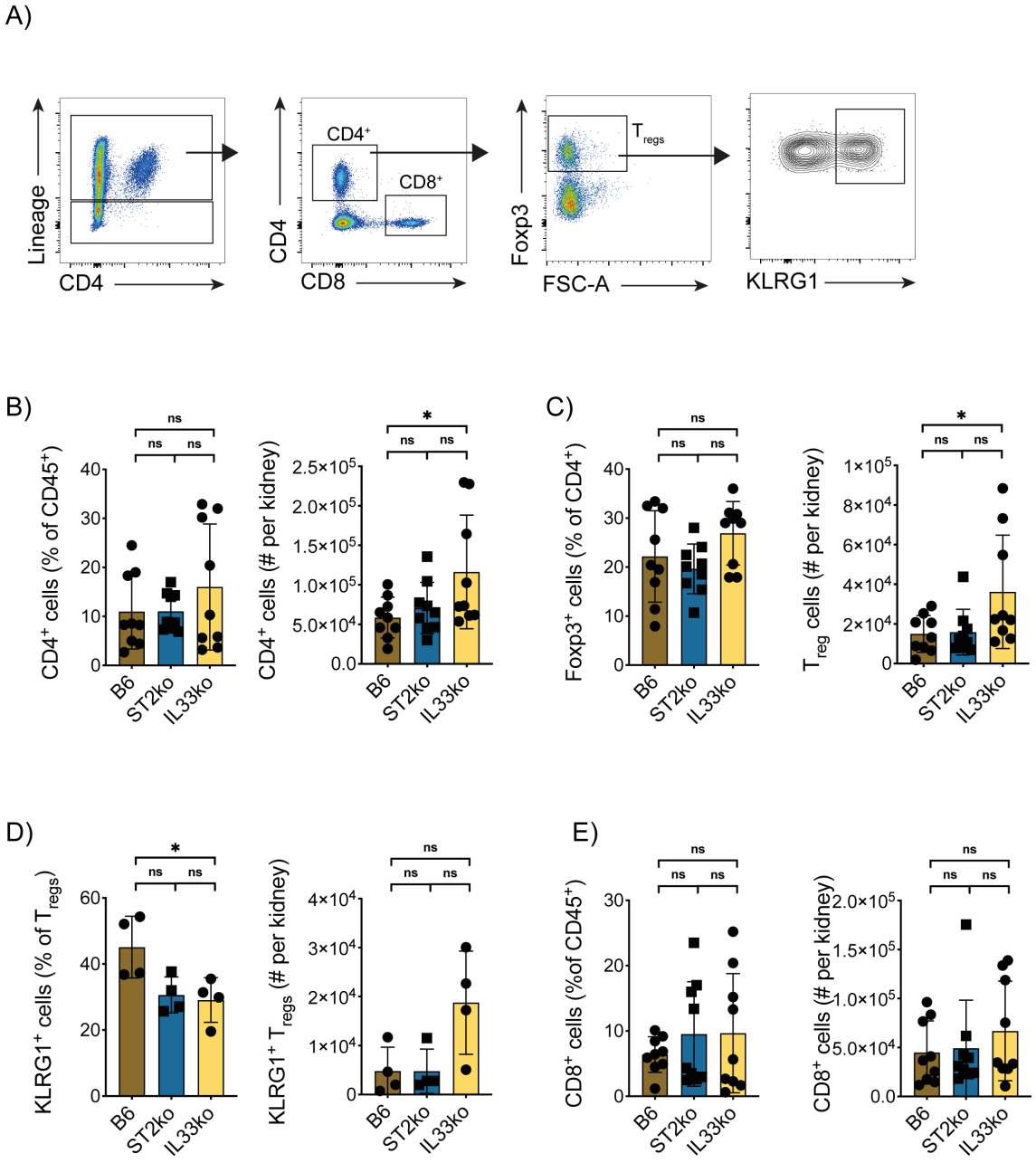


Figure 4-10 IL33ko mice show increased CD4 T cell numbers during mild-onset chronic kidney disease.

Mice were fed with 0.15% adenine-enriched diet for 42 days. (**A**) T cells were gated as follows: Lineage$^+$ (CD3ε, CD11b, CD5, CD19, TCRβ, γδ TCR, CD11c), CD4 T cells (CD4$^+$), CD8 T cells (CD8$^+$), T_{regs} (CD4$^+$, Foxp3$^+$). Frequency and total numbers of (**B**) CD4 T cells, (**C**) Fopx3$^+$ cells of CD4 T cells, (**D**) KLRG1$^+$ T_{regs} (**E**) CD8 T cells. Data represent the mean ± SD (n=4 mice per group, (B, D, E) Pooled data from two independent experiments, (D)representative for two independent experiments). Asterisks indicate statistically significant differences assessed by one-way ANOVA (* p<0.05, ** p<0.01, *** p<0.001).

Next, we analyzed the ILC2 compartment (Lineage$^-$GATA3$^+$CD90$^+$, gating strategy see Figure 4-11, A). Here, we found no significantly differences in frequencies between the three groups. Surprisingly, the numbers of ILC2s per kidney in IL33ko and ST2ko mice were significantly increased compared to the B6 group (Figure 4-11, B). Frequencies of KLRG1$^+$ ILC2s were significantly decreased in ST2ko mice, both compared to B6 and IL33ko mice. No differences between B6 and IL33ko mice was observed in of KLRG1$^+$ ILC2s frequency. However, IL33ko mice showed significantly increased numbers in KLRG1$^+$ ILC2s compared to B6 mice (Figure 4-11, C). We detected no significant differences between wild type and ST2ko mice, even though the total cell numbers of ST2ko showed a similar tendency as IL33ko mice. Interestingly, we saw significantly elevated numbers of ST2$^+$ ILC2s in IL33ko mice compared to B6 mice (Figure 4-11, *D*).

Together, our results show that the deficiency of the ST2 receptor only has minor effects in the CKD model. The amount of CD4 T cells, T_{regs}, and effector T_{regs} did not differ significantly compared to wild type mice. However, ST2ko mice showed significant differences in frequencies of KLRG1$^+$ T_{regs} and in total cell number of ILC2 compared to wild type mice. Furthermore, we showed that CD4 T cells, T_{regs}, ILC2s and KLRG1$^+$ ILC2s increased in cell numbers in the absence of IL-33. This was associated with increased pathology. Considering it had been shown that IL-33 has the potential to locally expand T_{regs} and ILC2s, our results are puzzling. Especially the significant higher number of ILC2s in both ST2ko and IL33ko kidneys indicates that these cells might not depend on IL-33 for their local proliferation or accumulation. The significant increase of ST2$^+$ ILC2s in IL33ko mice might suggest that those cells accumulate during enhanced inflammation. Higher number of activated (KLRG1$^+$) ILC2s in ST2ko and IL33ko might also be an indicator for higher inflammation. Maybe due to reduced frequency of KLRG1$^+$ T_{regs}. However, the role of ST2 and IL-33 in this model remains elusive. We will address these open questions with mixed bone marrow chimera (ST2ko) and a more detailed characterization of T_{regs} and ILC2s in this model.

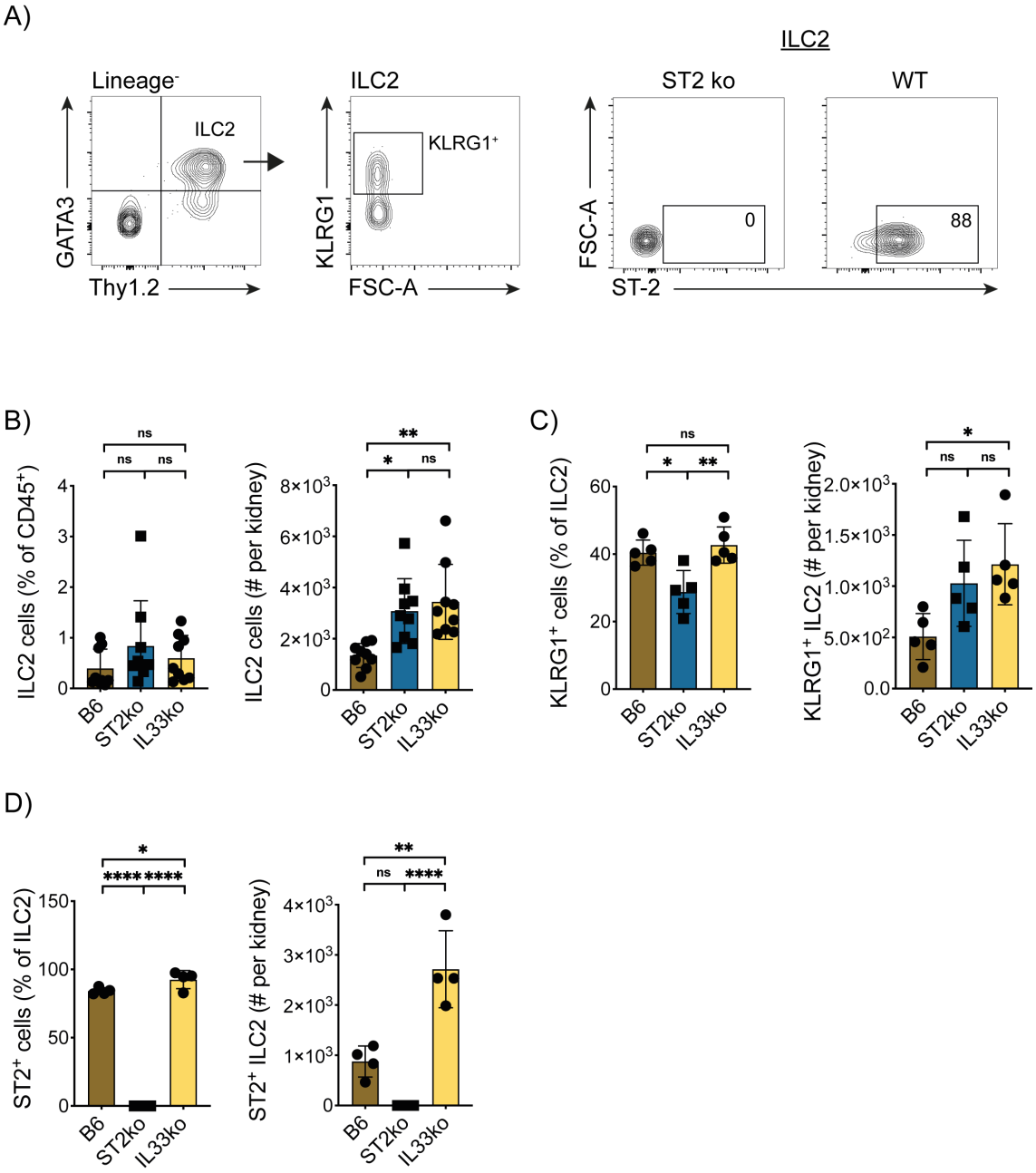


Figure 4-11 ILC2s during mild onset chronic kidney disease.

Mice were fed with 0.15% adenine-enriched diet for 42 days. (**A**) ILC2s were gated as follows: Lineage$^-$ (CD3ε, CD11b, CD5, CD19, TCRβ, γδTCR, CD11c), Gata3$^+$, Thy1.2$^+$. (**B**) Frequency and total numbers of ILC2 cells in kidney. (**C**) Frequency of KLRG1$^+$ ILC2s and cell numbers of ILC2s. (**D**) Frequency of ST2$^+$ ILC2s and cell numbers of ST2$^+$ ILC2s per kidney. Data represent the mean ± SD (4-5 mice per group, (B) Pooled data from two independent experiments (C, D) Representative data from at least two independent experiments). Asterisks indicate statistically significant differences assessed by one-way ANOVA (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$).

4.2.5 Influence of short-term IL-33 injections in crystal-induced kidney injury.

The above presented findings showed that the absence of the alarmin IL-33 leads to an increased number of several immune cells, most significantly an increase of macrophages. Other studies showed (Riedel et al. 2017; Vasanthakumar et al. 2015) that the administration of recombinant IL-33 can expand regulatory T cells or ILC2s and therefore leads to disease reduction. Here, we investigated if the administration of IL-33 (0.4 µg/injection) alters kidney damage. To align with the above-mentioned studies, we reduced our model to a time frame of 14 days. Mice were fed a 0.2% adenine-enriched diet and interperitoneally injected with IL-33 (0.4 µg) at days 3/5/7/9 and 11 (Figure 4-12, A). Using the same indicators for kidney damage as applied in 4.2.3 and 4.2.4, we found that the injection of IL-33 did not alter the concentration of KIM-1 (Figure 4-12, B) but showed a significant increase in TIMP-1 concentrations (Figure 4-12, C) compared to PBS injected controls. We found no significant differences in pathohistological analysis, using staining for αSMA or collagen III (Figure 4-12, D and E). Similarly, we found no significant difference in mRNA expression for αSMA, Collagen I, Fibronectin, TGFβ, TNFα and Vimentin in IL-33 injected mice compared to PBS controls; however, Collagen III showed a significant increase.

These data show that the injection of IL-33 did not lead to significant reduction in kidney damage. Rather IL-33 seemed to result in disease aggravation.

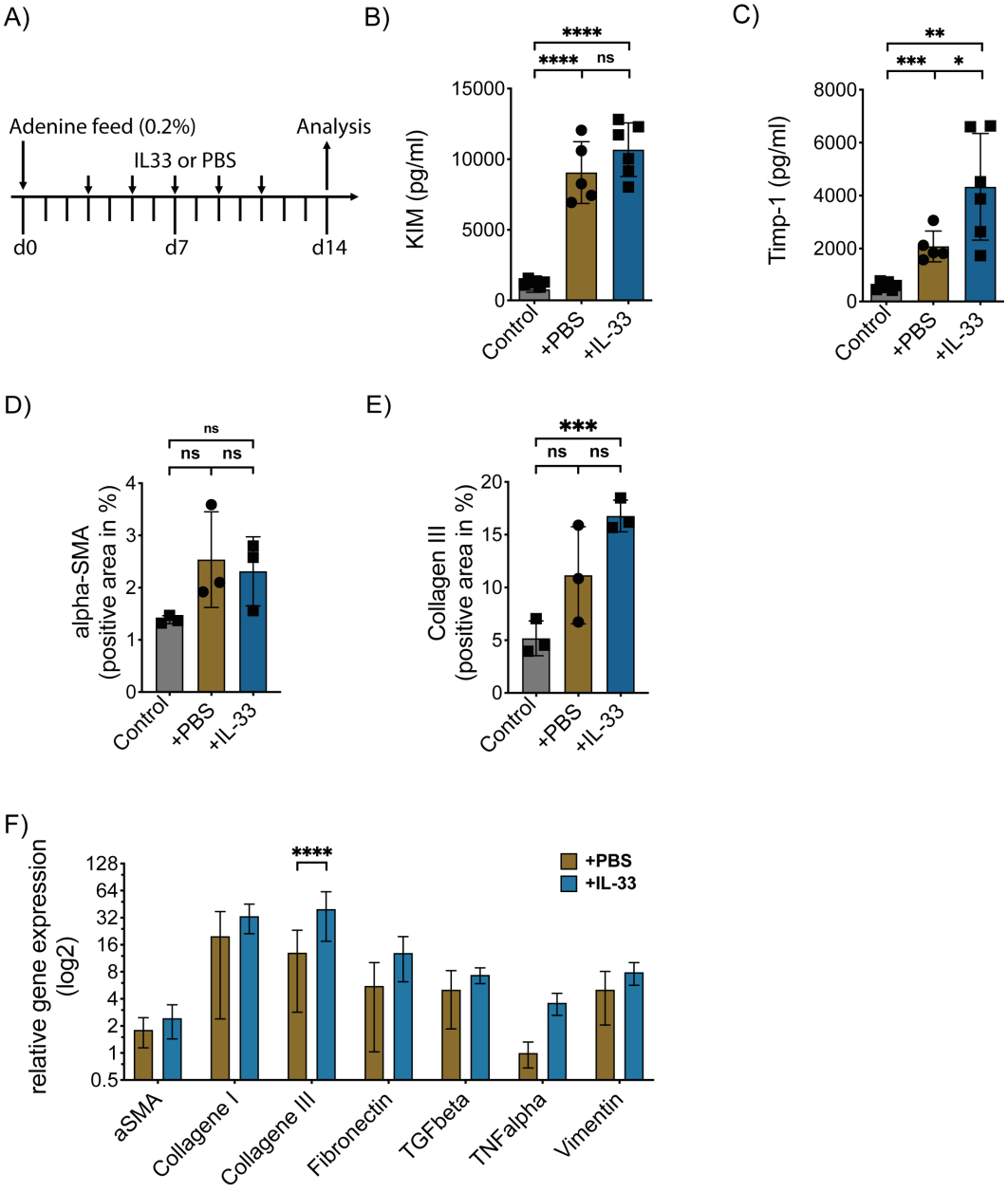


Figure 4-12 Physiological and cellular changes due to IL-33 injections during adenine induced CKD

(**A**) Mice were fed a 0.2% adenine-enriched or a control diet for 14 days. Additionally, mice were injected with 0.4 µg IL-33 or PBS at day 3, 5, 7, 9 and 11. (**B, C**) ELISA quantification of concentrations of KIM (urine) and Timp-1 (serum). (**D+E**) Pathohistological data for αSMA (**D**) and Collagen III (**E**) positive stained area per kidney section. (**F**) RT-qPCR data for several fibrosis linked genes relative to untreated control B6 mice. Data shown represent the mean ± SD. (B, C) 5-6 mice per group, (D+E) 3 mice pre group, (F) 4 mice per group. All data are representative for two independent experiments. Asterisks indicate statistically significant differences as assessed by one-way ANOVA (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$).

To investigate the underlying reasons for this aggravation at the cellular level, we applied multicolor flow cytometry and analyzed the immune cell compartments of the kidneys.

We found that macrophages, cDC2, eosinophils, monocytes and PMNs significantly increased in total numbers in adenine-enriched diet fed mice compared to control diet fed mice. Further, we found that macrophages significantly increased in total numbers to IL-33 injection, but not in frequency (Figure 4-13, A and B). Dendritic cells decreased in frequency when mice were injected with IL-33; however, total numbers did not differ between the PBS and the IL-33 groups. We did not observe significant differences in cDC1s nor cDC2s due to IL-33 injection. Also did the injection of IL-33 not alter the number of eosinophils per kidney (Figure 4-13, E and F). Monocytes increased nearly two-fold between PBS group and IL-33 treated mice. We did not detect differences in monocytic or PMN MDSCs (IFNyR2^{+} monocytes, IFNyR2^{+} PMNs) between the groups. Analyzing PMNs, we found an increase in the IL-33 treated group in frequency and numbers.

Our findings demonstrate that the intraperitonially injection of IL-33 leads to an increase of macrophages but not DCs. Furthermore, we found an increase in total cell numbers of monocytes and PMNs, but not their MDSC counter parts. These results indicate that IL-33 per se does not reduce the infiltration of immune cells which are connected to kidney damage.

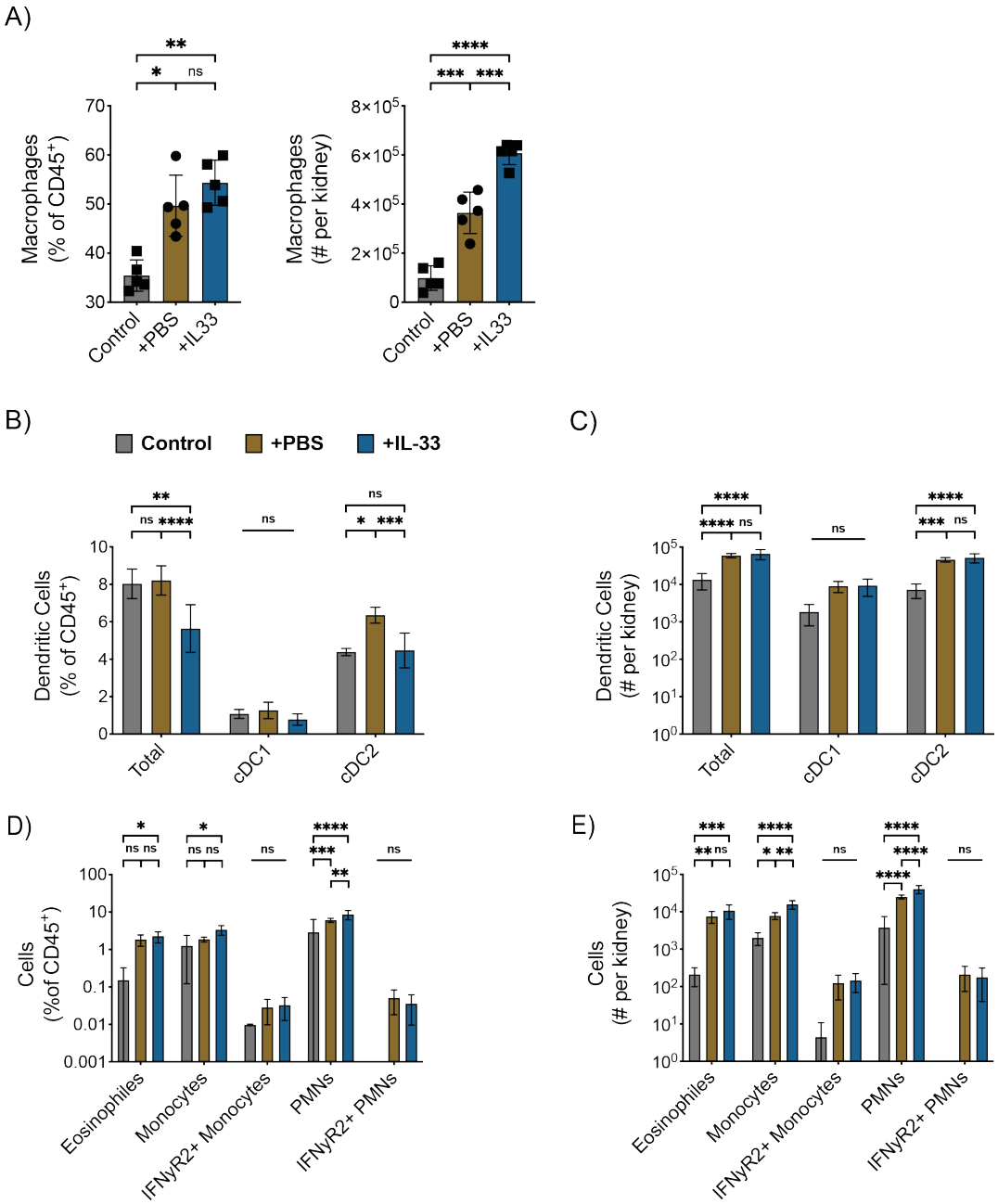


Figure 4-13 Effect of IL-33 administration on different myeloid cell subsets. (A-F)

Mice were fed a normal diet (control) or 0.2% adenine-enriched diet for 14 days. Additionally, they were injected with PBS or IL-33 (0.4 µg) at days 3/5/7/9/11. Gating strategy is shown in Figure 4-2 and Figure 4-3, A. (**A**) Frequencies and (**B**) total cell numbers of macrophages (CD64$^+$ F4/80$^+$) (**C**) Frequencies or (**D**) numbers of total dendritic cells or two different subsets, cDC1s (XCR1$^+$, CD11b$^-$) and cDC2s (XCR1$^-$, CD11b$^+$). (**E**) Frequencies or (**F**) total cell numbers of different myeloid cell types: eosinophils (CD11b$^+$ SiglecF$^+$), monocytes (CD11b$^+$ Ly6C$^+$ Ly6G$^-$) and PMNs (CD11b$^+$ Ly6C^{int} Ly6G$^+$). Data represent the mean ± SD (3-5 mice per group, representative for two independent experiments). Asterisks indicate statistically significant differences as assessed by two-way ANOVA test (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

A previous report showed that the administration of IL-33 leads to the expansion of alternative activated macrophages in a colitis model (Tu et al. 2017). Therefore, we were interested if the administration of IL-33 leads to the expansion of $CD38^+$ classical activated macrophages (CAM) or $CD206^+$ alternatively activated macrophages (AAM) during kidney disease. Using flow cytometry, we analyzed the macrophage compartment using the expression of the surface markers CD38 and CD206 (Figure 4-14, A). Interestingly, we found very little expression of CD38 or CD206 in control mice (Figure 4-14, B+C), suggesting that no CAM or AAM are present in mouse kidneys during homeostasis. Mice fed a 0.2% adenine-enriched diet for 14 days showed an increase in $CD38^+$ and $CD206^+$ cells; however, the most prominent population was double positive for both markers (Figure 4-14, A,B,C). The $CD38^+$ and $CD206^+$ populations did not show significant differences in frequencies of $CD45^+$ cells; however, the double positive cells are significantly increased in IL-33 treated mice (Figure 4-14, B). Also, total cell numbers of single positive cells (CD38, CD206) showed no significant differences, whereas double positive cells significantly increased in IL-33 treated mice compared to PBS treated mice. Thus, we found that IL-33 administration leads a more than two-fold expansion of $CD38^+$ $CD206^+$ macrophages. Additionally, we found a significant increase in $ST2^+$ macrophages in the kidneys of IL-33 injected mice (Figure 4-14, D, E).

To examine if those macrophages are classically or alternatively activated, we used RT-qPCR to analyze total kidney lysate for specific genes related to those activation states. Three genes related to AAMs, namely *Arginase-1* and *Fizz-1* both showed a significant increase, whereas *YM-1* increased in a not significant manner in IL-33 treated mice. Further, *iNos* a marker for classical activated macrophages, did not differ between the PBS and IL-33 treated group (Figure 4-14, F).

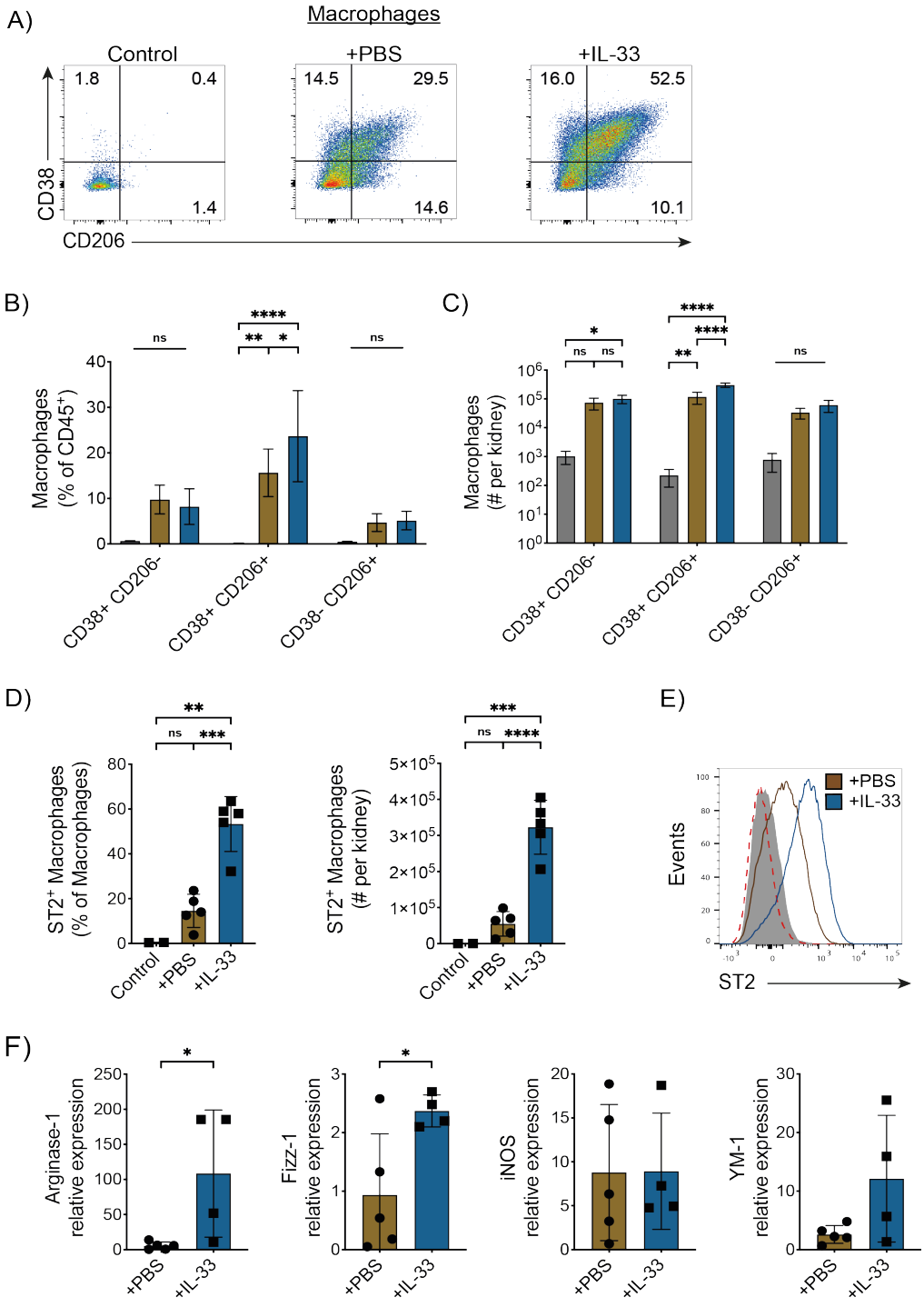


Figure 4-14 IL-33 administration leads to increased numbers of CD38+ CD206+ macrophages in kidney.

Mice were fed a normal diet (control) or 0.2% adenine-enriched diet for 14 days and administered PBS or IL-33 (0.4 µg) at days 3/5/7/9/11 (Figure 4-12,A). (**A**) Representative FACS plots pre-gated on macrophages ($CD45^+TCRb^-CD19^-CD64^+F4/80^+$). (figure legend continues next page)

Our results showed that IL-33 administration significantly increased $ST2^+$ macrophages. Additionally, we saw a significant increase in $CD38^+$ $CD206^+$ macrophages in IL-33 treated mice compared to PBS treated mice. Examination of genes related to macrophage activation states determined that genes for AAMs are significant upregulated in IL-33 treated mice. Together our data suggest that the administration of IL-33 leads to an accumulation of AAM in the kidney of crystal-induced nephropathy mice.

IL-33 has been reported to expand suppressive cells, foremost T_{regs} and ILC2s. Applying the gating strategy shown in Figure 4-10, A, we analyzed these cell compartments for compositional changes. We found first a significant reduction in the frequencies of CD4 T cells in the IL-33 treated group; however, the total cell count did not differ significantly between PBS and IL-33 treated group (Figure 4-15, A). T_{reg} numbers on the other hand did increase significantly in numbers per kidney (Figure 4-15, B). Additionally, we examined if the T_{reg} phenotype was influenced due to IL-33 injection. Therefore, we further gated T_{regs} on KLRG1, a marker for effector T_{regs}. We found a non-significant increase in numbers of $KLRG1^+$ T_{regs} per kidney. Next, we turned our attention to the second group of cells reported to expand upon IL-33 injection: ILC2s. Applying the same gating strategy from Figure 4-11 ($Lineage^-$ $GATA3^+$ $CD90^+$), we found increased ILC2 numbers per kidney and increased frequencies of $KLRG1^+$ ILC2 in the IL-33 treated group compared to the PBS group (Figure 4-15, E).

Taken together, we found that the short-term treatment of B6 mice with IL-33 leads to higher accumulation of T_{regs} and ILC2s. Furthermore, both cell types show higher level of KLRG1 expression, indicating a more activated phenotype.

(**B**) Percentages and numbers (**C**) of macrophages based on differential expression of CD206 and CD38. (**D**) Percentages and numbers of $ST2^+$ macrophages. (**E**) Representative histogram of ST2 cell-surface expression on macrophages of control (grey), PBS (blue) or IL-33 injected mice (red). Red dotted line represents FMO control. (**F**) mRNA expression of different M1/M2 related genes in total tissue lysate. Data represent the mean ± SD (3-5 mice per group, representative for two independent experiments). Asterisks indicate statistically significant differences as assessed by two-way ANOVA test (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$).

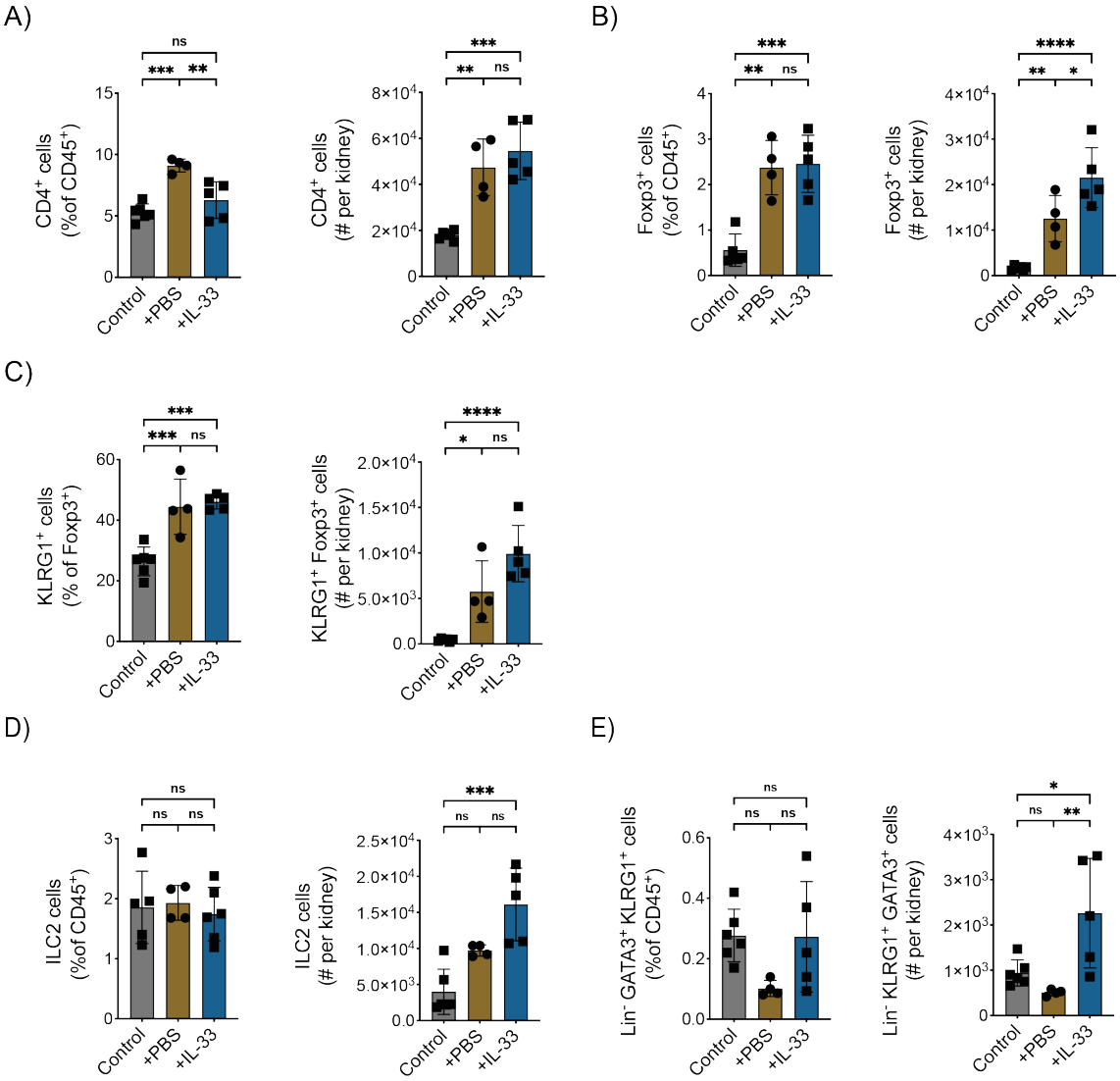


Figure 4-15 Effect of IL-33 administration on different non-myeloid cell subsets.

(**A-F**) Mice were fed a normal diet (control) or 0.2% adenine-enriched diet for 14 days, and additionally were injected with PBS or IL-33 (0.4 µg) at days 3/5/7/9/11. Data shown for kidney cells. Gating strategy is shown in Figure 4-10 and Figure 4-11, A. (**A**) Percentage and total numbers of CD4 T cells. (**B**) Percentage and total numbers of T_{regs}. (**C**) Percentage and total numbers of KLRG1$^+$ Foxp3$^+$ cells. (**D**) Percentage and total numbers of Lineage$^-$ Gata3$^+$ CD90$^+$ cells. (**E**) Percentage and total numbers of KLRG1$^+$ ILC2 cells. Data shown represent the mean ± SD (n=3-5 mice per group, representative for two independent experiments). Asterisks indicate statistically significant differences as assessed by one-way ANOVA (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

4.3 Discussion

This study has investigated the role of the IL-33/ST2 axis in chronic kidney disease. Several studies investigated the impact of IL-33 and its receptor ST2 in other disease and organs and showed a specific or unique role of the IL-33/ST2 axis in organ-specific disease progression (Liew et al. 2016). Our results show that the major source of *Il33* in the kidney are non-hematopoietic cells and that $CD31^+$ endothelial cells are the dominant source. This is contrast to adipose tissue, where $CD31^-$ stromal cells were the main producers of *Il33* (Moussion et al. 2008; Pichery et al. 2012; Rana et al. 2019). Even though we observed a significantly higher proportion of $Il33^+$ $CD31^+$ cells under inflammatory conditions, the total cell number of $Il33^+$ non-hematopoietic cells did not change. Recognized as nuclear alarmin, IL-33 is located in the cell nucleolus and is released into the extracellular space after cell injury to signal tissue damage (Cayrol and Girard 2014). The observation that the number of *Il33* producing cells stayed similar during inflammation might indicate a higher rate of cell death and correlates with higher levels of extracellular IL-33 protein. Notably, we detected significant elevated *Il33* expression in cDC2s, macrophages, monocytes and granulocytes. Consistent with recent reports the observed level of expression was considerably lower compared to non-hematopoietic cells (Byers et al. 2013; Talabot-Ayer et al. 2012). However, the specific role of *Il33* produced by $CD45^+$ cells has not yet been fully addressed. Fock *et al.* reported a crucial role of macrophage-derived IL-33 in placental growth indicating potential importance of immune cell derived IL-33 (Fock et al. 2013). Furthermore, it has been shown that DCs produce IL-33 after stimulation with LPS and that this is enhanced via adrenoreceptor-mediated stimulation (Yanagawa, Matsumoto, and Togashi 2011). Further analysis is necessary to address if IL-33 has cell intrinsic effects, especially during inflammation, when IL-33 is upregulated in immune cells. Since IL-33 is also produced from stromal cells in the bone marrow, mice deficient for IL-33 might possess immune cells which egress in an already altered phenotype due to differences in their microenvironment (Mager et al. 2015). Interestingly, a recent study showed the accumulation of ILC2 progenitor cells inside the bone marrow

of IL-33 knockout mice (Stier et al. 2018). It has been shown that ILC2s establish their niches in the perinatal period and get replenished *in situ* in peripheral tissues (Gasteiger et al. 2015; O'Sullivan et al. 2016). In the lung ILC2 niche establishment happens in an IL-33 dependent manner, however, if kidney ILC2s are dependent on IL-33 remains elusive (de Kleer et al. 2016; Steer et al. 2017). To our surprise we found elevated numbers of ILC2s in the kidneys of ST2 and IL-33 deficient mice. Recently, another group reported similar findings in the lung of ST2 knockout mice (Verma et al. 2018). They discuss that the deficiency of ST2 leads to the accumulation of $ST2^-$ ILC2s which are positive for IL-9 and IL-13. If this is the case in our model and how those ILC2s interfere with disease progression has to be further investigated. To answer this question, we will utilize ST2 deficient mixed bone marrow chimera and closer investigate the phenotype of $ST2^-$ ILC2s.

Our data also show that monocytes, granulocytes, macrophages and cDCs express the IL-33 receptor ST2. Similar, to *Il33* the ST2 expression was elevated during kidney inflammation on the before mentioned cells, indicating that IL-33 in the inflamed kidney acts on several myeloid cell types. $ST2^+$ AAMs have been linked to tissue repair in the lung (Dagher et al. 2017); however, we did not further investigate the specific role of $ST2^+$ myeloid cells. However, we demonstrate that deficiency in ST2 had no significant impact on overall disease severity in our severe model of adenine induced nephropathy. However, adapting our model to a more chronic disease by reducing the concentration of adenine in the diet and therefore prolonging the treatment time to 42 days showed increased fibrotic parameters due to ST2 deficiency. In this 'mild-onset' model, we also demonstrated that the deficiency in *Il33* leads to more severe physiological kidney damage as well as higher expression of fibrosis associated genes. We found no striking differences between adenine treated wild type mice and ST2-ko mice, suggesting that the increased fibrosis in ST2 deficient mice was at least unrelated to increased accumulation of immune cells. A recent study suggested a role for IL-33 in an ST2-independent manner (Nishizaki 2018). ST2-ko mice may show high levels of "free" IL-33 as neither membrane-bound nor the decoy

receptor sST2 is present. Thus, free IL-33 may potentiate ST2 independent IL-33 effects. However, the finding that the deficiency for ST2 leads the accumulation of IL-9^{+} ILC2s and IL-9 is known to expand mast cells (Tete et al. 2012; Verma et al. 2018; Zhang et al. 2017) To this extent there is a body of literature linking mast cells to the progression of kidney disease (Owens et al. 2019). We did not yet address mast cells in our analysis, neither did we in depth characterize cytokine profiles of specific cell types, such as T_H9 cells. These analyses will be included in further experiments to answer the involvement of mast cells and ILC2 phenotypes in our model of chronic kidney disease.

The exogenous administration of IL-33 has been a widely used approach in the last years. Especially, in a model of renal ischemia-reperfusion injury (IRI) it was shown that the expansion of regulatory T cells and ILC2s upon IL-33 injection promoted renoprotective functions (Cao et al. 2018). However, in our severe model of adenine induced nephropathy, we did not observe protective properties of exogenous IL-33; to the contrary, we obtained higher level of timp-1 and collagen III, suggesting increased disease activity. However, studies showed that IL-33 can have direct effects on timp-1 and collagen and lead to their upregulation, which might explain our findings that both are increased (Millar et al. 2015; Wu et al. 2018). Most strikingly, we found a significant increase in macrophage numbers in IL-33 treated mice. Whereas, the number of cDCs and eosinophils and MDSCs did not differ, we found higher numbers of monocytes and PMNs. The remarkable increase in macrophages led us to further characterize their phenotype. We showed that in steady state, macrophages do barely express the “M1” associated marker CD38 nor the “M2” associated marker CD206 (Amici et al. 2018; Jablonski et al. 2015). However, during inflammation we found an increase in CD38 and CD206 expressing macrophages, in particular CD38/CD206 double positive macrophages. Further, we showed that the expression of ST2 was significantly elevated on macrophages, indicating an important role for macrophages in this model. Analysis of total tissue mRNA showed that the M2 associated genes such as *Arginase1* and *Fizz1* were significantly increased upon IL-33 administration, but the M1 related gene *iNOS*

did not show differences between PBS and IL-33 group. These data suggest a M2-like polarization of macrophages. Tu *et al.* reported the induction of AAMs due to IL-33 administration and a protective effect in a colitis model (Tu et al. 2017). Interestingly, it had been shown that the deletion of arginase-1 in macrophages, a hallmark enzyme in AAMs, leads to a stronger development of fibrosis in the liver, indicating that AAMs can suppress fibrosis (Pesce et al. 2009). However, it also had been reported that the accumulation of AAMs in the lung leads to tissue fibrosis (Li et al. 2014). In our model, the injection of IL-33 led to increased influx of several immune cells, what correlates with enhanced tissue damage. This influx indicates that IL-33 injection and the accompanied accumulation of AAMs is not sufficient to protect the kidney. Moreover, we see elevated fibrotic markers, which argues more in favor of the observations of Li *et al.*, that the accumulation of AAMs exacerbates fibrosis. However, since IL-33 can directly act on fibrocytes, a cell type involved in the development of fibrosis, the accumulation of collagen III might be an AAM independent effect, triggered by non-hematopoietic cells (Hayashi et al. 2014).

IL-33 is a known mediator for T_{reg} and ILC2 expansion and therefore provides tissue protective effects (Besnard et al. 2015; Riedel et al. 2017). Consistent with those reports we found increased cell numbers of T_{regs} and ILC2s. Yet, we did not observe direct renoprotecive effects in this severe CKD model; however, we cannot exclude that we will see those effects in the mild onset model. Therefore, we plan to perform the administration of IL-33 in the mild onset model which will allow a direct comparison to the IL33ko phenotype than using the IL-33 administrations in the severe model. That the deficiency and the administration of IL-33 leads both to higher accumulation of immune cells and also induces higher gene expression of fibrosis related genes is an interesting finding. We cannot exclude that the available concentration of IL-33 inside the inflamed kidney plays a critical role. Other studies already reported and discussed dose dependent effects of IL-33 on different cell types, such as lymphatic endothelial cells (LECs) or human umbilical vein endothelial cells (HUVECs)(Choi et al. 2009; Han et al. 2017; Hazlett et al. 2010). The administration of IL-33 in the severe kidney model together with the high

concentration of IL-33 released from non-hematopoietic cells might explain a different outcome in the here used model compared to other models or investigated organs. We might address these effects in further studies. Therefore, the higher fibrotic gene expression in the IL-33 administered kidney compared to the loss of IL-33 in IL33ko mice, might underlayer two separate causes. Further studies might elaborate those different effects.

Taken together, this study demonstrates the complexity of the IL-33/ST2 axis. Whereas, ST2-deficiency only led to moderate changes, the deficiency for IL-33 led to massive influx of several immune cells of myeloid and lymphoid origin. This indicates that IL-33 has protective properties by regulating inflammation and immune cell influx into the kidney. Furthermore, the discrepancy between cytokine and receptor knockout might point out that IL-33 is able to directly act in a non-ST2-dependent manner on cells. The administration of IL-33 led to accumulation of macrophages and the upregulation of “M2” associated genes; however, this had no effect of immune cell influx or kidney damage. Future experiments will address if the deficiency for ST2 or IL-33 leads to cell specific phenotype changes. The understanding if and how IL-33 influences specific cells and their phenotype is an important step to further predict disease outcome and might shape the future use of IL-33 as a therapeutic agent.

Chapter 5 - Transcription factors of the IRF and BATF family and their role in chronic inflammations

5.1 Introduction:

Different studies showed the role of the interferon-regulatory factor (IRF) transcription factor family in immune cell regulation (Platanitis and Decker 2018; Tamura et al. 2008; Yanai et al. 2012). The finding that different subsets of dendritic cells can be classified by the expression of IRF4 or IRF8 is of specific interest for this work. Whereas IRF4 is mainly expressed in $CD4^+$ DCs and pDCs, IRF8 expression had been found in the $CD8^+$ DCs and pDC subset. The use of knockout mice (IRF4-ko or IRF8-ko) demonstrated the developmental dependency of those DC subtypes on these transcription factors (Schiavoni et al. 2002; Tamura et al. 2005). Whereas, IRF8-ko mice have a deficiency for cDC1 cells, cDC2 cell development is not impaired (Sichien, Charlotte L Scott, et al. 2016) In contrast, cDC2 depend of IRF4. However, several studies showed that cDC2s are comprised of a heterogenous group of cells with different characteristics and tissue specialization (Alcantara-Hernandez et al. 2017; See et al. 2017; Villani et al. 2017). This is also reflected in their dependency on IRF4. Schlitzer *et al.* identified an IRF4-dependent $CD103^-$ $CD11b^+$ $CD24^+$ DC population (cDC2 subset) in the lung, which was necessary for the induction of IL-17 producing T helper cells. In their study they showed reduced numbers in the cDC2 compartment, but not a total ablation (Flores-Langarica et al. 2018; Persson et al. 2013; Schlitzer et al. 2013)

However, IRF TFs have not only been reported to be important in DCs but were also described to be relevant for macrophages and monocytes. Monocytes deficient for IRF4 showed reduced capacity to develop into monocyte-derived DCs, instead these monocytes develop into macrophages in response to GM-CSF and IL-4 (Briseño et al. 2016).

IRF4 has been shown to regulate the polarization between M1 and M2 in macrophages. Several studies reported that IRF4 deficient mice showed a skewing to the M1 phenotype in macrophages (Bruns et al. 2016; El Chartouni, Schwarzfischer, and Rehli 2010; Satoh et al. 2010).

Transcription factors of the BATF family play important roles in immune cells and form heterodimers with members of the IRF family. In homeostatic conditions,

high levels of BATF3 have been reported in cDCs, whereas BATF and BATF2 get upregulated during inflammatory conditions (Tussiwand et al. 2012). *Batf3* knockout mice lacking $CD8^+$ DCs, revealing their dependency on this TF (Hildner et al. 2008). In a murine crescentic glomerulonephritis (cGN) model Evers et al. showed that the lack of *Batf3* leads to reduced number of $CD103^+$ DCs (cDC1) accompanied by a reduction of regulatory T cells and therefore, concluded that cDC1s foster intrarenal T_{reg} accumulation (Evers et al. 2016). Interestingly, Li et al. reported an inhibitory effect of BATF3 in T_{reg} differentiation. They showed that BATF3 binds to the CNS1 region of the *Foxp3* locus which lead to a reduction in gene expression (Li et al. 2012). Furthermore, in a model of experimental autoimmune encephalomyelitis the lack of BATF expression (*Batf* knockout mice) showed reduced T_H17 immune cell polarization and resistance to inflammation (Schraml et al. 2009). A functional cooperation of the transcription factor BATF and IRF4 has been found to promote *IL10* transcription in T cells (Li et al. 2012). Additionally, IRF4 is responsible for Blimp-1 expression and therefore plays a critical role in the establishment of functional aspects in regulatory T cells (Cretney et al. 2011).

The phenotypes of *Irf4* deficient and *Batf* deficient mice are very similar suggesting their regulation of common genes (Murphy et al. 2013). Both, IRF4 and BATF, are linked had been linked to T_H2 associated gene expression, such as *Il4* and *Il10* (Ahyi et al. 2009; Tussiwand et al. 2012). Several reports showed that the lack of IRF4 in CD4 T cells results in a block of T_H2, T_H9, T_H17 and T_{FH} cell development (Bollig et al. 2012; Brüstle et al. 2007; Huber et al. 2008; Lohoff et al. 2002; Rengarajan et al. 2002; Staudt et al. 2010; Tamiya et al. 2013; Tominaga et al. 2003).

Foxp3 directly regulates IRF4 in T_{regs}, which express high amounts of IRF4 (Zheng et al. 2009). Vasanthakumar *et al*. showed that T_{regs} in the adipose tissue require IRF4 and BATF for their differentiation, which is directly regulated through the IL-33 receptor ST2 and the PPAR-γ (Vasanthakumar et al. 2015).

IRF1 is another member of the IRF family and has also been identified to play crucial roles in myeloid cell development (Zhao, Jiang, and Li 2015). Macrophage and DC cells in IRF1 knockout mice display immature phenotypes,

indicating developmental requirements for IRF1 (Hoffman et al. 1991; Testa et al. 2004). Furthermore, polarization of macrophages to either M1 or M2 phenotype were connected to several members of the IRF family, such as Irf1, Irf3, Irf4, Irf5 and Irf8, underlying the importance of IRFs in regulation of immune responses (Günthner et al. 2013).

Taken together, this body of literature identifies important functions of IRF4 and IRF8 in lymphocytes but also myeloid cells. Whereas, IRF8 seems to be important in DC development and a terminal selector for cDC1 cells, IRF4 controls cDC2 but also other immune cells, such as B cells, $CD4^+$ and $CD8^+$ T cells and (Betz et al. 2010; Nam and Lim 2016). BATF and IRF4 cooperate functionally e.g. in Th17 cells and T_{regs}. However, the specific roles of these different TF in chronic disease are not fully addressed yet.

The aim of the following chapter was to identify the contribution of different myeloid and lymphoid cell subsets in a chronic model of crystal-induced nephritis and to determine their regulation by members of the IRF and BATF families.

5.2 Results

5.2.1 IRF4 and BATF deficiency result in a dysregulated myeloid cell compartment

Our data showed a correlation between accumulation of myeloid cells and disease progression. As IRF4 and BATF have been identified to support the differentiation of specific lymphoid and myeloid cell types (Liao *et al.*, 2011), we aimed to investigate if the knockout of these TFs influences the development and progression of chronic kidney disease.

Here, we applied the crystal-induced nephropathy model with 0.2% adenine-enriched diet described in chapter 4 (4.2.1). *Irf4-ko* and *Batf-ko* mice were analyzed after 21 days of treatment and myeloid cell populations in the kidney were defined by flow cytometry. In comparison to wildtype controls, we

identified a proportional increase in macrophages in both knockouts; however, we only observed a significant increase in macrophage numbers in *Irf4-ko* (Figure 5-1, *A*). Using the previously introduced staining and gating of CD38 and CD206 expression, we further investigated changes in macrophage polarization (described in detail in chapter 1.2.3). Here, we detected a modestly increased frequency of $CD206^+$ $CD38^+$ macrophages and decreased frequency of $CD206^+$ $CD38^-$ macrophages in the absence of *Irf4* but those changes were not significant compared to WT (Figure 5-1, *B-D*). These findings were further supported by the significantly increase of total cell numbers of $CD206^-$ $CD38^+$ and $CD206^+$ $CD38^+$ macrophages in the *Irf4*-ko mice. No differences in the frequencies and numbers of macrophages were observed comparing *Batf*-ko to B6 mice (Figure 5-1, *B-D*). In addition, we observed a significant increase in the number and proportion of dendritic cells in the kidneys of *Irf4*-ko relative to control kidneys, while loss of *Batf* did not impact DC frequencies (Figure 5-1, *E*). *Irf4* deficiency led to a shift from cDC1 cells to cDC2 cells, and numbers of cDC2s were increased comparing *Irf4*-ko mice to wild type mice. *Batf* deficiency did not lead to significant changes in DCs composition (Figure 5-1, *F* and *G*).

Taken together, we found significantly more macrophages and dendritic cells, specifically cDC2s, due to *Irf4* deficiency and a tendency to more $CD206^+$ $CD38^+$ macrophages. The deficiency for *Batf* resulted in only a modest proportional increase in kidney macrophages. However, these are preliminary data, and further experiments have to be conducted to confirm our findings.

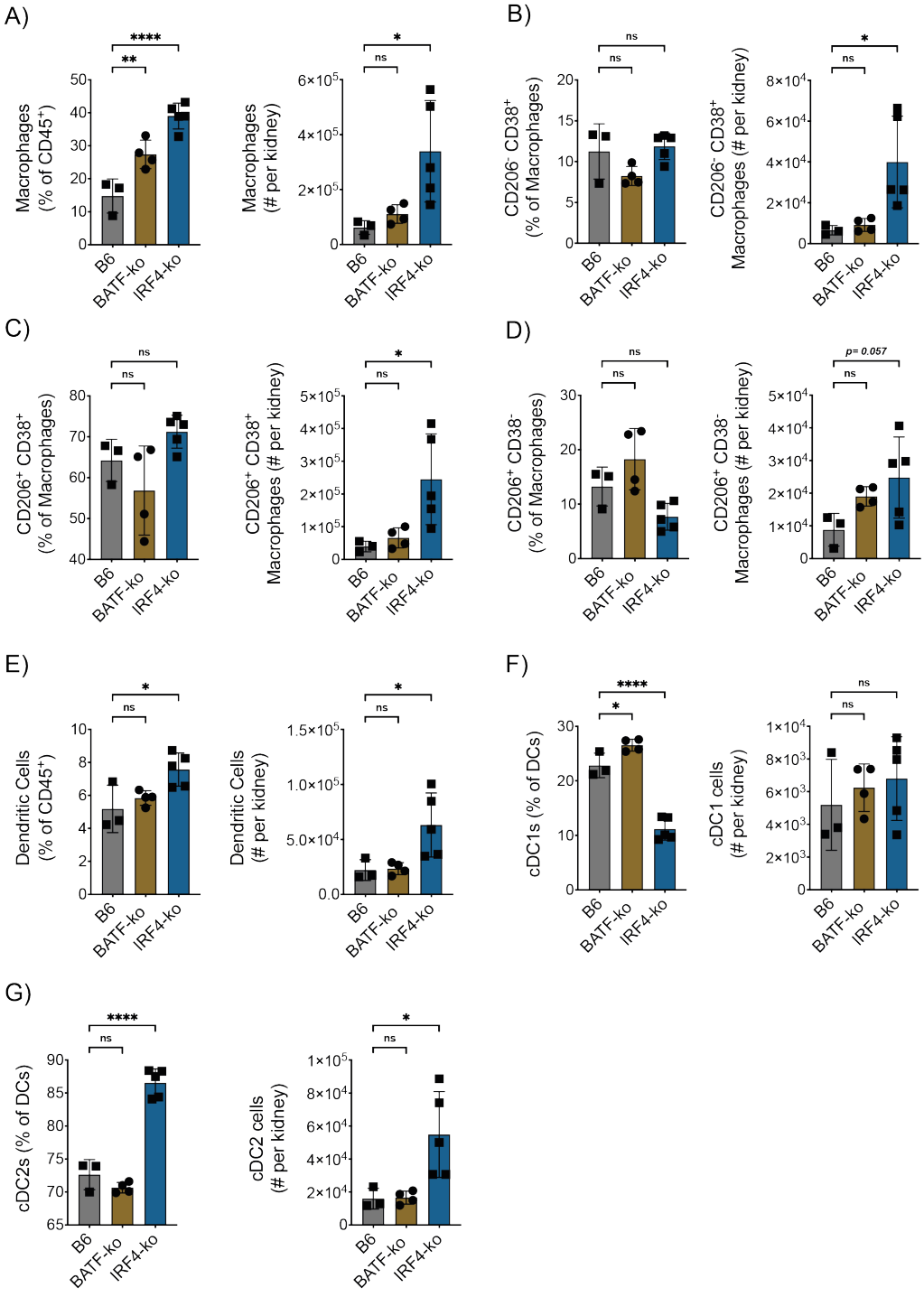


Figure 5-1 IRF4 knockout mice show increased accumulation of macrophages and cDC2 in CKD model.

Mice were fed a 0.2% adenine-enriched diet for a period of 21 days. Frequencies and numbers in the kidney of (**A**) macrophages, (**B-D**) macrophage subsets, (**E**) dendritic cells, (**F**) cDC1, (**G**) cDC2. Data represent the mean ± SD (3-5 mice per group, n=1). Asterisks indicate statistically significant differences between AF groups as assessed by one-way ANOVA (* p<0.05, ** p<0.01, **** p<0.0001).

5.2.2 IRF4 and BATF deficiency lead to decreased numbers of kidney regulatory T cells and CD8 T cells in chronic kidney disease

As *Irf4* and *Batf* have well characterized roles in T cell differentiation (Grusdat et al. 2014; Hagman 2017; Li et al. 2012; Vasanthakumar et al. 2015), we next investigated the kidney T cell compartment for changes due to *Irf4* and *Batf* deficiency in our kidney disease model. Here, we found reduced numbers of CD4 T cells in *Batf*-deficient mice. However, *Irf4-ko* mice did not show significantly reduced CD4 T cell numbers (Figure 5-2, *A*). In contrast to the total CD4 T cell population, Tregs showed a significant reduction in the frequency as well as in total cell numbers in the kidneys of both knockout genotypes compared to WT controls (Figure 5-2, *B*). Further, both knockouts had a similar reduction in T_{regs} frequencies. Interestingly, the expression of killer cell lectin-like receptor G (KLRG)-1, expressed by effector T_{reg} cells, showed barely any expression in *Irf4*-ko mice, whereas *Batf*-ko mice still expressed it in lower amounts. However, both are significantly reduced compared to wild type mice (Figure 5-2, *C*). Lastly, we found lower total $CD8^+$ T cell numbers in *Batf*- and *Irf4*- deficient mice, though only the latter was significantly reduced compared to B6 mice (Figure 5-2, *D*).

Together, these results indicate that the loss of the *Irf4* leads to reduced numbers of T_{reg} cells and specifically $KLRG1^+$ effector T_{reg} cells in the kidney T cell compartment. Though *Batf* deficiency resulted in a similar extent of T_{reg} cell loss, we observed significant differences in $KLRG1^+$ Treg cell frequencies compared to *Irf4* deficiency. Furthermore, loss of either factor resulted in decreased numbers of kidney CD8 T cells.

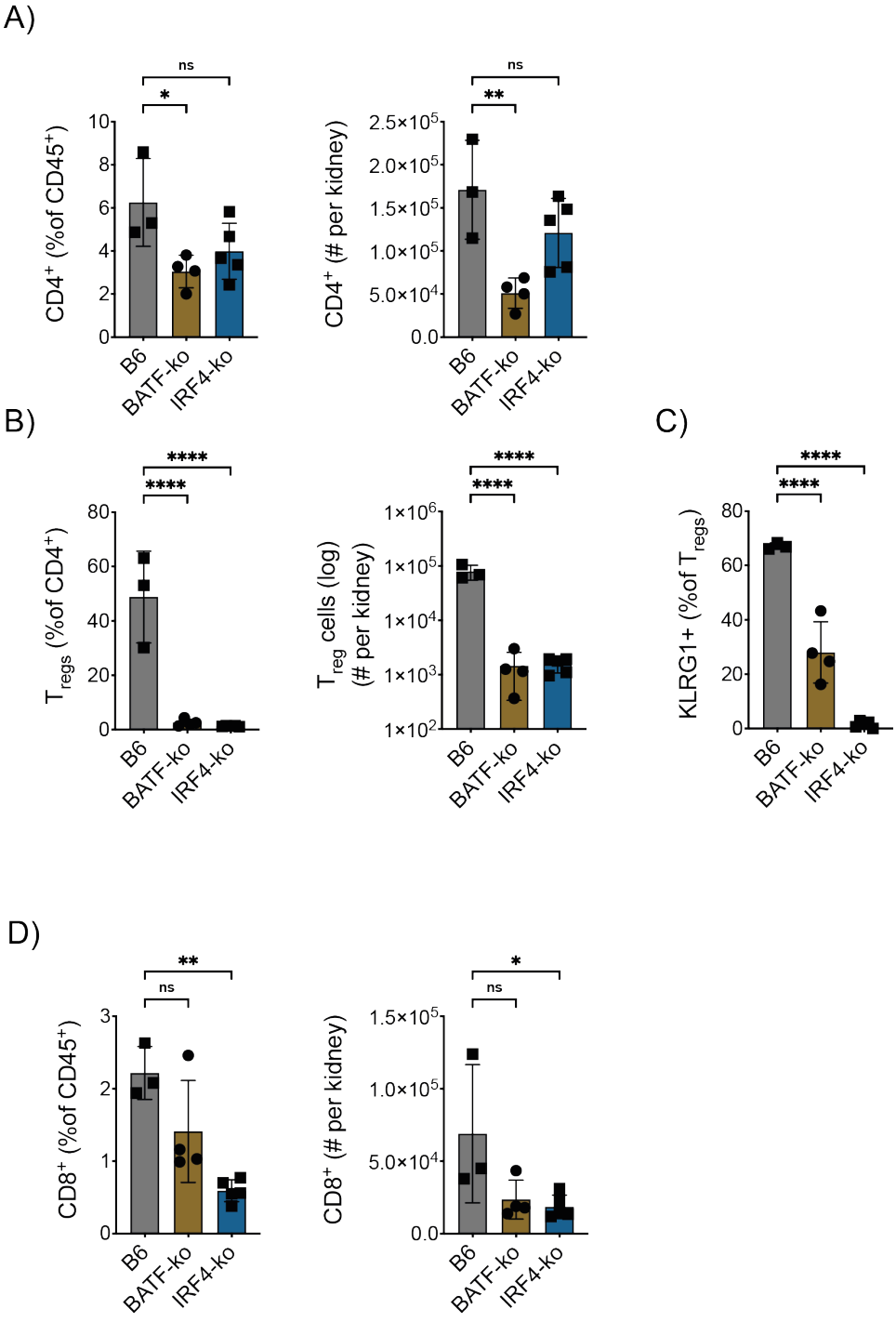


Figure 5-2 BATF and IRF4 knockout leads to loss of Tregs in the kidney.

Mice were fed for 21 days with 0.2% adenine-enriched diet. Frequency and numbers in the kidney of (**A**) CD4 T cells and (**B**) regulatory T cells. (**C**) Frequency of KLRG1$^+$ T_{regs}. (**D**) Frequency and total cell number of CD8 T cells. Data shown represents the mean ± SD (3-5 mice per group, n=1). Asterisks indicate statistically significant differences between AF groups as assessed by one-way ANOVA (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$).

5.2.3 Unique patterns of IRF4 and IRF8 expression identify specific macrophage and DC populations

Having observed that *IRF4* deficiency leads to changes in macrophages and DCs, we wanted to examine the expression of IRF4 in those cell types. Since it also has been reported that the transcription factor IRF8 plays important roles in myeloid cell differentiation and cell lineage commitment (Sichien, Charlotte L Scott, et al. 2016) we asked whether distinct patterns of expression of these factors might elucidate specific myeloid populations. To this end, we utilized a novel double reporter mouse for IRF4 and IRF8 developed by the Kallies lab. The IRF4 reporter allele was made by knocking the coding sequence for a tomato fluorescent protein 3' into the IRF4 locus, which results in the generation of an IRF4-tomato fusion protein (Kallies et al, unpublished). This mouse was crossed to mouse line expressing an IRF8-GFP fusion protein described earlier (Wang et al. 2014) to generate the IRF4/IRF8 double reporter mouse line. In order to assess reporter expression in a range of mature myeloid populations, we characterized reporter expression by VAT and Kidney resident cells using flow cytometry. Pre-gating on macrophages ($CD64^+$ $F4/80^+$) helped to resolve two distinct populations by reporter expression. One population was double negative (DN) for both transcription factors, whereas the other population showed an intermediate expression of both *Irf4* and *Irf8* (D^{int}) (Figure 5-3, A upper). Interestingly, comparing VAT and kidney we identified a clear DN population in the VAT, whereas the kidney showed a more diverse expression pattern, with a low expression of *Irf4,* nevertheless both organs showed similar distribution in frequencies. The dendritic cell compartment resolved into five different populations based on reporter expression. Here, we can clearly distinguish the $Irf8^+$ population which are the cDC1 DCs (Sichien, Charlotte L Scott, et al. 2016). The $Irf8^+$ population showed in both tissues a similar frequency (ca 28% VAT to ca 23% kidney).

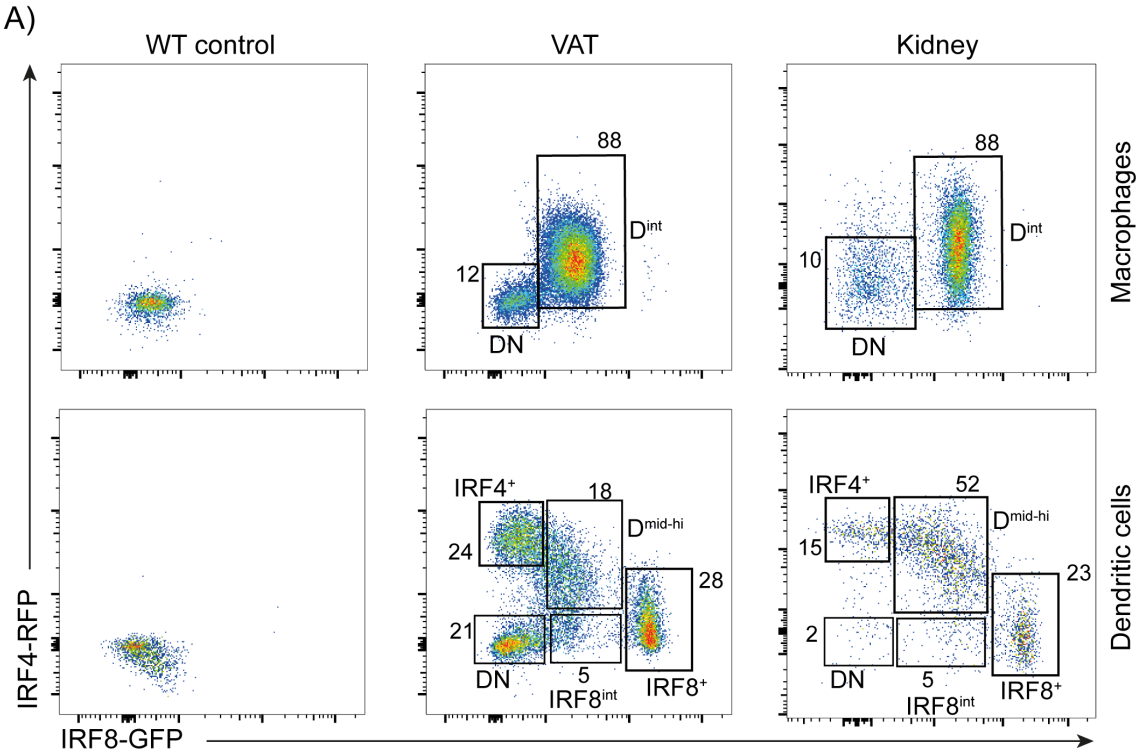


Figure 5-3 IRF4/IRF8 double reporter distinguishes different myeloid cell populations across tissues.

(**A**) Left plots show B6 control mouse (VAT), middle plots show adipose tissue and right plots kidney cells. Upper plots are pre-gated on macrophages (F4/80^{+} CD64^{+}), lower panel on DCs (F4/80^{lo} CD64^{-}, MHC-II^{+} CD11c^{+}). Numbers indicate frequencies of each population.

Similar to the macrophages, we observed a double negative population, which was only found in the adipose tissue (21%) but barely in the kidney (2%) (DN). Also, the *Irf4*$^{+}$ DC population was more pronounced in the VAT (24%) compared to kidney (15%). The *Irf8*^{int} populations did not differ between VAT and kidney, both showed a frequency of 5%. The main population in the kidney was the double mid-hi positive population (52%) (D$^{mid\text{-}hi}$), which displayed only a small population in the VAT (18%) (Figure 5-3, A lower).

These data identify distinct patterns of *Irf4* and *Irf8* expression in different myeloid populations. Furthermore, we found populations in macrophages and dendritic cells which do not express *IRF4* and *IRF8*. Additionally, our reporter showed a clear population of IRF8^{+} DCs, indicating cDC1s (see chapter 1.7).Several studies showed involvement of IRF4 and IRF8 in the development and differentiation of myeloid cells, especially of pre-DCs into specific subsets of cDCs (Bajaña et al. 2016). Together with established cell surface marker, this double reporter mouse will enable us to clearly identify subsets of the myeloid lineage. Further, this novel tool will provide the means to investigate the commitment of cells to IRF4 or IRF8 in homeostasis and even more striking during the course of disease development.

5.2.4 The role of IRF4 in CD4 T cells in kidney disease

To test the requirement of IRF4 in T cells in kidney disease, we utilized a mouse line in which T cells conditionally lose IRF4 expression (IRF4$^{fl/fl}$xCD4^{cre} mice, described previously (Man et al. 2017)). To be able to examine effects of IRF4 intrinsic to Tregs and conventional effector T cells we generated mixed bone marrow chimeric mice. To this end, we obtained bone marrow (BM) from IRF4$^{fl/fl}$xCD4^{cre} donor mice and mixed it with BM from Ly5.1 donor mice in a ratio of 2 to 1 (see experimental protocol Figure 5-4, *A*). As a control group we isolated BM from Ly5.2 wild type donor mice (Figure 5-4, *A*). The cells were injected into

lethally irradiated Ly5.1 recipient mice (hosts). After 6 weeks of reconstitution recipient mice were fed with 0.15% adenine-enriched diet for 42 days (model description see chapter 4.2.4). In the interests of clarity (if not directly labelled with Ly5.1 or Ly5.2) $IRF4^{fl/fl}xCD4^{cre}$ describes the hosts containing the $IRF4^{fl/fl}xCD4^{cre}$ cells.

5.2.5 IRF4 is required for kidney Tregs

Having observed significant differences in the kidney T cell compartments of IRF4ko and wildtype controls (Figure 5-2, *D*), we assessed the intrinsic requirement for IRF4 in their generation by assessing the capacity of $IRF4^{fl/fl}xCD4^{cre}$ donor bone marrow to regenerate these populations in a competitive mixed bone marrow chimeric model (Figure 5-4, A). Analyzing the spleen of WT and $IRF4^{fl/fl}xCD4^{cre}$ chimeric mice, we found CD4+ T cells showed an expected distribution of $Ly5.1^+$ and $Ly5.2^+$ cells (Figure 5-4, B). Investigating the spleen of WT chimeras, we found as expected that T_{regs} in the spleen were reconstituted similarly from the Ly5.1 and Ly5.2 bone marrow compartments (Figure 5-4, C, D). Furthermore, we found in the spleens from $IRF4^{fl/fl}xCD4^{cre}$ chimeric mice a reduced frequency of T_{reg} cells of the knock-out ($Ly5.2^+$) compartment compared to WT (Ly5.1) cells (Figure 5-4, D). These data identify an *Irf4*-dependent T_{reg} deficiency in the splenic Ly5.2 compartment.

Reduced frequencies of $Ly5.2^+$ T_{regs} in the $IRF4^{fl}xCD4^{cre}$ BM compared to WT BM cells proves the intrinsic defect of T_{reg} differentiation of the IRF4 deficient CD4 T cell compartment.

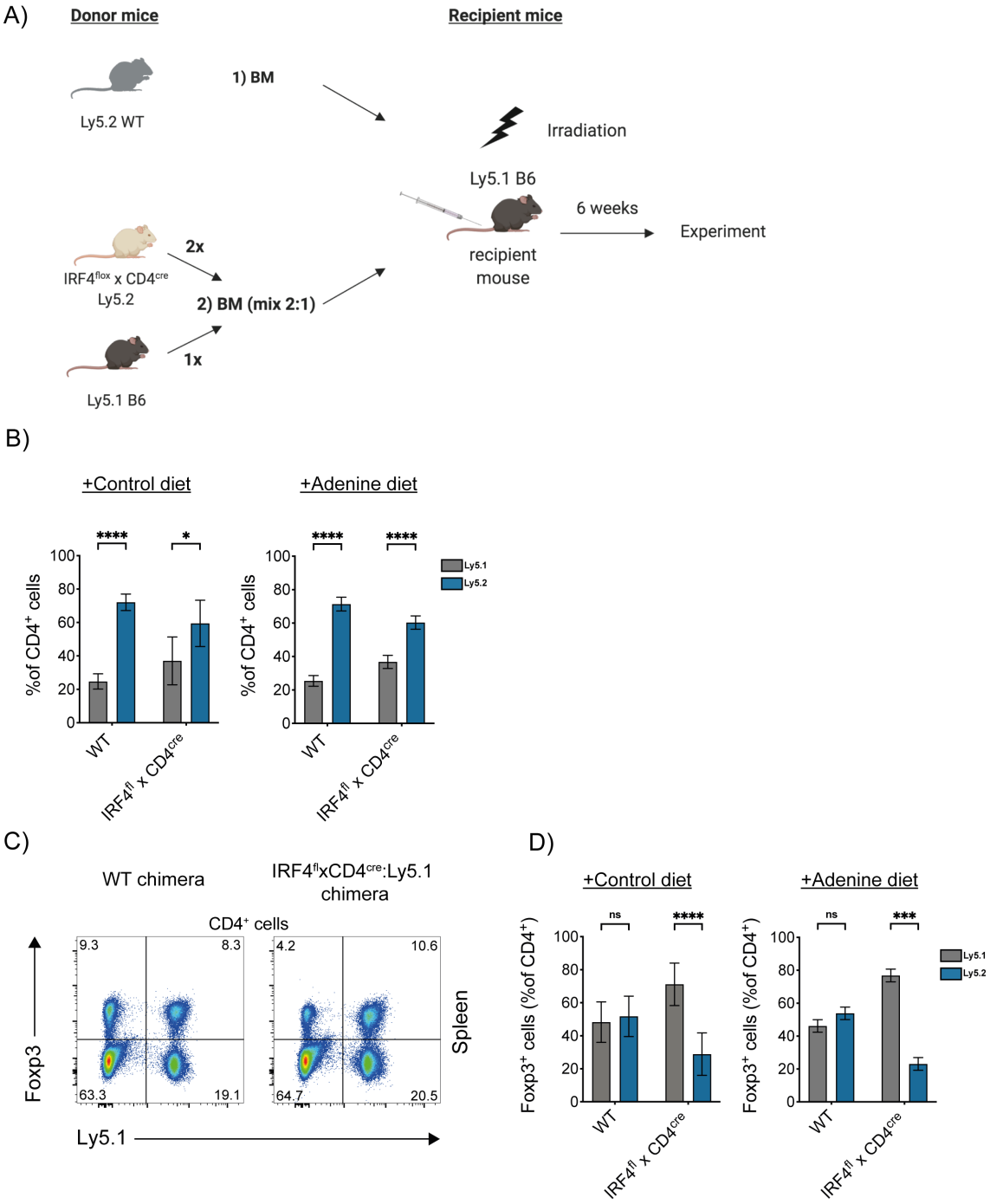


Figure 5-4 IRF4fl/flxCD4cre + Ly5.1 chimeras show a deficient T_{reg} compartment.

(**A**) Generation of BM chimera: Ly5.1 WT chimera received Ly5.2 BM (WT) + Ly5.1 (WT) or IRF4^{fl}xCD4^{cre} (KO, 2x) + Ly5.1 (WT, 1x) mixed BM. (**B**) Splenic frequencies of CD4^{+} cells of WT chimera and IRF4^{fl}xCD4^{cre} chimera with control diet (left) or adenine-enriched diet (right). (**C**) Representative plot showing CD4^{+} cells gated on Foxp3 and Ly5.1. (**D**) Splenic frequencies of Foxp3^{+} cells of WT chimera and IRF4^{fl}xCD4^{cre} chimera with control diet (left) or adenine-enriched diet (right). Data shown represents the mean ± SD (4-5 mice per group, n=1). Asterisks indicate statistically significant differences assessed by one-way ANOVA (* p<0.05, **** p<0.0001).

We then analyzed the composition of the kidney T cell compartments by flow cytometry following CKD induction. In WT chimeric mice more than half of the CD4 T cells had been replenished by transferred BM cells ($Ly5.2^+$). This was in contrast to $IRF4^{fl}xCD4^{cre}$ chimeric mice, in which Ly5.2 (KO) cells contributed much less to the kidney T cells, indicating that *Irf4* deficient CD4 T cells have a reduced capacity to reconstitute the CD4 T cell compartments of the kidney. (Figure 5-5, A).

Next, we analyzed the compartment of regulatory T cells. While WT chimeric mice showed a similar $Ly5.1^+$ to $Ly5.2^+$ T_{reg} cell distribution (Figure 5-5, A and B), we observed a nearly total loss of the $Ly5.2^+$ (KO) Treg compartment of mice that received $IRF4^{fl}xCD4^{cre}$ BM (Figure 5-5, B). Furthermore, $KLRG1^+$ T_{regs} had been completely vanished in the *Irf4*-deficient T_{reg} compartment (Figure 5-5, *C)*. As expected, in the WT chimeric mice we observed no significant changes (Figure 5-5, *C)*.

Using a cell specific gene knockout system ($IRF4^{fl}$ x $CD4^{cre}$) shows a T cell intrinsic requirement for IRF4 both in conventional and regulatory T cells in the kidney. Compared to the mildly reduced T_{regs} numbers in the splenic IRF4-deficient Treg compartment, we found a near complete loss of IRF4-deficient T_{reg} in the kidney. This indicates significant differences of IRF4 and T_{reg} development in lymphoid and non-lymphoid organs. Further, we found no $KLRG1^+$ T_{regs} in the *Irf4*-deficent compartment, confirming that effector T_{reg} generation requires IRF4.

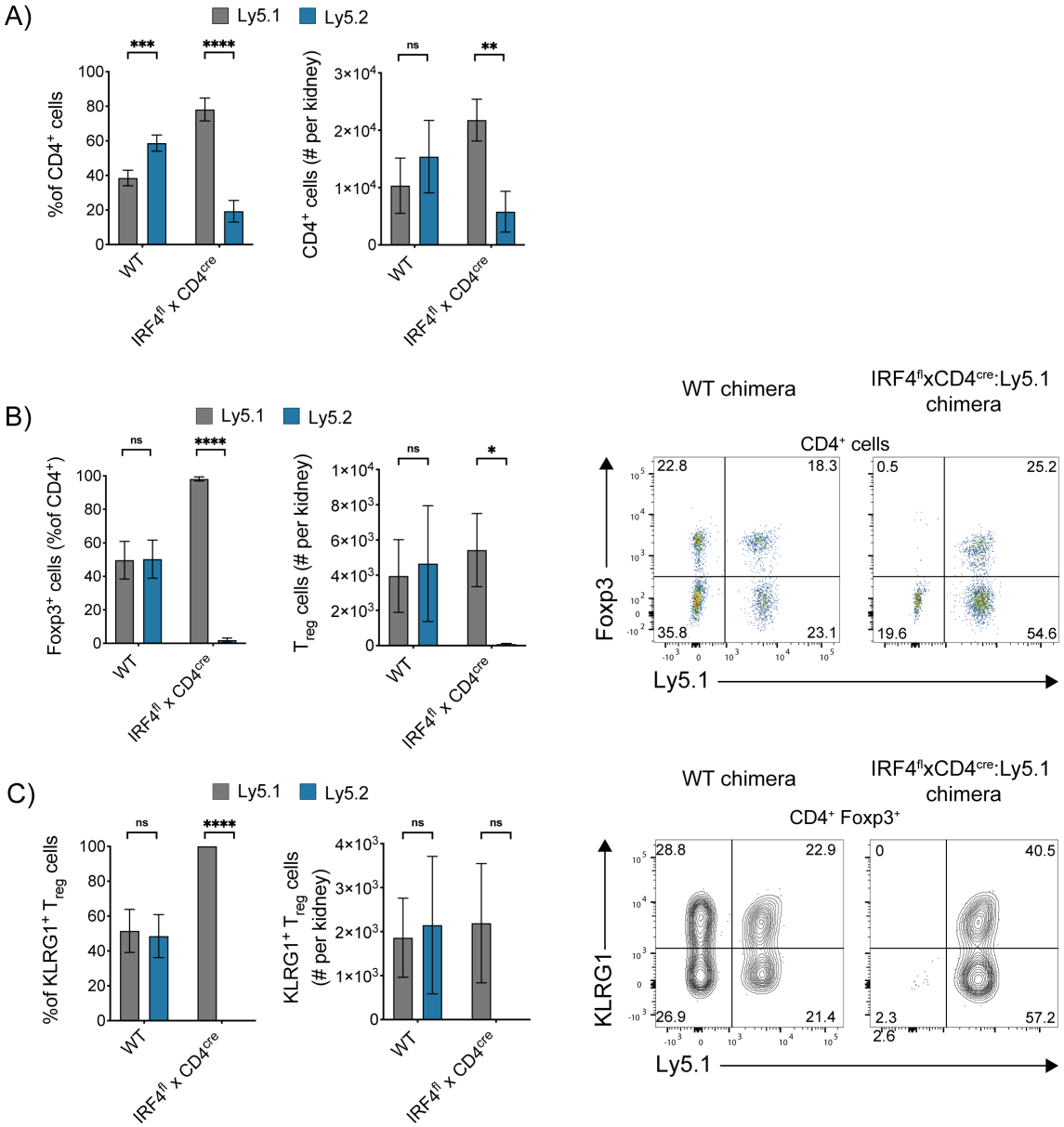


Figure 5-5 IRF4 deficiency in T cells leads to loss of Tregs in the kidney.

Bone marrow chimeric mice containing Ly5.1+ WT mixed with either Ly5.2+ WT or IRF4fl/flxCDCre bone marrow cells were fed for 42 days with 0.15% adenine-enriched diet. Frequency and numbers of CD4 T cells (**A**), regulatory T cells (**B**, left) and KLRG1$^+$ regulatory T cells (**C**, left). (**B+C**, right) Representative facs plots of Tregs and KLRG1$^+$ Tregs and Ly5.1 expression. Data shown represents the mean ± SD (4-5 mice per group, n=1). Asterisks indicate statistically significant differences assessed by one-way ANOVA (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

5.2.6 Irf4-deficiency in T cells alters myeloid infiltration in CKD

Next, we wanted to assess whether loss of IRF4 in T cells and impaired Treg development resulted in alteration in the myeloid compartment. We found a significant increase in frequency and total cell numbers of kidney macrophages in hosts that received $IRF4^{fl}xCD4^{cre}$ relative to control bone marrow (Figure 5-6, *A*). We identified a similar increase in kidney dendritic cells following reconstitution with IRF4 conditionally deleting bone marrow (Figure 5-6, *B*). There was no change in relative distribution of cDC1s and cDC2s: both cell types did increase in a similar manner in total numbers (Figure 5-6, *C* and *D*). Interestingly, we observed a slight increase in monocytes both in frequency and total cell numbers among recipients of $IRF4^{fl}xCD4^{cre}$ cells even though these increases were not significant (Figure 5-6, *E*). Numerically, PMN accumulated significant more among recipients of $IRF4^{fl}xCD4^{cre}$ compared to those of B6 marrow (Figure 5-6, *F*).

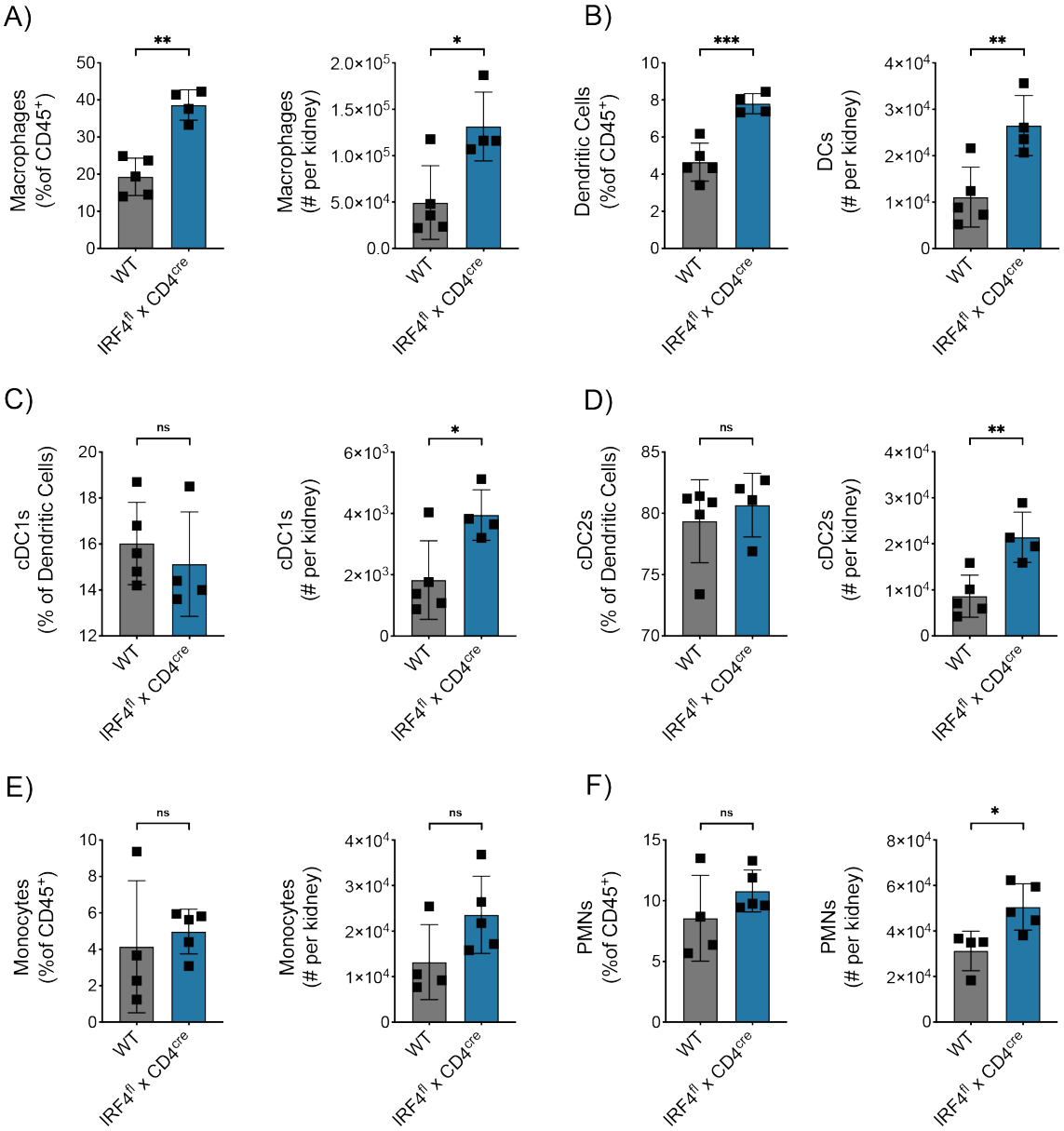


Figure 5-6 IRF4 deficiency in CD4 T cells leads to increased accumulation of myeloid cells.

Bone marrow chimeric mice (described in Figure 3) were fed for 42 days with 0.15% adenine-enriched diet. Frequencies and numbers of macrophages (**A**), dendritic cells (**B**), cDC1 DCs (**C**), cDC2 DCs (**D**), monocytes (**E**) and PMNs (**F**). Data represent the mean ± SD (4-5 mice per group, n=1). Asterisks indicate statistically significant differences between AF groups as assessed by two tailed students T test (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

Here we have found that deficiency of *Irf4* in T cells leads to a loss of kidney T_{regs}. Moreover, we found a significant increase in the amount of myeloid infiltration observed in the kidney following CKD induction. We identified a significant increase in total cell numbers of macrophages, DCs and PMNs, and a trend toward increased numbers of monocytes when IRF4 is absent from the T cell compartment. This indicates a requirement for IRF4-sufficient T cells to control tissue infiltration of myeloid cells in kidney disease.

5.2.7 Loss of IRF1 ameliorates pathology in crystal-induced chronic kidney disease

Our previous data showed the influence of IRF4 deficiency on conventional and regulatory T cell infiltration and accumulation of myeloid cells in the kidney of adenine-enriched diet fed mice. Recent publications have identified IRF1, another IRF family member, to be important for the regulation of macrophage M1/M2 polarization (Günthner et al. 2013). Specifically, IRF1 was identified to be important for nitric oxide (NO) synthesis in macrophages, making it a candidate regulator of effector function in classically activated macrophages (CAMs) (Kamijo et al. 1994).

In order to more specifically characterize the role of macrophages, in particular CAMs, in our CKD model, we utilized *Irf1* knockout mice. Analyzing the urine and blood serum after three weeks of feeding with 0.2% adenine-enriched diet (severe CKD model), we found decreased levels of urea KIM-1 and serum Timp-1 in Irf1ko mice compared with B6 mice (Figure 5-7, *A*).

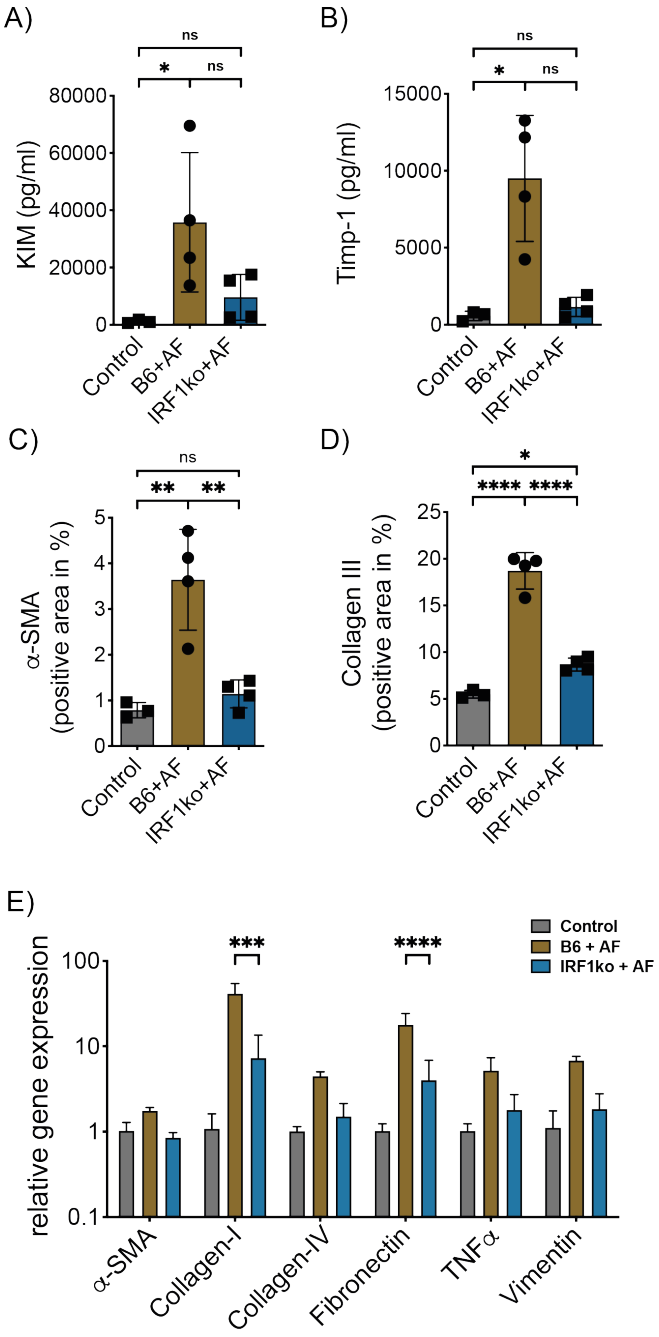


Figure 5-7 *Irf1* deficiency results in reduced kidney damage in the CKD model.

Mice were fed with 0.2% adenine-enriched diet for 21 days. (**A**) Concentration of urine KIM. (**B**) Concentration of serum TIMP-1. Patho-histologically analysis of alpha-SMA (**C**) and collagen III (**D**). Relative expression of different genes related to kidney fibrosis. Data represent the mean ± SD (3-4 mice per group, n=1). Asterisks indicate statistically significant differences between AF groups as assessed by two tailed students T test (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$).

Histopathological analysis revealed a significant reduction in for alpha-SMA expression (Figure 5-7, *B*) and an even more pronounced reduction in collagen III (Figure 5-7, *D*) in the kidneys of IRF1ko mice compared with treated B6 mice. To further investigate the impact of Irf1 deficiency we analyzed total kidney mRNA levels for genes related to kidney fibrosis. In line with the reduced disease, genes encoding alpha-SMA, collagen I and IV, fibronectin, TNFα and vimentin were expressed at lower levels in the kidneys of *Irf1*ko versus B6 CKD mice, though only the differences in collagen I and fibronectin transcript levels reached statistical significance (Figure 5-7, *E*).

Together our data identify significantly reduced kidney damage and fibrosis progression in the absence of Irf1. Strikingly, Irf1-ko animals were largely protected from CKD, appearing more similar to untreated B6 controls than treated B6 controls in each marker of pathology tested. These data identify a clear role for IRF1 in mediating the pathology observed in our experimental CKD system.

5.2.8 IRF1 deficiency leads to reduced accumulation of macrophages and monocytes in crystal-induced chronic kidney disease

Having observed a striking amelioration of pathology in crystal induced chronic kidney disease in IRF1-deficient animals, we sought to assess whether the myeloid compartment was a mediator of this phenotype. To this end, we analyzed the kidneys of IRF1ko and B6 CKD and untreated control B6 mice by flow cytometry for the composition of the myeloid compartment. We observed significantly reduced frequencies of macrophages (MHC-II$^+$ F4/80$^+$ CD11b$^+$) in the kidneys of treated IRF1ko compared to treated B6 mice, though total numbers of macrophages did not significantly differ between groups (Figure 5-8, *A*).

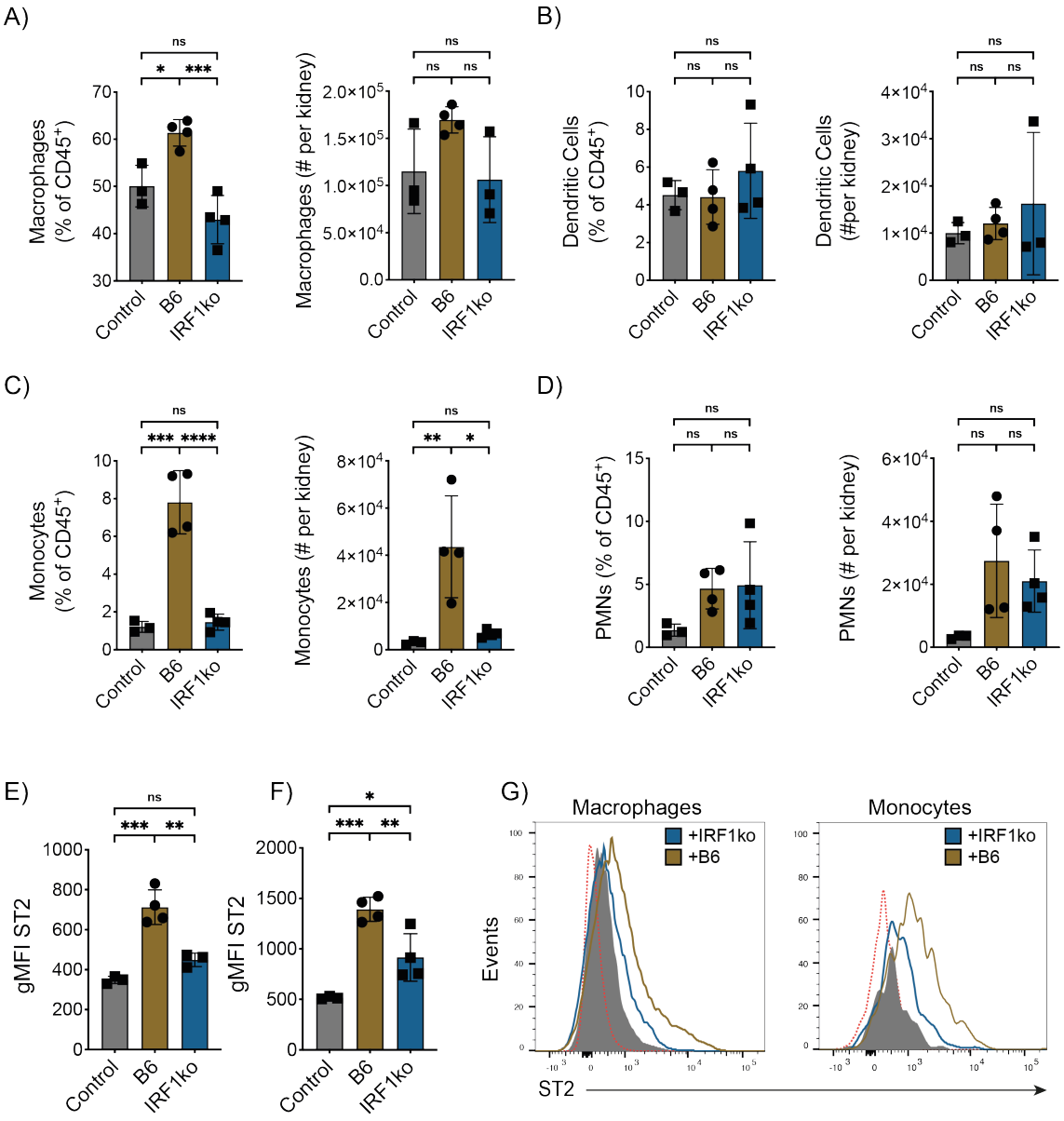


Figure 5-8 IRF1 deficiency leads to reduced accumulation of macrophages and monocytes in CKD.

Mice were fed with 0.2% adenine-enriched diet for 21 days. Frequencies and numbers of macrophages (**A**), dendritic cells (**B**), monocytes (**C**) and PMNs (**D**). Geometric MFI of ST2 expression on macrophages (**E**) and monocytes (**F**). (**G**) Representative histogram showing expression of ST2 receptor on macrophages (left) and monocytes (right). Red dotted line shows ST2-FMO control, grey population control group fed with normal diet, blue line IRF1ko mice and red line are B6 mice. Data represent the mean ± SD (3-4 mice per group, n=1). Asterisks indicate statistically significant differences between groups as assessed by one-way ANOVA test (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

Interestingly, we did not observe any differences in frequencies or numbers of DCs (MHC-II^{+} F4/80^{lo} CD11c^{+}, (Figure 5-8, *B*). Next, we analyzed kidney monocytes (CD11b^{+} Ly6C^{+} Ly6G^{-}) and found a significant reduction of monocytes in the kidneys of IRF1ko mice, in both frequencies and numbers, relative to treated B6 mice (Figure 5-8, *C*). Analysis of PMNs (CD11b^{+} Ly6C^{int} Ly6G^{+}) showed no differences between treated IRF1ko and B6 mice (Figure 5-8, *D*). Last, we investigated the expression of the IL-33 receptor ST2 and found that the kidney macrophages of IRF1ko mice showed significantly less ST2 expression compared to those of B6 treated mice (Figure 5-8, *E*). Similar results were obtained investigating the ST2 expression on monocytes, with monocytes from IRF1-ko mice showing a significantly lower MFI of ST2 (Figure 5-8, *F, G*).

Taken together, our data showed that the deficiency of IRF1 impaired the infiltration of monocytes and macrophages into the inflamed kidney. These cells were less activated in the absence of IRF1, with reduced expression of the IL-33 receptor ST2. Furthermore, we found strikingly reduced inflammation in the absence of IRF1, which correlated with reduced kidney damage and fibrosis.

5.3 Discussion

In this chapter we investigated the role of the transcription factors *Irf4* and *Batf* in a crystal-induced chronic kidney disease model. IRF4 plays important roles in T and B lymphocytes, dendritic cells and macrophages where it regulates activation, differentiation and function (Nam and Lim 2016). BATF is binding partner of IRF4 and both factors control similar regulatory pathways, at least in T cells (Huber and Lohoff 2014).

In our study we found a strong influence of *Irf4* on the lymphoid and myeloid compartment, whereas *Batf* deficiency mainly affected the T cell compartment. Using conditional knockout mice we confirmed that IRF4 is required for kidney Tregs and that the loss of IRF4 in T cells leads to accumulation of myeloid cells. Further, we used a novel reporter mouse to show distinct expression pattern of IRF4 and IRF8 in myeloid cells. Finally, we found that IRF1 is essential for monocytes and macrophage accumulation in the inflamed kidney and its ablation led to reduced kidney damage and fibrosis.

Our results showed that kidneys of *Irf4*-ko mice fed with 0.2% adenine-enriched diet had significantly more macrophages and dendritic cells than B6 or *Batf*-ko mice. This might indicate more tissue damage, which would be in line with the notion that accumulation of macrophages correlates with increased kidney damage (Eddy 2014; Meng et al. 2014; Wang and Harris 2011). However, analysis for tissue damage have still to be performed to show conclusive results.

In Chapter 3 and 4 we used the concept of the classically activated “M1” macrophage (CAM) marker CD38 and CD206 as alternatively activated “M2” macrophage (AAM) marker. Previously, IRF4 had been described to be a required transcription factor for AAM polarization and to induce signature gene transcription (Satoh et al. 2010). However, analyzing the macrophage compartment of *Irf4*-ko kidneys, we found elevated cell numbers of $CD38^+$ and $CD206^+$ macrophages, especially double positive (DP) cells accumulated in high numbers. This observation would either argue against the concept that *Irf4* is

required for AAM polarization or question that CD206 is a marker for AAMs in the kidney. However, we have to analyze this population in more depth to test if it has other AAM properties, such as arginase-1, IL-10 or TGF-β (Gordon and Martinez 2010). Further, we found a significant shift from cDC1s to cDC2s in *Irf4*-ko mice. This was surprising as IRF4 has been described to be required for cDC2 differentiation (Bajaña et al. 2016). cDC2s however, are a heterogenous group of cells with clear DC characteristics and their role especially in the kidney was shown to be mainly pro-inflammatory (Alcantara-Hernandez et al. 2017; Gottschalk and Kurts 2015; Guilliams et al. 2016). In a model of cGN it had been shown that the ablation of cDC1s directly correlated with reduced T_{reg} number in the kidney and disease aggravation. Further that study showed that cDC1 can produce IL-10 but also CCL20, a chemoattractant for T_{regs}, indicating a direct and indirect protective role for cDC1 (Evers et al. 2016). The shift from cDC1 to cDC2 cells in *Irf4* deficient mice might therefore indicate more inflammation due to missing regulation. Our study supports this idea as IRF4Ko mice have very limited T_{reg} numbers and completely lack effector ($KLRG1^+$) T_{reg}.

The role of IRF4 in myeloid cells is well documented (Yamamoto et al. 2011), whereas, only a handful of reports link BATF to the myeloid cell compartment. One is the compensatory role of BATF for BATF3 in cDC1s, (Guler et al. 2015; Tussiwand et al. 2012). However, the role for Batf in lymphoid cells is well accepted (Betz et al. 2010; Li et al. 2012; Schraml et al. 2009). Studies showed that BATF and IRF4 form cooperative complexes to promote chromatin accessibility to other TFs, and in this regard *Batf*- and *Irf4*-ko mice are very similar in their phenotype (Huber and Lohoff 2014). In line with this model, Vasanthakumar *et al*. demonstrated that VAT T_{reg} cells are dependent on IRF4 and BATF (Vasanthakumar et al. 2015). We reported here a similar phenotype regarding the T cell compartment. *Batf*-ko mice showed reduced frequencies of $CD4^+$ T cells; however, in contrast to *Irf4*-ko mice, also significantly reduced total cell numbers. Further, both knockouts displayed similar reductions in Tregs and CD8 T cells. The only difference was that *Baft*-ko mice still possessed some KLRG1+ T_{regs} in the inflamed kidney, while this populations was absent from

IRF4-KO. This might indicate that both TFs share same pathways for T_{reg} differentiation in the kidney but only IRF4 is indispensable for effector T_{reg} differentiation. In contrast to Irf4-ko mice, the deficiency of *Batf* did not lead to a significant accumulation of myeloid cell numbers in the kidney. However, we found a significant increase in cDC1 frequencies, indicating a potential role in cDC regulation for BATF as described by others (Tussiwand et al. 2012). Taking the dual role of IRF4 in lymphoid and myeloid cells into consideration and comparing the *Batf*-ko data with the *Irf4*-ko data, we cannot exclude a suppressive role for *Irf4* dependent myeloid cells in the CKD model. However, this experiment has only be performed once and we did not further investigate specific cDC2 subsets nor did we investigate the macrophages for M2 gene expression or cytokine profile. Therefore, further experiments with higher mouse numbers have to be performed to thoroughly test the role of *Irf4* in myeloid cells during CKD.

To investigate the role of IRF4 in kidney CD4 T cells in more detail, we generated mixed $IRF4^{fl}xCD4^{cre}$ bone marrow chimeric mice. With this conditional *Irf4* knockout we were able to confirm that kidney T_{reg} depend on IRF4, which goes in line with already reported IRF4 dependency in tissue T_{regs} (Burzyn et al. 2013; Cretney et al. 2011; Panduro et al. 2016; Teh et al. 2015). Further, the diminished T_{reg} population leads to increased accumulation of several myeloid cell populations, such as macrophages, DCs, monocytes and PMNs. This suggests an important role for T_{regs} to suppress kidney inflammation, which is well in line with reports for other kidney disease models (Alikhan et al. 2018). However, also this experiment needs repetition to confirm our findings. To fully understand the role of IRF4 in the myeloid cell compartment, we will use the IRF4 floxed mice and cross them with CD11c-cre or LysM-cre mice, to generate conditional knockouts in DCs and macrophages. IRF4 deficiency in DCs had been shown to deplete $CD24^{+}$ $CD11b^{+}$ cDC2s, which resulted in a reduced T_H17 response in the lung tissue (Schlitzer et al. 2013). Since cDC2 cells increased significantly in our model, this would allow us to further differentiate between the heterogenic cDC populations and further identify their specific role in CKD.

Further, IRF4 is upregulated in IL-4 treated macrophages and is associated with AAM polarization (El Chartouni et al. 2010). The conditional deletion of IRF4 with LysM-cre mice might answer our question which role AAMs play in our model.

Further we addressed the expression of IRF4 and IRF8 in myeloid cells using a novel Irf4/Irf8 double reporter mouse. We identified two clearly distinguishable macrophage populations. A recent paper reported that tissues contain two macrophage populations: Lyve1^{lo}MHC-II^{hi}Cx3CR1^{hi} and Lyve1^{hi}MHC-II^{lo}Cx3CR1^{lo} (Chakarov et al. 2019). Further flow cytometry analysis is needed to review whether these two reported populations correlate with the two populations we found. Furthermore, analysis of the cDC compartment of the double reporter mice showed at least four different populations. High expression of IRF8 has been reported for cDC1s (Guilliams et al. 2016), therefore we can identify the IRF8^{+} IRF4^{-} population as cDC1. Further, Guilliams et al showed that cDC2s express IRF4^{+}. Indeed, we identify a single IRF4 positive population, which we conclude represent cDC2. We also identify a population that expresses low amounts of both IRF4 and IRF8. Comparing VAT to kidney, we found that this double positive population contains the highest frequency of cDCs in the kidney, whereas the lowest frequency in the VAT. These cells may represent transitional cDC types. But more work is required to examine their identity and function. These results showed that the double reporter is a state-of-the-art tool and can further be used to explore different myeloid cell populations and in combination with the right cell surface marker be able to resolve everlasting questions of myeloid cell ontogeny.

Finally, we found another IRF family member to be important in chronic kidney disease. Applying our severe CKD model to *Irf1*-ko mice, we found amelioration of kidney inflammation and fibrosis. Deficiency in Irf1 led to reduced accumulation of macrophages and monocytes in the kidney. Resting DCs and macrophages only express low level of Irf1, whereas an increase of expression is detectable after stimulation of macrophages (Martinez et al. 2006). It has been shown before that IRF1 interacts with MyD88 and leads to TLR-mediated transcription of pro-

inflammatory genes (Liu et al. 2003; Negishi et al. 2006). *Irf1* deficient macrophages lack the production of inducible nitric oxide synthase (iNOS) upon LPS stimulation (Kamijo et al. 1994) and moreover IRF1 intrinsically represses IL-4 promotor activity (Elser et al. 2002). In this way IRF1 is a modulator of M1 polarization of macrophages. In our model adenine-diet treated *Irf1*-ko mice showed impaired recruitment of macrophages and monocytes into the kidney. Their numbers were similar to those in WT mice on control diet. From previous experiments we know that WT kidney macrophages under homeostatic conditions do not express CD38 or CD206, and it is very likely that these cells represent mainly tissue resident macrophages. In wildtype mice, the high influx of monocytes leads to the accumulation of pro-inflammatory monocytes and monocyte derived macrophages, both of which promote kidney inflammation (Kratofil, Kubes, and Deniset 2017; Shi and Pamer 2011). The few monocytes infiltrating the kidneys in *Irf1*-ko mice might be restricted to differentiate into M2 macrophages, which are able to suppress the ongoing inflammation. However, to investigate this point in more detail, we have to conduct further experiments. Interestingly, we found higher expression of ST2 on monocytes and macrophages in B6 mice with CKD compared to Irf1-ko mice, suggesting that IRF1 regulates expression of ST2 on myeloid cells. Moreover, we did not yet examine the lymphoid cell compartment, where *Irf1* is known to play a role in T cell differentiation (Brien et al. 2011; Giang and La Cava 2017). Unfortunately, our experiments were limited by mouse numbers and therefore we were not able yet to further investigate the role of IRF1 in more detail. Additional experiments will be necessary to more closely examine IRF1-dependent gene expression and cytokine profiles of macrophages and monocytes. Further, the use of conditional *Irf1* knockout mice will help to understand the roles of IRF1 in different cell types.

Chapter 6 - General discussion

The alarmin IL-33 is ubiquitously expressed in several tissues of the body, such as lung, skin, liver, adipose and kidney (Liew et al. 2016; Rana et al. 2019). Its role has been described in several diseases, and depending on tissue and condition, the IL-33/ST2 axis can have an immunostimulant or an immunosuppressive function and therefore attenuate or exacerbate disease progression (rev. in (Liew et al. 2016)). In the respiratory system insults to the airway epithelium releases IL-33 and initiate a T_H2 immune response (Islam and Luster 2012). In line with this notion, IL-33 promoted disease progression and fibrosis in bleomycin- and rhinovirus-induced injury models (Jackson et al. 2014; Li et al. 2014). However, due to its dual functions several studies reported protective effects of IL-33. A paper reported that in a house dust mite model of airway hyperresponsiveness IL-33 signaling can have a protective effect (Zoltowska Nilsson et al. 2018). IL-33 has also indications in different cancer, where it has mainly pro-tumorigenic effects mostly by regulating the accumulation of immunosuppressive cells (reviewed in: Larsen et al., 2018). Further, in a model of myocardial infarction IL-33 attenuated inflammation and reduced fibrosis by promoting M2 macrophages (Li et al. 2019). However, it was also shown that IL-33 exerts a protective effect due to the stimulation of $CCR2^+$ infiltrating myeloid cells that produce iNOS to fight the inflammation in the central nervous system due to *Toxoplasma gondii* infection (Still et al. 2018). Especially, in the adipose tissue IL-33 exhibits protective functions by promoting ILC2s and T_{regs} (Brestoff et al. 2015; Han et al. 2015; Vasanthakumar et al. 2015). The decline in ILC2s and T_{regs} during the chronic state of obesity is linked to development of type 2 diabetes, indicating their protective role (Brestoff et al. 2015; Shu, Benoist, and Mathis 2012). In acute kidney injury, IL-33 expression correlated with exacerbated kidney disease (Akcay et al. 2011; Park et al. 2016). In addition to these studies, it had been shown that IL-33 can promote ILC2s and be protective in a model of adriamycin-induced glomerulosclerosis (Riedel et al. 2017). However, the role of IL-33 in chronic kidney disease is not yet intensively investigated. Furthermore, it has been shown that in both diseases, adipose tissue inflammation and in chronic kidney disease, macrophage accumulation and phenotype correlate with disease progression (Anders and Ryu 2011; Zeyda

and Stulnig 2009). Here, we wanted to investigate the diversity and function of myeloid cells in two different chronic inflammatory microenvironments.

In this study, we show that high amounts of Il33 is expressed in kidney endothelia cells during homeostasis and inflammation. Furthermore, we found that *Il33* is expressed at low amounts in monocytes, PMNs, macrophages and DCs during kidney inflammation, similar to reports in lung inflammation (Hardman et al. 2013). IL-33 is stored in the nucleolus of barriers cells, such as endo- or epithelia cells. Upon tissue insult or necroptosis of these cells, IL-33 gets released into the extracellular space and activates other cells via its receptor ST2 (Cayrol and Girard 2009; Lüthi et al. 2009).

We found in a severe onset model of crystal-induced nephropathy that deficiency in ST2 does not alter the disease outcome in a significant way. However, using a mild onset model with a prolonged exposure to the insult, we found that ST2 deficient mice showed exacerbated disease. We show that during inflammatory conditions the ST2 receptor is upregulated on myeloid cells, such as macrophages, DCs, monocytes and PMNs, which is consistent with other reports (reviewed in Griesenauer and Paczesny, 2017). It has been reported that $ST2^+$ microglia have an more pronounced pro-inflammatory phenotype and that ST2 deficiency leads to exacerbated brain injury (Yang et al. 2017). In our study, we did not further characterize the specific phenotype of $ST2^+$ macrophages or other myeloid cells. However, also ILC2s and T_{regs} express the ST2 receptor and are known for their anti-inflammatory properties. Interestingly, we did not detect significant differences in T_{reg} numbers, but an increase in ILC2 numbers in ST2ko compared to WT mice. The accumulation of ILC2s in ST2 deficient mice had recently been reported in an asthma model (Verma et al. 2018). In further studies, we will more closely characterize the phenotype of $ST2^+$ myeloid cells but also investigate if the ST2 deficiency leads to the accumulation of IL-9^+ ILC2s, which further promote kidney inflammation. The induction of kidney inflammation in the mild onset model led to a significantly increased influx of immune cells of the innate and adaptive lineages into the kidneys of IL-33 deficient mice compared to WT mice.

However, we did not clarify yet whether this accumulation of immune cells was caused by the lack of IL-33-dependent suppressive cell populations, such as ILC2 or T_{regs}, or was mediated by other mechanisms. Furthermore, we did not investigate the role of mast cells, which are another cell type with high expression of ST2.

Furthermore, IL-33 has been shown to act in two different ways, either as extracellular cytokine or as an endogenous nuclear factor with transcriptional properties (Carriere et al. 2007; Gautier et al. 2016). It has been reported that endogenous IL-33 negatively modulates the NF-κB pathway and therefore is able to dampen pro-inflammatory cytokine production (Ali et al. 2011). Taking this into consideration, the knockout of *Il33* might enhance pro-inflammatory cytokine production. If this is the case, upregulation of *Il33* in both epithelial and myeloid immune cells would have a self-intrinsic regulation to attenuate immune responses. Therefore, it is necessary to evaluate our data and to further investigate the phenotype of specific immune cells in IL-33 knockout mice.

Importantly, we show that the deficiency of ST2 can have significant differences depending on disease onset. The two kidney models applied here follow the same principle and only differ in the adenine concentrations used. IL-33 is a potent immunostimulant and therefore tightly regulated. In the extracellular space the decoy receptor sST2 dampens IL-33 signaling (Akimoto et al. 2016), as well as rapid oxidation and the forming of disulfide-bridges (Cohen et al. 2015). It is important to consider, that due to this restricted availability in the onset of IL-33 release, the tissue location of immune cells might play a critical role. ILC2, tissue T_{regs} and mast cells express constitutively high level of ST2 on their surface and are therefore likely to be first to respond to IL-33 release (Cayrol and Girard 2014; Lunderius-Andersson, Enoksson, and Nilsson 2012; Turnquist et al. 2011). Similar, to the suppressive mechanism of IL2/CD25 on T_{regs}, this might reduce the availability of IL-33 to other cells. Therefore, the tissue concentration of IL-33 might play a crucial role and is likely a determining factor for disease progression.

We found that the short-term injection of IL-33 increased the numbers of ILC2s, Tregs and CD206$^+$ macrophages. However, in contrast to a kidney model of adriamycin-induced glomerulosclerosis, we found only a moderate effect of IL-33 on ILC2s (Riedel et al. 2017). Whether this is an effect of local IL-33 concentration, location of tissue insult or location of immune cell remains to be shown. However, an important factor is the use of mice on different genetic backgrounds. Riedel et al. injected IL-33 into BALB/c mice, whereas we used C57BL/6 mice. It has been shown that BALB/c mice exhibit a T_H2 dominant, whereas C57BL/6 mice exhibit a T_H1 dominant phenotype (Nemanja Jovicic et al. 2015; Watanabe et al. 2004). Thus, the use of different mouse strains has important implications for further studies. Interestingly, in our short-term model, the injection of IL-33 led to increased levels of timp-1 and collagen-III. These findings are in line with a liver fibrosis model, where the authors reported that IL-33 acted directly on hepatic stellate cells to produce IL-6, TGF-β, α-SMA and collagen (Tan et al. 2018). This direct effect of IL-33 on non-hematopoietic cells has additionally to be considered, especially if fibrosis is a part of the disease readout. Injection of IL-33 might lead to the additional induction of M2 macrophages, which play important roles in wound healing and tissue repair, but in an uncontrolled environment may also promote kidney fibrosis (Wynn and Vannella 2016).

To address the question if M2 macrophages accumulate in our model we used the mannose receptor (CD206), which has been identified in different studies as a marker for M2 macrophages (Bellón et al. 2011; Li et al. 2014; Nawaz et al. 2017). Furthermore, we used CD38 to identify M1 macrophages (Amici et al. 2018; Jablonski et al. 2015). Under homeostatic conditions, we found no expression of either marker on macrophages in the kidney. After induction of inflammation, however, we found a robust accumulation of CD38$^+$, CD206$^+$ and double positive macrophages. Having found expression of these markers in the inflamed kidney, we further wanted to know if we find similar expression pattern in the chronic inflamed adipose tissue.

Analyzing the adipose tissue, we found again both markers expressed on macrophages. Notably, our study indicates higher accumulation of $CD206^+$ macrophages in the adipose tissue of HFD fed male mice, but also in male mice compared with female mice. This correlated both with impaired metabolic fitness as examined by glucose and insulin tolerance tests (Vasanthakumar *et al.*, under review). In this study the Kallies lab also found an increased amount of Tregs in male adipose tissue and propose that they are recruited to restrain inflammation. A similar model could envisaged for M2 macrophages. However, at present we don't know the function of CD206+ macrophages in the adipose tissue. Importantly, we observed large numbers of macrophages expressing both CD206 and CD38, questioning the use of CD38 as proinflammatory marker. Here, we observed fewer $CD38^+$ cells under more inflammatory conditions. Our results contribute to the discussion of the concept of M1 and M2 macrophages and further to their identification with specific cell surface marker. More studies have to be conducted to clearly identify the heterogenous groups of macrophages and their individual contribution to disease progression in the kidney and in adipose tissue.

As outlined above, myeloid cell identification by surface markers is inherently difficult, as they often share similar patterns of marker expression. Furthermore, cell surface marker can be differentially expressed or even downregulated in different tissues (Gurka et al. 2015; Singh-Jasuja et al. 2013). Therefore, we generated a genetic mouse model which reports the expression of the lineage defining transcription factors *Irf4* and *Irf8*. Both transcription factors are expressed by different myeloid immune cells, such as macrophages and DCs. This reporter mouse together with some already known cell surface marker might aid to identify novel cell specific marker and unique cell populations across tissues. Even though, the expression of *Irf4* and *Irf8* might vary, we strongly believe that their expression is more stable, and this tool will enable us to identify novel aspects of myeloid cell development and their contribution to health and disease.

We have shown that the immune cell composition in male and female adipose tissue differs, which is paralleled by differences in the metabolic fitness (Vasanthakumar et al. 2019, under review). This study shows a pronounced sexual dimorphism of Treg cells accompanied by differences in the stroma cell compartment. Since IL-33 is a driving factor of this dimorphism in the adipose tissue, it will be important to investigate if sex specific differences also apply to the kidney model. Identifying these differences between sexes is of upmost importance, especially in regard of disease prevention and treatment (Neugarten, Golestaneh, and Kolhe 2018).

The alarmin IL-33 plays manifold roles in a cell- and tissue specific manner. Many studies show its involvement in various aspects of health and disease. However, there are still many unanswered questions. Especially, the fact that IL-33 responses do not only differ between tissues, but even in the same tissue depend on the kind of insult. Here, we report that the absence of ST2 has different implications depending on fast or slow disease onset in the kidney. Furthermore, we found differences between IL-33 deficiency and ST2 deficiency. Both findings add another layer of complexity to fully understand the mechanisms of IL-33 and future studies have to address these different outcomes. Besides that, we examined sexual dimorphism of macrophages and dendritic cells in the adipose tissue. Here, we found that male mice accumulated more macrophages and DCs in the adipose tissue in comparison to female. Additionally, we showed that male and female DCs and macrophages differ in their genetic profile, with a more pronounces pro-inflammatory phenotype in male isolated cells. However, further investigations have to show which exact role these cells play during VAT inflammation. To further characterize the phenotype of macrophages we used the marker CD206 (M2) and CD38 (M1) in our study. The accumulation of $CD206^+$ macrophages and the decline in $CD38^+$ $CD206^+$ macrophages during inflammation indicated that surface markers can vary depending on tissue and condition. The use of the here generated IRF4/IRF8 double reporter mouse might help to circumvent the use of varying cell surface marker and aid to identify specific cell populations across tissues during health and disease.

Chapter 7 - References

Ahyi, Ayele-Nati N., Hua-Chen Chang, Alexander L. Dent, Stephen L. Nutt, and Mark H. Kaplan. 2009. “IFN Regulatory Factor 4 Regulates the Expression of a Subset of Th2 Cytokines.” *Journal of Immunology (Baltimore, Md. : 1950)* 183(3):1598–1606.

Akbari, Omid, Rosemarie H. DeKruyff, and Dale T. Umetsu. 2001. “Pulmonary Dendritic Cells Producing IL-10 Mediate Tolerance Induced by Respiratory Exposure to Antigen.” *Nature Immunology* 2(8):725–31.

Akcay, Ali, Quocan Nguyen, Zhibin He, Kultigin Turkmen, Dong Won Lee, Ana Andres Hernando, Christopher Altmann, Aysun Toker, Arijana Pacic, Danica Galesic Ljubanovic, Alkesh Jani, Sarah Faubel, and Charles L. Edelstein. 2011. “IL-33 Exacerbates Acute Kidney Injury.” *Journal of the American Society of Nephrology* 22(11):2057–67.

Akimoto, Miho, Riruke Maruyama, Hiroyuki Takamaru, Takahiro Ochiya, and Keizo Takenaga. 2016. “Soluble IL-33 Receptor SST2 Inhibits Colorectal Cancer Malignant Growth by Modifying the Tumour Microenvironment.” *Nature Communications* 7(1):13589.

Alcantara-Hernandez, Marcela, Rebecca Leylek, Lisa E. Wagar, Mark M. Davis, Garry P. Nolan, and Juliana Idoyaga Correspondence. 2017. “High-Dimensional Phenotypic Mapping of Human Dendritic Cells Reveals Interindividual Variation and Tissue Specialization.” *Immunity* 47:1037-1050.e6.

Ali, Shafaqat, Antje Mohs, Meike Thomas, Jan Klare, Ralf Ross, Michael Lienhard Schmitz, and Michael Uwe Martin. 2011. “The Dual Function Cytokine IL-33 Interacts with the Transcription Factor NF-KB to Dampen NF-KB-Stimulated Gene Transcription.” *Journal of Immunology (Baltimore, Md. : 1950)* 187(4):1609–16.

Alikhan, Maliha A., Megan Huynh, A. Richard Kitching, and Joshua D. Ooi. 2018. “Regulatory T Cells in Renal Disease.” *Clinical & Translational Immunology* 7(1):e1004.

Alisi, Anna, Guido Carpino, Felipe L. Oliveira, Nadia Panera, Valerio Nobili, and Eugenio Gaudio. 2017. “The Role of Tissue Macrophage-Mediated Inflammation on NAFLD Pathogenesis and Its Clinical Implications.”

Mediators of Inflammation 2017:1–15.

Almand, Bond, Joseph I. Clark, Ekaterina Nikitina, James van Beynen, Nicholas R. English, Stella C. Knight, David P. Carbone, and Dmitry I. Gabrilovich. 2001. “Increased Production of Immature Myeloid Cells in Cancer Patients: A Mechanism of Immunosuppression in Cancer.” *The Journal of Immunology* 166(1):678–89.

Almeida, F. F. and G. T. Belz. 2016. “Innate Lymphoid Cells: Models of Plasticity for Immune Homeostasis and Rapid Responsiveness in Protection.” *Mucosal Immunology* 9(5):1103–12.

Amici, Stephanie A., Nicholas A. Young, Janiret Narvaez-Miranda, Kyle A. Jablonski, Jesus Arcos, Lucia Rosas, Tracey L. Papenfuss, Jordi B. Torrelles, Wael N. Jarjour, and Mireia Guerau-de-Arellano. 2018. “CD38 Is Robustly Induced in Human Macrophages and Monocytes in Inflammatory Conditions.” *Frontiers in Immunology* 9:1593.

Anders, Hans-Joachim and Mi Ryu. 2011. “Renal Microenvironments and Macrophage Phenotypes Determine Progression or Resolution of Renal Inflammation and Fibrosis.” *Kidney International* 80(9):915–25.

Anon. 2013. “Immunity in the Tissues.” *Nature Immunology* 14(10):977–977.

Arpaia, Nicholas, Jesse A. Green, Bruno Moltedo, Aaron Arvey, Saskia Hemmers, Shaopeng Yuan, Piper M. Treuting, and Alexander Y. Rudensky. 2015. “A Distinct Function of Regulatory T Cells in Tissue Protection.” *Cell* 162(5):1078–89.

Austyn, Jonathan M. and Siamon Gordon. 1981. “F4/80, a Monoclonal Antibody Directed Specifically against the Mouse Macrophage.” *European Journal of Immunology* 11(10):805–15.

Awad, Alaa S., Michael Rouse, Liping Huang, Amy L. Vergis, Jörg Reutershan, Helen P. Cathro, Joel Linden, and Mark D. Okusa. 2009. “Compartmentalization of Neutrophils in the Kidney and Lung Following Acute Ischemic Kidney Injury.” *Kidney International* 75(7):689–98.

Bajaña, Sandra, Sean Turner, Jinny Paul, Erola Ainsua-Enrich, and Susan Kovats. 2016. “IRF4 and IRF8 Act in CD11c+ Cells To Regulate Terminal Differentiation of Lung Tissue Dendritic Cells.” *Journal of Immunology*

(Baltimore, Md. : 1950) 196(4):1666–77.

Barbosa, Maria Thereza P., Sandra M. Soares, Colleen M. Novak, David Sinclair, James A. Levine, Pinar Aksoy, and Eduardo Nunes Chini. 2007. "The Enzyme CD38 (a NAD Glycohydrolase, EC 3.2.2.5) Is Necessary for the Development of Diet-Induced Obesity." *The FASEB Journal* 21(13):3629–39.

Barlow, Jillian L., Samantha Peel, Jane Fox, Veera Panova, Clare S. Hardman, Ana Camelo, Christine Bucks, Xiaoying Wu, Colleen M. Kane, Daniel R. Neill, Robin J. Flynn, Ian Sayers, Ian P. Hall, and Andrew N. J. McKenzie. 2013. "IL-33 Is More Potent than IL-25 in Provoking IL-13–Producing Nuocytes (Type 2 Innate Lymphoid Cells) and Airway Contraction." *Journal of Allergy and Clinical Immunology* 132(4):933–41.

Bastard, J. P., C. Jardel, J. Delattre, B. Hainque, E. Bruckert, and F. Oberlin. 1999. "Evidence for a Link between Adipose Tissue Interleukin-6 Content and Serum C-Reactive Protein Concentrations in Obese Subjects." *Circulation* 99(16):2221–22.

Bateman, A., Lachlan Coin, Richard Durbin, Robert D. Finn, Volker Hollich, Sam Griffiths-Jones, Ajay Khanna, Mhairi Marshall, Simon Moxon, Erik L. L. Sonnhammer, David J. Studholme, Corin Yeats, and Sean R. Eddy. 2004. "The Pfam Protein Families Database." *Nucleic Acids Research* 32(90001):138D – 141.

Becerra-Díaz, Mireya, Ashley B. Strickland, Aleksander Keselman, and Nicola M. Heller. 2018. "Androgen and Androgen Receptor as Enhancers of M2 Macrophage Polarization in Allergic Lung Inflammation." *The Journal of Immunology* 201(10):2923–33.

Bellón, Teresa, Virginia Martínez, Baltasar Lucendo, Gloria del Peso, María José Castro, Luiz S. Aroeira, Aranzazu Rodríguez-Sanz, Marta Ossorio, Rafael Sánchez-Villanueva, Rafael Selgas, and María Auxiliadora Bajo. 2011. "Alternative Activation of Macrophages in Human Peritoneum: Implications for Peritoneal Fibrosis." *Nephrology Dialysis Transplantation* 26(9):2995–3005.

Berg, Anders H. and Philipp E. Scherer. 2005. "Adipose Tissue, Inflammation,

and Cardiovascular Disease." *Circulation Research* 96(9):939–49.

Besnard, Anne-Gaelle, Rodrigo Guabiraba, Wanda Niedbala, Jennifer Palomo, Flora Reverchon, Tovah N. Shaw, Kevin N. Couper, Bernhard Ryffel, and Foo Y. Liew. 2015. "IL-33-Mediated Protection against Experimental Cerebral Malaria Is Linked to Induction of Type 2 Innate Lymphoid Cells, M2 Macrophages and Regulatory T Cells" edited by P. Loke. *PLOS Pathogens* 11(2):e1004607.

Betz, Briana C., Kimberly L. Jordan-Williams, Chuanwu Wang, Seung Goo Kang, Juan Liao, Michael R. Logan, Chang H. Kim, and Elizabeth J. Taparowsky. 2010. "Batf Coordinates Multiple Aspects of B and T Cell Function Required for Normal Antibody Responses." *The Journal of Experimental Medicine* 207(5):933–42.

Bhan, A. K., E. L. Reinherz, S. Poppema, R. T. McCluskey, and S. F. Schlossman. 1980. "Location of T Cell and Major Histocompatibility Complex Antigens in the Human Thymus." *Journal of Experimental Medicine* 152(4):771–82.

Bhattacharya, Palash, Isadore Budnick, Medha Singh, Muthusamy Thiruppathi, Khaled Alharshawi, Hatem Elshabrawy, Mark J. Holterman, and Bellur S. Prabhakar. 2015. "Dual Role of GM-CSF as a Pro-Inflammatory and a Regulatory Cytokine: Implications for Immune Therapy." *Journal of Interferon & Cytokine Research : The Official Journal of the International Society for Interferon and Cytokine Research* 35(8):585–99.

Bianchi, Marco E. 2007. "DAMPs, PAMPs and Alarmins: All We Need to Know about Danger." *Journal of Leukocyte Biology* 81(1):1–5.

Bianchi, Vittorio Emanuele. 2019. "The Anti-Inflammatory Effects of Testosterone." *Journal of the Endocrine Society* 3(1):91–107.

Birbrair, Alexander and Paul S. Frenette. 2016. "Niche Heterogeneity in the Bone Marrow." *Annals of the New York Academy of Sciences* 1370(1):82–96.

Birbrair, Alexander, Tan Zhang, Zhong-Min Wang, Maria Laura Messi, Grigori N. Enikolopov, Akiva Mintz, and Osvaldo Delbono. 2013. "Role of Pericytes in Skeletal Muscle Regeneration and Fat Accumulation." *Stem Cells and Development* 22(16):2298–2314.

Blum, Janice S., Pamela A. Wearsch, and Peter Cresswell. 2013. “Pathways of Antigen Processing.” *Annual Review of Immunology* 31(1):443–73.

Bogunovic, Milena, Florent Ginhoux, Amy Wagers, Martine Loubeau, Luis M. Isola, Lauren Lubrano, Vesna Najfeld, Robert G. Phelps, Celia Grosskreutz, Eilleen Scigliano, Paul S. Frenette, and Miriam Merad. 2006. “Identification of a Radio-Resistant and Cycling Dermal Dendritic Cell Population in Mice and Men.” *The Journal of Experimental Medicine* 203(12):2627–38.

Bollig, N., A. Brustle, K. Kellner, W. Ackermann, E. Abass, H. Raifer, B. Camara, C. Brendel, G. Giel, E. Bothur, M. Huber, C. Paul, A. Elli, R. A. Kroczek, R. Nurieva, C. Dong, R. Jacob, T. W. Mak, and M. Lohoff. 2012. “Transcription Factor IRF4 Determines Germinal Center Formation through Follicular T-Helper Cell Differentiation.” *Proceedings of the National Academy of Sciences* 109(22):8664–69.

Bonilla, Francisco A. and Hans C. Oettgen. 2010. “Adaptive Immunity.” *Journal of Allergy and Clinical Immunology* 125(2):S33–40.

Braga, Tarcio Teodoro, Matheus Correa-Costa, Reinaldo Correia Silva, Mario Costa Cruz, Meire Ioshie Hiyane, Joao Santana da Silva, Katia Regina Perez, Iolanda Midea Cuccovia, and Niels Olsen Saraiva Camara. 2018. “CCR2 Contributes to the Recruitment of Monocytes and Leads to Kidney Inflammation and Fibrosis Development.” *Inflammopharmacology* 26(2):403–11.

Brestoff, Jonathan R., Brian S. Kim, Steven A. Saenz, Rachel R. Stine, Laurel A. Monticelli, Gregory F. Sonnenberg, Joseph J. Thome, Donna L. Farber, Kabirullah Lutfy, Patrick Seale, and David Artis. 2015. “Group 2 Innate Lymphoid Cells Promote Beiging of White Adipose Tissue and Limit Obesity.” *Nature* 519(7542):242–46.

Brien, James D., Stephane Daffis, Helen M. Lazear, Hyelim Cho, Mehul S. Suthar, Michael Gale, and Michael S. Diamond. 2011. “Interferon Regulatory Factor-1 (IRF-1) Shapes Both Innate and CD8+ T Cell Immune Responses against West Nile Virus Infection” edited by J. K. Lim. *PLoS Pathogens* 7(9):e1002230.

Brinkmann, V., Ulrike Reichard, Christian Goosmann, Beatrix Fauler, Yvonne

Uhlemann, David S. Weiss, Yvette Weinrauch, and Arturo Zychlinsky. 2004. “Neutrophil Extracellular Traps Kill Bacteria.” *Science* 303(5663):1532–35.

Briseño, Carlos G., Malay Haldar, Nicole M. Kretzer, Xiaodi Wu, Derek J. Theisen, Wumesh KC, Vivek Durai, Gary E. Grajales-Reyes, Arifumi Iwata, Prachi Bagadia, Theresa L. Murphy, and Kenneth M. Murphy. 2016. “Distinct Transcriptional Programs Control Cross-Priming in Classical and Monocyte-Derived Dendritic Cells.” *Cell Reports* 15(11):2462–74.

Bronte, V., D. B. Chappell, E. Apolloni, A. Cabrelle, M. Wang, P. Hwu, and N. P. Restifo. 1999. “Unopposed Production of Granulocyte-Macrophage Colony-Stimulating Factor by Tumors Inhibits CD8+ T Cell Responses by Dysregulating Antigen-Presenting Cell Maturation.” *Journal of Immunology (Baltimore, Md. : 1950)* 162(10):5728–37.

Bruns, Heiko, Dimitrios Mougiakakos, Christian Bach, Martin Böttcher, Joerg Thomas Bittenbring, Maike Büttner, Kolja Gelse, Michael Rehli, Julia Wimmer, Fabian Beier, Deniz Gezer, Jan Krönke, Heidi Balzer, Stephanie Moi, Bernd Spriewald, Andreas Mackensen, and Leonhard Busch. 2016. “The IKZF1-IRF4 Axis Regulates Macrophage Polarization and Macrophage-Mediated Anti-Tumor Immunity.” *Blood* 128(22).

Brüstle, Anne, Sylvia Heink, Magdalena Huber, Christine Rosenplänter, Christine Stadelmann, Philipp Yu, Enrico Arpaia, Tak W. Mak, Thomas Kamradt, and Michael Lohoff. 2007. “The Development of Inflammatory TH-17 Cells Requires Interferon-Regulatory Factor 4.” *Nature Immunology* 8(9):958–66.

Burzyn, Dalia, Christophe Benoist, and Diane Mathis. 2013. “Regulatory T Cells in Nonlymphoid Tissues.” *Nature Immunology* 14(10):1007–13.

Buscher, Konrad, Erik Ehinger, Pritha Gupta, Akula Bala Pramod, Dennis Wolf, George Tweet, Calvin Pan, Charles D. Mills, Aldons J. Lusis, and Klaus Ley. 2017. “Natural Variation of Macrophage Activation as Disease-Relevant Phenotype Predictive of Inflammation and Cancer Survival.” *Nature Communications* 8(1):16041.

Byers, Derek E., Jennifer Alexander-Brett, Anand C. Patel, Eugene Agapov, Geoffrey Dang-Vu, Xiaohua Jin, Kangyun Wu, Yingjian You, Yael Alevy, Jean-Philippe Girard, Thaddeus S. Stappenbeck, G. Alexander Patterson,

Richard A. Pierce, Steven L. Brody, and Michael J. Holtzman. 2013. “Long-Term IL-33–Producing Epithelial Progenitor Cells in Chronic Obstructive Lung Disease.” *Journal of Clinical Investigation* 123(9):3967–82.

Cai, Dongsheng, Minsheng Yuan, Daniel F. Frantz, Peter A. Melendez, Lone Hansen, Jongsoon Lee, and Steven E. Shoelson. 2005. “Local and Systemic Insulin Resistance Resulting from Hepatic Activation of IKK-β and NF-KB.” *Nature Medicine* 11(2):183–90.

Camacho-Pereira, Juliana, Mariana G. Tarragó, Claudia C. S. Chini, Veronica Nin, Carlos Escande, Gina M. Warner, Amrutesh S. Puranik, Renee A. Schoon, Joel M. Reid, Antonio Galina, and Eduardo N. Chini. 2016. “CD38 Dictates Age-Related NAD Decline and Mitochondrial Dysfunction through an SIRT3-Dependent Mechanism.” *Cell Metabolism* 23(6):1127–39.

Campfield, L., F. Smith, Y. Guisez, R. Devos, and P. Burn. 1995. “Recombinant Mouse OB Protein: Evidence for a Peripheral Signal Linking Adiposity and Central Neural Networks.” *Science* 269(5223):546–49.

Cao, Qi, Yiping Wang, Zhiguo Niu, Chengshi Wang, Ruifeng Wang, Zhiqiang Zhang, Titi Chen, Xin Maggie Wang, Qing Li, Vincent W. S. Lee, Qingsong Huang, Jing Tan, Minghao Guo, Yuan Min Wang, Guoping Zheng, Di Yu, Stephen I. Alexander, Hui Wang, and David C. H. Harris. 2018. “Potentiating Tissue-Resident Type 2 Innate Lymphoid Cells by IL-33 to Prevent Renal Ischemia-Reperfusion Injury.” *Journal of the American Society of Nephrology* ASN.2017070774.

Carreras, Esther, Sean Turner, Vladislava Paharkova-Vatchkova, Allen Mao, Christopher Dascher, and Susan Kovats. 2008. “Estradiol Acts Directly on Bone Marrow Myeloid Progenitors to Differentially Regulate GM-CSF or Flt3 Ligand-Mediated Dendritic Cell Differentiation.” *Journal of Immunology (Baltimore, Md. : 1950)* 180(2):727–38.

Carriere, V., L. Roussel, N. Ortega, D. A. Lacorre, L. Americh, L. Aguilar, G. Bouche, and J. P. Girard. 2007. “IL-33, the IL-1-like Cytokine Ligand for ST2 Receptor, Is a Chromatin-Associated Nuclear Factor in Vivo.” *Proceedings of the National Academy of Sciences* 104(1):282–87.

Castell-Rodríguez, Andrés, Gabriela Piñón-Zárate, Miguel Herrera-Enríquez,

Katia Jarquín-Yáñez, and Iliana Medina-Solares. 2017. “Dendritic Cells: Location, Function, and Clinical Implications.” in *Biology of Myelomonocytic Cells*. InTech.

Castoldi, Angela, Cristiane Naffah de Souza, Niels Olsen Saraiva Câmara, and Pedro M. Moraes-Vieira. 2015. “The Macrophage Switch in Obesity Development.” *Frontiers in Immunology* 6:637.

Cavallo, Federica, Carla De Giovanni, Patrizia Nanni, Guido Forni, and Pier Luigi Lollini. 2011. “2011: The Immune Hallmarks of Cancer.” Pp. 319–26 in *Cancer Immunology, Immunotherapy*. Vol. 60.

Cayrol, Corinne and Jean-Philippe Girard. 2009. “The IL-1-like Cytokine IL-33 Is Inactivated after Maturation by Caspase-1.” *Proceedings of the National Academy of Sciences* 106(22):9021–26.

Cayrol, Corinne and Jean-Philippe Girard. 2014. “IL-33: An Alarmin Cytokine with Crucial Roles in Innate Immunity, Inflammation and Allergy.” *Current Opinion in Immunology* 31:31–37.

Chackerian, Alissa A., Elizabeth R. Oldham, Erin E. Murphy, Jochen Schmitz, Stefan Pflanz, and Robert A. Kastelein. 2007. “IL-1 Receptor Accessory Protein and ST2 Comprise the IL-33 Receptor Complex.” *Journal of Immunology (Baltimore, Md. : 1950)* 179(4):2551–55.

Chakarov, Svetoslav, Hwee Ying Lim, Leonard Tan, Sheau Yng Lim, Peter See, Josephine Lum, Xiao-Meng Zhang, Shihui Foo, Satoshi Nakamizo, Kaibo Duan, Wan Ting Kong, Rebecca Gentek, Akhila Balachander, Daniel Carbajo, Camille Bleriot, Benoit Malleret, John Kit Chung Tam, Sonia Baig, Muhammad Shabeer, Sue-Anne Ee Shiow Toh, Andreas Schlitzer, Anis Larbi, Thomas Marichal, Bernard Malissen, Jinmiao Chen, Michael Poidinger, Kenji Kabashima, Marc Bajenoff, Lai Guan Ng, Veronique Angeli, and Florent Ginhoux. 2019. “Two Distinct Interstitial Macrophage Populations Coexist across Tissues in Specific Subtissular Niches.” *Science (New York, N.Y.)* 363(6432):eaau0964.

Chang, Yiming K., Zheng Zuo, and Gary D. Stormo. 2018. “Quantitative Profiling of BATF Family Proteins/JUNB/IRF Hetero-Trimers Using Spec-Seq.” *BMC Molecular Biology* 19(1):5.

Chaplin, H. 2005. “Review: The Burgeoning History of the Complement System 1888-2005.” *Immunohematology* 21(3):85–93.

El Chartouni, Carol, Lucia Schwarzfischer, and Michael Rehli. 2010. “Interleukin-4 Induced Interferon Regulatory Factor (Irf) 4 Participates in the Regulation of Alternative Macrophage Priming.” *Immunobiology* 215(9–10):821–25.

Chatzigeorgiou, Antonios, Maria Lyberi, Gregorios Chatzilymperis, Adrianos Nezos, and Elli Kamper. 2009. “CD40/CD40L Signaling and Its Implication in Health and Disease.” *BioFactors* 35(6):474–83.

Chen, C., A. Gault, L. Shen, and N. Nabavi. 1994. “Molecular Cloning and Expression of Early T Cell Costimulatory Molecule-1 and Its Characterization as B7-2 Molecule.” *Journal of Immunology (Baltimore, Md.: 1950)* 152(10):4929–36.

Cheung, Clement C., Donald K. Clifton, and Robert A. Steiner. 1997. “Proopiomelanocortin Neurons Are Direct Targets for Leptin in the Hypothalamus.” *Endocrinology* 138(10):4489–92.

Chinenov, Yurii and Tom K. Kerppola. 2001. “Close Encounters of Many Kinds: Fos-Jun Interactions That Mediate Transcription Regulatory Specificity.” *Oncogene* 20(19):2438–52.

Chini, Eduardo. 2009. “CD38 as a Regulator of Cellular NAD: A Novel Potential Pharmacological Target for Metabolic Conditions.” *Current Pharmaceutical Design* 15(1):57–63.

Cho, Kae Won, Brian F. Zamarron, Lindsey A. Muir, Kanakadurga Singer, Cara E. Porsche, Jennifer B. DelProposto, Lynn Geletka, Kevin A. Meyer, Robert W. O’Rourke, and Carey N. Lumeng. 2016. “Adipose Tissue Dendritic Cells Are Independent Contributors to Obesity-Induced Inflammation and Insulin Resistance.” *The Journal of Immunology* 197(9):3650–61.

Choi, Yeon Sook, Hyun Jung Choi, Jeong Ki Min, Bo Jeong Pyun, Yong Sun Maeng, Hongryeol Park, Jihye Kim, Young Myeong Kim, and Young Guen Kwon. 2009. “Interleukin-33 Induces Angiogenesis and Vascular Permeability through ST2/TRAF6-Mediated Endothelial Nitric Oxide Production.” *Blood* 114(14):3117–26.

Christ, Anette, Patrick Günther, Mario A. R. Lauterbach, Peter Duewell, Debjani

Biswas, Karin Pelka, Claus J. Scholz, Marije Oosting, Kristian Haendler, Kevin Baßler, Kathrin Klee, Jonas Schulte-Schrepping, Thomas Ulas, Simone J. C. F. M. Moorlag, Vinod Kumar, Min Hi Park, Leo A. B. Joosten, Laszlo A. Groh, Niels P. Riksen, Terje Espevik, Andreas Schlitzer, Yang Li, Michael L. Fitzgerald, Mihai G. Netea, Joachim L. Schultze, and Eicke Latz. 2018. "Western Diet Triggers NLRP3-Dependent Innate Immune Reprogramming." *Cell* 172(1–2):162-175.e14.

Christ, Anette and Eicke Latz. 2019. "The Western Lifestyle Has Lasting Effects on Metaflammation." *Nature Reviews Immunology* 19(5):267–68.

Chylikova, Jaroslava, Jana Dvorackova, Zdenek Tauber, and Vojtech Kamarad. 2018. "M1/M2 Macrophage Polarization in Human Obese Adipose Tissue." *Biomedical Papers* 162(2):79–82.

Cipolletta, Daniela. 2014. "Adipose Tissue-Resident Regulatory T Cells: Phenotypic Specialization, Functions and Therapeutic Potential." *Immunology* 142(4):517–25.

Clark, Stephen R., Adrienne C. Ma, Samantha A. Tavener, Braedon McDonald, Zahra Goodarzi, Margaret M. Kelly, Kamala D. Patel, Subhadeep Chakrabarti, Erin McAvoy, Gary D. Sinclair, Elizabeth M. Keys, Emma Allen-Vercoe, Rebekah DeVinney, Christopher J. Doig, Francis H. Y. Green, and Paul Kubes. 2007. "Platelet TLR4 Activates Neutrophil Extracellular Traps to Ensnare Bacteria in Septic Blood." *Nature Medicine* 13(4):463–69.

Clocchiatti, Andrea, Elisa Cora, Yosra Zhang, and G. Paolo Dotto. 2016. "Sexual Dimorphism in Cancer." *Nature Reviews Cancer* 16(5):330–39.

Cohen, E. Suzanne, Ian C. Scott, Jayesh B. Majithiya, Laura Rapley, Benjamin P. Kemp, Elizabeth England, D. Gareth Rees, Catherine L. Overed-Sayer, Joanne Woods, Nicholas J. Bond, Christel Séguy Veyssier, Kevin J. Embrey, Dorothy A. Sims, Michael R. Snaith, Katherine A. Vousden, Martin D. Strain, Denice T. Y. Chan, Sara Carmen, Catherine E. Huntington, Liz Flavell, Jianqing Xu, Bojana Popovic, Christopher E. Brightling, Tristan J. Vaughan, Robin Butler, David C. Lowe, Daniel R. Higazi, Dominic J. Corkill, Richard D. May, Matthew A. Sleeman, and Tomas Mustelin. 2015. "Oxidation of the Alarmin IL-33 Regulates ST2-Dependent Inflammation." *Nature*

Communications 6(1):8327.

Collaborators, The GBD 2015 Obesity. 2017. “Health Effects of Overweight and Obesity in 195 Countries over 25 Years.” *New England Journal of Medicine* 377(1):13–27.

Coppack, Simon W. 2001. “Pro-Inflammatory Cytokines and Adipose Tissue.” *Proceedings of the Nutrition Society* 60(3):349–56.

Coppola, Antonino, Raffaele Marfella, Ludovico Coppola, Ercole Tagliamonte, Dario Fontana, Erminio Liguori, Teresa Cirillo, Maria Cafiero, Silvana Natale, and Costantino Astarita. 2009. “Effect of Weight Loss on Coronary Circulation and Adiponectin Levels in Obese Women.” *International Journal of Cardiology* 134(3):414–16.

Correa-Costa, Matheus, Tarcio Teodoro Braga, Patricia Semedo, Caroline Yuri Hayashida, Luiz Roberto Grassmann Bechara, Rosa Maria Elias, Claudiene Rodrigues Barreto, Claudia Silva-Cunha, Meire Ioshie Hyane, Giselle Martins Gonçalves, Patricia Chakur Brum, Clarice Fujihara, Roberto Zatz, Alvaro Pacheco-Silva, Dario S. Zamboni, and Niels Olsen Saraiva Camara. 2011. “Pivotal Role of Toll-Like Receptors 2 and 4, Its Adaptor Molecule MyD88, and Inflammasome Complex in Experimental Tubule-Interstitial Nephritis” edited by J. Coers. *PLoS ONE* 6(12):e29004.

Cretney, Erika, Axel Kallies, and Stephen L. Nutt. 2013. “Differentiation and Function of Foxp3+ Effector Regulatory T Cells.” *Trends in Immunology* 34(2):74–80.

Cretney, Erika, Annie Xin, Wei Shi, Martina Minnich, Frederick Masson, Maria Miasari, Gabrielle T. Belz, Gordon K. Smyth, Meinrad Busslinger, Stephen L. Nutt, and Axel Kallies. 2011. “The Transcription Factors Blimp-1 and IRF4 Jointly Control the Differentiation and Function of Effector Regulatory T Cells.” *Nature Immunology* 12(4):304–11.

Dagher, Rania, Alan M. Copenhaver, Valerie Besnard, Marielle Maret, Fatima Hamidi, Aaron A. Berlin, Michel Aubier, Roland Kolbeck, Alison A. Humbles, and Marina Pretolani. 2017. “IL-33/ST2 Macrophage Crosstalk Promotes Lung Epithelial Repair.” *The Journal of Immunology* 198(1 Supplement).

Dalmas, Elise, Nicolas Venteclef, Charles Caer, Christine Poitou, Isabelle

Cremer, Judith Aron-Wisnewsky, Sébastien Lacroix-Desmazes, Jagadeesh Bayry, Srinivas V. Kaveri, Karine Clément, Sébastien André, and Michèle Guerre-Millo. 2014. “T Cell–Derived IL-22 Amplifies IL-1β–Driven Inflammation in Human Adipose Tissue: Relevance to Obesity and Type 2 Diabetes.” *Diabetes* 63(6):1966–77.

Das, Amitava, Mithun Sinha, Soma Datta, Motaz Abas, Scott Chaffee, Chandan K. Sen, and Sashwati Roy. 2015. “Monocyte and Macrophage Plasticity in Tissue Repair and Regeneration.” *The American Journal of Pathology* 185(10):2596–2606.

Davis, Kathryn E., Michael D. Neinast, Kai Sun, William M. Skiles, Jessica D. Bills, Jordan A. Zehr, Daniel Zeve, Lisa D. Hahner, Derek W. Cox, Lana M. Gent, Yong Xu, Zhao V. Wang, Sohaib A. Khan, and Deborah J. Clegg. 2013. “The Sexually Dimorphic Role of Adipose and Adipocyte Estrogen Receptors in Modulating Adipose Tissue Expansion, Inflammation, and Fibrosis.” *Molecular Metabolism* 2(3):227–42.

Deaglio, Silvia, Karen M. Dwyer, Wenda Gao, David Friedman, Anny Usheva, Anna Erat, Jiang-Fan Chen, Keiichii Enjyoji, Joel Linden, Mohamed Oukka, Vijay K. Kuchroo, Terry B. Strom, and Simon C. Robson. 2007. “Adenosine Generation Catalyzed by CD39 and CD73 Expressed on Regulatory T Cells Mediates Immune Suppression.” *The Journal of Experimental Medicine* 204(6):1257–65.

Díez, Juan J. and Pedro Iglesias. 2003. “The Role of the Novel Adipocyte-Derived Hormone Adiponectin in Human Disease.” *European Journal of Endocrinology* 148(3):293–300.

Dubiński, Andrzej and Zygmunt Zdrojewicz. 2007. “[The Role of Interleukin-6 in Development and Progression of Atherosclerosis].” *Polski Merkuriusz Lekarski : Organ Polskiego Towarzystwa Lekarskiego* 22(130):291–94.

Duffield, Jeremy S, Stuart J. Forbes, Christothea M. Constandinou, Spike Clay, Marina Partolina, Srilatha Vuthoori, Shengji Wu, Richard Lang, and John P. Iredale. 2005. “Selective Depletion of Macrophages Reveals Distinct, Opposing Roles during Liver Injury and Repair.” *The Journal of Clinical Investigation* 115(1):56–65.

Duffield, Jeremy S., Peter G. Tipping, Tiina Kipari, Jean-François Cailhier, Spike Clay, Richard Lang, Joseph V. Bonventre, and Jeremy Hughes. 2005. “Conditional Ablation of Macrophages Halts Progression of Crescentic Glomerulonephritis.” *The American Journal of Pathology* 167(5):1207–19.

Eberl, Gérard, James P. Di Santo, and Eric Vivier. 2015. “The Brave New World of Innate Lymphoid Cells.” *Nature Immunology* 16(1):1–5.

Eddy, Allison A. 2014. “Overview of the Cellular and Molecular Basis of Kidney Fibrosis.” *Kidney International Supplements* 4(1):2–8.

Ehses, J. A., A. Perren, E. Eppler, P. Ribaux, J. A. Pospisilik, R. Maor-Cahn, X. Gueripel, H. Ellingsgaard, M. K. J. Schneider, G. Biollaz, A. Fontana, M. Reinecke, F. Homo-Delarche, and M. Y. Donath. 2007. “Increased Number of Islet-Associated Macrophages in Type 2 Diabetes.” *Diabetes* 56(9):2356–70.

Elser, Bernd, Michael Lohoff, Sonja Kock, Marco Giaisi, Sabine Kirchhoff, Peter H. Krammer, and Min Li-Weber. 2002. “IFN-γ Represses IL-4 Expression via IRF-1 and IRF-2.” *Immunity* 17(6):703–12.

Ermert, David, Maria J. Niemiec, Marc Röhm, Andreas Glenthøj, Niels Borregaard, and Constantin F. Urban. 2013. “*Candida Albicans* Escapes from Mouse Neutrophils.” *Journal of Leukocyte Biology* 94(2):223–36.

Estrany, Maria E., Ana M. Proenza, Magdalena Gianotti, and Isabel Lladó. 2013. “High-Fat Diet Feeding Induces Sex-Dependent Changes in Inflammatory and Insulin Sensitivity Profiles of Rat Adipose Tissue.” *Cell Biochemistry and Function* 31(6):504–10.

Evers, Beatrix D. G., Daniel R. Engel, Alexander M. C. Böhner, André P. Tittel, Torsten A. Krause, Christoph Heuser, Natalio Garbi, Wolfgang Kastenmüller, Matthias Mack, Gisa Tiegs, Ulf Panzer, Peter Boor, Isis Ludwig-Portugall, and Christian Kurts. 2016. “CD103+ Kidney Dendritic Cells Protect against Crescentic GN by Maintaining IL-10-Producing Regulatory T Cells.” *Journal of the American Society of Nephrology : JASN*.

Fang, Difeng and Jinfang Zhu. 2017. “Dynamic Balance between Master Transcription Factors Determines the Fates and Functions of CD4 T Cell and Innate Lymphoid Cell Subsets.” *The Journal of Experimental Medicine*

214(7):1861–76.

Farber, Donna L., Naomi A. Yudanin, and Nicholas P. Restifo. 2014. “Human Memory T Cells: Generation, Compartmentalization and Homeostasis.” *Nature Reviews Immunology* 14(1):24–35.

Ferenbach, David A., Tara A. Sheldrake, Kevin Dhaliwal, Tiina M. J. Kipari, Lorna P. Marson, David C. Kluth, and Jeremy Hughes. 2012. “Macrophage/Monocyte Depletion by Clodronate, but Not Diphtheria Toxin, Improves Renal Ischemia/Reperfusion Injury in Mice.” *Kidney International* 82(8):928–33.

Ferrante, A. W. Jr. 2013. “The Immune Cells in Adipose Tissue.” *Diabetes, Obesity & Metabolism* 15 Suppl 3(0 3):34–38.

Feuerer, Markus, Laura Herrero, Daniela Cipolletta, Afia Naaz, Jamie Wong, Ali Nayer, Jongsoon Lee, Allison B. Goldfine, Christophe Benoist, Steven Shoelson, and Diane Mathis. 2009. “Lean, but Not Obese, Fat Is Enriched for a Unique Population of Regulatory T Cells That Affect Metabolic Parameters.” *Nature Medicine* 15(8):930–39.

Feuerer, Markus, Jonathan A. Hill, Diane Mathis, and Christophe Benoist. 2009. “Foxp3+ Regulatory T Cells: Differentiation, Specification, Subphenotypes.” *Nature Immunology* 10(7):689–95.

Finkelstein, Joel S., Hang Lee, Sherri-Ann M. Burnett-Bowie, J. Carl Pallais, Elaine W. Yu, Lawrence F. Borges, Brent F. Jones, Christopher V. Barry, Kendra E. Wulczyn, Bijoy J. Thomas, and Benjamin Z. Leder. 2013. “Gonadal Steroids and Body Composition, Strength, and Sexual Function in Men.” *New England Journal of Medicine* 369(11):1011–22.

Fitzgerald, Sarah Jayne, Amol Vijay Janorkar, Allison Barnes, and Rodrigo Oscar Maranon. 2018. “A New Approach to Study the Sex Differences in Adipose Tissue.” *Journal of Biomedical Science* 25(1):89.

Flier, Jeffrey S. 2004. “Obesity Wars: Molecular Progress Confronts an Expanding Epidemic.” *Cell* 116(2):337–50.

Flores-Langarica, Adriana, Charlotte Cook, Katarzyna Müller Luda, Emma K. Persson, Jennifer L. Marshall, Nonantzin Beristain-Covarrubias, Juan Carlos Yam-Puc, Madelene Dahlgren, Jenny J. Persson, Satoshi Uematsu, Shizuo

Akira, Ian R. Henderson, Bengt Johansson Lindbom, William Agace, and Adam F. Cunningham. 2018. “Intestinal CD103+CD11b+ CDC2 Conventional Dendritic Cells Are Required for Primary CD4+ T and B Cell Responses to Soluble Flagellin.” *Frontiers in Immunology* 9:2409.

Fock, Valerie, Mario Mairhofer, Gerlinde R. Otti, Ursula Hiden, Andreas Spittler, Harald Zeisler, Christian Fiala, Martin Knöfler, and Jürgen Pollheimer. 2013. “Macrophage-Derived IL-33 Is a Critical Factor for Placental Growth.” *The Journal of Immunology* 191(7):3734–43.

Fujisaka, S., I. Usui, A. Bukhari, M. Ikutani, T. Oya, Y. Kanatani, K. Tsuneyama, Y. Nagai, K. Takatsu, M. Urakaze, M. Kobayashi, and K. Tobe. 2009. “Regulatory Mechanisms for Adipose Tissue M1 and M2 Macrophages in Diet-Induced Obese Mice.” *Diabetes* 58(11):2574–82.

Gabrilovich, Dmitry I. 2017. “Myeloid-Derived Suppressor Cells.” *Cancer Immunology Research* 5(1):3–8.

Gabrilovich, Dmitry I., Suzanne Ostrand-Rosenberg, and Vincenzo Bronte. 2012. “Coordinated Regulation of Myeloid Cells by Tumours.” *Nature Reviews Immunology* 12(4):253–68.

Gadó, K., G. Domján, H. Hegyesi, and A. Falus. 2000. “ROLE OF INTERLEUKIN-6 IN THE PATHOGENESIS OF MULTIPLE MYELOMA.” *Cell Biology International* 24(4):195–209.

Gasteiger, Georg, Xiying Fan, Stanislav Dikiy, Sue Y. Lee, and Alexander Y. Rudensky. 2015. “Tissue Residency of Innate Lymphoid Cells in Lymphoid and Nonlymphoid Organs.” *Science (New York, N.Y.)* 350(6263):981–85.

Gautier, Emmanuel L., Tal Shay, Jennifer Miller, Melanie Greter, Claudia Jakubzick, Stoyan Ivanov, Julie Helft, Andrew Chow, Kutlu G. Elpek, Simon Gordonov, Amin R. Mazloom, Avi Ma’ayan, Wei-Jen Chua, Ted H. Hansen, Shannon J. Turley, Miriam Merad, Gwendalyn J. Randolph, the Immunological Genome Consortium, Emmanuel L. Gautier, Claudia Jakubzick, Gwendalyn J. Randolph, Adam J. Best, Jamie Knell, Ananda Goldrath, Jennifer Miller, Brian Brown, Miriam Merad, Vladimir Jojic, Daphne Koller, Nadia Cohen, Patrick Brennan, Michael Brenner, Tal Shay, Aviv Regev, Anne Fletcher, Kutlu Elpek, Angelique Bellemare-Pelletier, Deepali

Malhotra, Shannon Turley, Radu Jianu, David Laidlaw, Jim Collins, Kavitha Narayan, Katelyn Sylvia, Joonsoo Kang, Roi Gazit, Brian S. Garrison, Derrick J. Rossi, Francis Kim, Tata Nageswara Rao, Amy Wagers, Susan A. Shinton, Richard R. Hardy, Paul Monach, Natalie A. Bezman, Joseph C. Sun, Charlie C. Kim, Lewis L. Lanier, Tracy Heng, Taras Kreslavsky, Michio Painter, Jeffrey Ericson, Scott Davis, Diane Mathis, and Christophe Benoist. 2012. “Gene-Expression Profiles and Transcriptional Regulatory Pathways That Underlie the Identity and Diversity of Mouse Tissue Macrophages.” *Nature Immunology* 13(11):1118–28.

Gautier, Violette, Corinne Cayrol, Dorian Farache, Stéphane Roga, Bernard Monsarrat, Odile Burlet-Schiltz, Anne Gonzalez de Peredo, and Jean-Philippe Girard. 2016. “Extracellular IL-33 Cytokine, but Not Endogenous Nuclear IL-33, Regulates Protein Expression in Endothelial Cells.” *Scientific Reports* 6(1):34255.

Gavin, Kathleen M., Elizabeth E. Cooper, and Robert C. Hickner. 2013. “Estrogen Receptor Protein Content Is Different in Abdominal than Gluteal Subcutaneous Adipose Tissue of Overweight-to-Obese Premenopausal Women.” *Metabolism* 62(8):1180–88.

Giang, Sophia and Antonio La Cava. 2017. “IRF1 and BATF: Key Drivers of Type 1 Regulatory T-Cell Differentiation.” *Cellular & Molecular Immunology* 14(8):652–54.

Gomes, Ana P., Nathan L. Price, Alvin J. Y. Ling, Javid J. Moslehi, Magdalene K. Montgomery, Luis Rajman, James P. White, João S. Teodoro, Christiane D. Wrann, Basil P. Hubbard, Evi M. Mercken, Carlos M. Palmeira, Rafael De Cabo, Anabela P. Rolo, Nigel Turner, Eric L. Bell, and David A. Sinclair. 2013. “Declining NAD+ Induces a Pseudohypoxic State Disrupting Nuclear-Mitochondrial Communication during Aging.” *Cell* 155(7):1624–38.

Gondek, David C., Li-Fan Lu, Sergio A. Quezada, Shimon Sakaguchi, and Randolph J. Noelle. 2005. “Cutting Edge: Contact-Mediated Suppression by CD4+CD25+ Regulatory Cells Involves a Granzyme B-Dependent, Perforin-Independent Mechanism.” *Journal of Immunology (Baltimore, Md.: 1950)* 174(4):1783–86.

Gordon, Siamon. 2003. “Alternative Activation of Macrophages.” *Nature Reviews Immunology* 3(1):23–35.

Gordon, Siamon and Fernando O. Martinez. 2010. “Alternative Activation of Macrophages: Mechanism and Functions.” *Immunity* 32(5):593–604.

Gosselin, David, Verena M. Link, Casey E. Romanoski, Gregory J. Fonseca, Dawn Z. Eichenfield, Nathanael J. Spann, Joshua D. Stender, Hyun B. Chun, Hannah Garner, Frederic Geissmann, and Christopher K. Glass. 2014. “Environment Drives Selection and Function of Enhancers Controlling Tissue-Specific Macrophage Identities.” *Cell* 159(6):1327–40.

Gottschalk, Catherine and Christian Kurts. 2015. “The Debate about Dendritic Cells and Macrophages in the Kidney.” *Frontiers in Immunology* 6:435.

Graeff, Richard, Qun Liu, Irina A. Kriksunov, Masayo Kotaka, Norman Oppenheimer, Quan Hao, and Hon Cheung Lee. 2009. “Mechanism of Cyclizing NAD to Cyclic ADP-Ribose by ADP-Ribosyl Cyclase and CD38.” *Journal of Biological Chemistry* 284(40):27629–36.

Gregor, Margaret F. and Gökhan S. Hotamisligil. 2011. “Inflammatory Mechanisms in Obesity.” *Annual Review of Immunology* 29(1):415–45.

Griesenauer, Brad and Sophie Paczesny. 2017. “The ST2/IL-33 Axis in Immune Cells during Inflammatory Diseases.” *Frontiers in Immunology* 8:475.

Grusdat, M., D. R. McIlwain, H. C. Xu, V. I. Pozdeev, J. Knievel, S. Q. Crome, C. Robert-Tissot, R. J. Dress, A. A. Pandyra, D. E. Speiser, E. Lang, S. K. Maney, A. R. Elford, S. R. Hamilton, S. Scheu, K. Pfeffer, J. Bode, H. W. Mittrücker, M. Lohoff, M. Huber, D. Häussinger, P. S. Ohashi, T. W. Mak, K. S. Lang, and P. A. Lang. 2014. “IRF4 and BATF Are Critical for CD8+ T-Cell Function Following Infection with LCMV.” *Cell Death & Differentiation* 21(7):1050–60.

Guilliams, Martin, Charles-Antoine Dutertre, Charlotte L. Scott, Naomi McGovern, Dorine Sichien, Svetoslav Chakarov, Sofie Van Gassen, Jinmiao Chen, Michael Poidinger, Sofie De Prijck, Simon J. Tavernier, Ivy Low, Sergio Erdal Irac, Citra Nurfarah Mattar, Hermi Rizal Sumatoh, Gillian Hui Ling Low, Tam John Kit Chung, Dedrick Kok Hong Chan, Ker Kan Tan, Tony Lim Kiat Hon, Even Fossum, Bjarne Bogen, Mahesh Choolani, Jerry Kok Yen Chan, Anis

Larbi, Hervé Luche, Sandrine Henri, Yvan Saeys, Evan William Newell, Bart N. Lambrecht, Bernard Malissen, and Florent Ginhoux. 2016. “Unsupervised High-Dimensional Analysis Aligns Dendritic Cells across Tissues and Species.” *Immunity* 45(3):669–84.

Guilliams, Martin, Florent Ginhoux, Claudia Jakubzick, Shalin H. Naik, Nobuyuki Onai, Barbara U. Schraml, Elodie Segura, Roxane Tussiwand, and Simon Yona. 2014. “Dendritic Cells, Monocytes and Macrophages: A Unified Nomenclature Based on Ontogeny.” *Nature Reviews. Immunology* 14(8):571–78.

Guler, Reto, Sugata Roy, Harukazu Suzuki, and Frank Brombacher. 2015. “Targeting Batf2 for Infectious Diseases and Cancer.” *Oncotarget* 6(29).

Günthner, Roman, Hans-Joachim Anders, and Hans-Joachim Anders. 2013. “Interferon-Regulatory Factors Determine Macrophage Phenotype Polarization.” *Mediators of Inflammation* 2013:731023.

Gurka, Stephanie, Evelyn Hartung, Martina Becker, and Richard A. Kroczek. 2015. “Mouse Conventional Dendritic Cells Can Be Universally Classified Based on the Mutually Exclusive Expression of XCR1 and SIRPÎ±.” *Frontiers in Immunology* 6:35.

Gwilz. 2017. “Mechanisms of Suppression by Regulatory T Cells.”

den Haan, Joke M. M., Ramon Arens, and Menno C. van Zelm. 2014. “The Activation of the Adaptive Immune System: Cross-Talk between Antigen-Presenting Cells, T Cells and B Cells.” *Immunology Letters* 162(2):103–12.

Hadad, Nurit, Olga Burgazliev, Vered Elgazar-Carmon, Yulia Solomonov, Stephan Wueest, Flurin Item, Daniel Konrad, Assaf Rudich, and Rachel Levy. 2013. “Induction of Cytosolic Phospholipase A $_2$ α Is Required for Adipose Neutrophil Infiltration and Hepatic Insulin Resistance Early in the Course of High-Fat Feeding.” *Diabetes* 62(9):3053–63.

Hagman, James. 2017. “Critical Functions of IRF4 in B and T Lymphocytes.” *Journal of Immunology (Baltimore, Md. : 1950)* 199(11):3715–16.

Haile, Lydia A., Reinhard von Wasielewski, Jaba Gamrekelashvili, Christine Krüger, Oliver Bachmann, Astrid M. Westendorf, Jan Buer, Roland Liblau, Michael P. Manns, Firouzeh Korangy, and Tim F. Greten. 2008. “Myeloid-

Derived Suppressor Cells in Inflammatory Bowel Disease: A New Immunoregulatory Pathway." *Gastroenterology* 135(3):871-881.e5.

Han, Jonathan M., Dan Wu, Heather C. Denroche, Yu Yao, C. Bruce Verchere, and Megan K. Levings. 2015. "IL-33 Reverses an Obesity-Induced Deficit in Visceral Adipose Tissue ST2+ T Regulatory Cells and Ameliorates Adipose Tissue Inflammation and Insulin Resistance." *Journal of Immunology (Baltimore, Md. : 1950)* 194(10):4777–83.

Han, Longhui, Minglian Zhang, Xu Liang, Xin Jia, Jinchen Jia, Miying Zhao, and Yiming Fan. 2017. "Interleukin-33 Promotes Inflammation-Induced Lymphangiogenesis via ST2/TRAF6-Mediated Akt/ENOS/NO Signalling Pathway." *Scientific Reports* 7(1).

Hardman, Clare S., Veera Panova, and Andrew N. J. McKenzie. 2013. "IL-33 Citrine Reporter Mice Reveal the Temporal and Spatial Expression of IL-33 during Allergic Lung Inflammation." *European Journal of Immunology* 43(2):488.

Hardy, Olga T., Richard A. Perugini, Sarah M. Nicoloro, Karen Gallagher-Dorval, Vishwajeet Puri, Juerg Straubhaar, and Michael P. Czech. 2011. "Body Mass Index-Independent Inflammation in Omental Adipose Tissue Associated with Insulin Resistance in Morbid Obesity." *Surgery for Obesity and Related Diseases* 7(1):60–67.

Hart, D. N. and J. W. Fabre. 1981. "Major Histocompatibility Complex Antigens in Rat Kidney, Ureter, and Bladder. Localization with Monoclonal Antibodies and Demonstration of Ia-Positive Dendritic Cells." *Transplantation* 31(5):318–25.

Harwood, Naomi E. and Facundo D. Batista. 2010. "Early Events in B Cell Activation." *Annual Review of Immunology* 28(1):185–210.

Hasan, Amal, Fahad Al-Ghimlas, Samia Warsame, Asma Al-Hubail, Rasheed Ahmad, Abdullah Bennakhi, Monira Al-Arouj, Kazem Behbehani, Mohammed Dehbi, and Said Dermime. 2014. "IL-33 Is Negatively Associated with the BMI and Confers a Protective Lipid/Metabolic Profile in Non-Diabetic but Not Diabetic Subjects." *BMC Immunology* 15:19.

Hayashi, Hisako, Akiko Kawakita, Shintaro Okazaki, Hiroki Murai, Motoko

Yasutomi, and Yusei Ohshima. 2014. “IL-33 Enhanced the Proliferation and Constitutive Production of IL-13 and IL-5 by Fibrocytes.” *BioMed Research International* 2014.

Hazlett, Linda D., Sharon A. McClellan, Ronald P. Barrett, Xi Huang, Yunfan Zhang, Minhao Wu, Nico van Rooijen, and Elizabeth Szliter. 2010. “IL-33 Shifts Macrophage Polarization, Promoting Resistance against Pseudomonas Aeruginosa Keratitis.” *Investigative Opthalmology & Visual Science* 51(3):1524.

Heath, William R. and Francis R. Carbone. 2001. “Cross-Presentation in Viral Immunity and Self-Tolerance.” *Nature Reviews Immunology* 1(2):126–34.

Hesketh, Mark, Katherine B. Sahin, Zoe E. West, and Rachael Z. Murray. 2017. “Macrophage Phenotypes Regulate Scar Formation and Chronic Wound Healing.” *International Journal of Molecular Sciences* 18(7).

Hildner, K., B. T. Edelson, W. E. Purtha, M. Diamond, H. Matsushita, M. Kohyama, B. Calderon, B. U. Schraml, E. R. Unanue, M. S. Diamond, R. D. Schreiber, T. L. Murphy, and K. M. Murphy. 2008. “Batf3 Deficiency Reveals a Critical Role for CD8 + Dendritic Cells in Cytotoxic T Cell Immunity.” *Science* 322(5904):1097–1100.

Hobert, O. 2008. “Regulatory Logic of Neuronal Diversity: Terminal Selector Genes and Selector Motifs.” *Proceedings of the National Academy of Sciences* 105(51):20067–71.

Hochheiser, Katharina, Daniel R. Engel, Linda Hammerich, Felix Heymann, Percy A. Knolle, Ulf Panzer, and Christian Kurts. 2011. “Kidney Dendritic Cells Become Pathogenic during Crescentic Glomerulonephritis with Proteinuria.” *Journal of the American Society of Nephrology* 22(2):306–16.

Höchst, Bastian, Julita Mikulec, Tania Baccega, Christina Metzger, Meike Welz, Julia Peusquens, Frank Tacke, Percy Knolle, Christian Kurts, Linda Diehl, and Isis Ludwig-Portugall. n.d. “Differential Induction of Ly6G and Ly6C Positive Myeloid Derived Suppressor Cells in Chronic Kidney and Liver Inflammation and Fibrosis.”

Hoeffel, Guillaume, Jinmiao Chen, Yonit Lavin, Donovan Low, Francisca F. Almeida, Peter See, Anna E. Beaudin, Josephine Lum, Ivy Low, E. Camilla

Forsberg, Michael Poidinger, Francesca Zolezzi, Anis Larbi, Lai Guan Ng, Jerry K. Y. Chan, Melanie Greter, Burkhard Becher, Igor M. Samokhvalov, Miriam Merad, and Florent Ginhoux. 2015. “C-Myb+ Erythro-Myeloid Progenitor-Derived Fetal Monocytes Give Rise to Adult Tissue-Resident Macrophages.” *Immunity* 42(4):665–78.

Hoffman, Barbara, Abbas Abdollahi, Kenneth A. Lord, Barbara Hoffman-Liebermann, and Dan A. Liebermann2. 1991. *Interferon Regulatory Factor-1 (IRF-1) Is a Primary Response Gene to IL-6 and Leukemia Inhibitory Factor (LIF) Induced Terminal Differentiation: Role in Growth Inhibition Interferon Regulatory Factor 1 Is a Myeloid Differentiation Primary Response Gene Induced by Interleukin 6 and Leukemia Inhibitory Factor: Role in Growth Inhibition’*.

Holmberg, Johan and Thomas Perlmann. 2012. “Maintaining Differentiated Cellular Identity.” *Nature Reviews Genetics* 13(6):429–39.

Holtschke, T., J. Löhler, Y. Kanno, T. Fehr, N. Giese, F. Rosenbauer, J. Lou, K. P. Knobeloch, L. Gabriele, J. F. Waring, M. F. Bachmann, R. M. Zinkernagel, H. C. Morse, K. Ozato, and I. Horak. 1996. “Immunodeficiency and Chronic Myelogenous Leukemia-like Syndrome in Mice with a Targeted Mutation of the ICSBP Gene.” *Cell* 87(2):307–17.

Honda, Kenya and Tadatsugu Taniguchi. 2006. “IRFs: Master Regulators of Signalling by Toll-like Receptors and Cytosolic Pattern-Recognition Receptors.” *Nature Reviews Immunology* 6(9):644–58.

Hotamisligil, G. S., P. Arner, J. F. Caro, R. L. Atkinson, and B. M. Spiegelman. 1995. “Increased Adipose Tissue Expression of Tumor Necrosis Factor-Alpha in Human Obesity and Insulin Resistance.” *Journal of Clinical Investigation* 95(5):2409–15.

Hotamisligil, G. S., N. S. Shargill, and B. M. Spiegelman. 1993. “Adipose Expression of Tumor Necrosis Factor-Alpha: Direct Role in Obesity-Linked Insulin Resistance.” *Science (New York, N.Y.)* 259(5091):87–91.

Hotamisligil, Gökhan S. 2006. “Inflammation and Metabolic Disorders.” *Nature* 444(7121):860–67.

Hoyer, Stefanie, Sabrina Prommersberger, Isabell A. Pfeiffer, Beatrice Schuler-

Thurner, Gerold Schuler, Jan Dörrie, and Niels Schaft. 2014. “Concurrent Interaction of DCs with CD4 + and CD8 + T Cells Improves Secondary CTL Expansion: It Takes Three to Tango.” *European Journal of Immunology* 44(12):3543–59.

Hu, Wei and Chandrashekhar Pasare. 2013. “Location, Location, Location: Tissue-Specific Regulation of Immune Responses.” *Journal of Leukocyte Biology* 94(3):409–21.

Huber, M., A. Brustle, K. Reinhard, A. Guralnik, G. Walter, A. Mahiny, E. von Low, and M. Lohoff. 2008. “IRF4 Is Essential for IL-21-Mediated Induction, Amplification, and Stabilization of the Th17 Phenotype.” *Proceedings of the National Academy of Sciences* 105(52):20846–51.

Huber, Magdalena and Michael Lohoff. 2014. “IRF4 at the Crossroads of Effector T-Cell Fate Decision.” *European Journal of Immunology* 44(7):1886–95.

Humphreys, Neil E., Damo Xu, Matthew R. Hepworth, Foo Y. Liew, and Richard K. Grencis. 2008. “IL-33, a Potent Inducer of Adaptive Immunity to Intestinal Nematodes.” *The Journal of Immunology* 180(4):2443–49.

Hung, L. Y., I. P. Lewkowich, L. A. Dawson, J. Downey, Y. Yang, D. E. Smith, and D. R. Herbert. 2013. “IL-33 Drives Biphasic IL-13 Production for Noncanonical Type 2 Immunity against Hookworms.” *Proceedings of the National Academy of Sciences* 110(1):282–87.

Islam, Sabina A. and Andrew D. Luster. 2012. “T Cell Homing to Epithelial Barriers in Allergic Disease.” *Nature Medicine* 18(5):705–15.

Iwasaki, Akiko and Ruslan Medzhitov. 2015. “Control of Adaptive Immunity by the Innate Immune System.” *Nature Immunology* 16(4):343–53.

Jablonski, Kyle A., Stephanie A. Amici, Lindsay M. Webb, Juan de Dios Ruiz-Rosado, Phillip G. Popovich, Santiago Partida-Sanchez, and Mireia Guerau-de-Arellano. 2015. “Novel Markers to Delineate Murine M1 and M2 Macrophages” edited by M. A. Olszewski. *PLOS ONE* 10(12):e0145342.

Jackson, David J., Heidi Makrinioti, Batika M. J. Rana, Betty W. H. Shamji, Maria-Belen Trujillo-Torralbo, Joseph Footitt, Jerico del-Rosario, Aurica G. Telcian, Alexandra Nikonova, Jie Zhu, Julia Aniscenko, Leila Gogsadze, Eteri Bakhsoliani, Stephanie Traub, Jaideep Dhariwal, James Porter, Duncan

Hunt, Toby Hunt, Trevor Hunt, Luminita A. Stanciu, Musa Khaitov, Nathan W. Bartlett, Michael R. Edwards, Onn Min Kon, Patrick Mallia, Nikolaos G. Papadopoulos, Cezmi A. Akdis, John Westwick, Matthew J. Edwards, David J. Cousins, Ross P. Walton, and Sebastian L. Johnston. 2014. “IL-33–Dependent Type 2 Inflammation during Rhinovirus-Induced Asthma Exacerbations *In Vivo*.” *American Journal of Respiratory and Critical Care Medicine* 190(12):1373–82.

Jacobs, Lotte, Tim S. Nawrot, Bas de Geus, Romain Meeusen, Bart Degraeuwe, Alfred Bernard, Muhammad Sughis, Benoit Nemery, and Luc Int Panis. 2010. “Subclinical Responses in Healthy Cyclists Briefly Exposed to Traffic-Related Air Pollution: An Intervention Study.” *Environmental Health* 9(1):64.

Jakubzick, Claudia, Emmanuel L. Gautier, Sophie L. Gibbings, Dorothy K. Sojka, Andreas Schlitzer, Theodore E. Johnson, Stoyan Ivanov, Qiaonan Duan, Shashi Bala, Tracy Condon, Nico van Rooijen, John R. Grainger, Yasmine Belkaid, Avi Ma’ayan, David W. H. Riches, Wayne M. Yokoyama, Florent Ginhoux, Peter M. Henson, and Gwendalyn J. Randolph. 2013. “Minimal Differentiation of Classical Monocytes as They Survey Steady-State Tissues and Transport Antigen to Lymph Nodes.” *Immunity* 39(3):599–610.

Jang, Hye Ryoun and Hamid Rabb. 2009. “The Innate Immune Response in Ischemic Acute Kidney Injury.” *Clinical Immunology* 130(1):41–50.

Janssen, Edith M., Edward E. Lemmens, Tom Wolfe, Urs Christen, Matthias G. von Herrath, and Stephen P. Schoenberger. 2003. “CD4+ T Cells Are Required for Secondary Expansion and Memory in CD8+ T Lymphocytes.” *Nature* 421(6925):852–56.

Jaryal, Ajay and Sanjay Vikrant. 2017. “Current Status of Lupus Nephritis.” *The Indian Journal of Medical Research* 145(2):167–78.

Johnson, J. D., E. L. Ford, E. Bernal-Mizrachi, K. L. Kusser, D. S. Luciani, Z. Han, H. Tran, T. D. Randall, F. E. Lund, and K. S. Polonsky. 2006. “Suppressed Insulin Signaling and Increased Apoptosis in Cd38-Null Islets.” *Diabetes* 55(10):2737–46.

Jovicic, N., I. Jeftic, M. Kovacevic, I. Tanaskovic, N. Arsenijevic, and N. Lukic, M. L., & Pejnovic. 2015. “ST2 Deficiency Ameliorates High Fat Diet-Induced

Liver Steatosis In BALB/c Mice." *Serbian Journal of Experimental and Clinical Research* 16(1):9–20.

Jovicic, Nemanja, Ilija Jeftic, Ivan Jovanovic, Gordana Radosavljevic, Nebojsa Arsenijevic, Miodrag L. Lukic, and Nada Pejnovic. 2015. "Differential Immunometabolic Phenotype in Th1 and Th2 Dominant Mouse Strains in Response to High-Fat Feeding" edited by P. V. Nerurkar. *PLOS ONE* 10(7):e0134089.

Kamijo, R., H. Harada, T. Matsuyama, M. Bosland, J. Gerecitano, D. Shapiro, J. Le, S. Koh, T. Kimura, S. Green, and al. et. 1994. "Requirement for Transcription Factor IRF-1 in NO Synthase Induction in Macrophages." *Science* 263(5153):1612–15.

Kang, Yea Eun, Ji Min Kim, Kyong Hye Joung, Ju Hee Lee, Bo Ram You, Min Jeong Choi, Min Jeong Ryu, Young Bok Ko, Min A. Lee, Junguee Lee, Bon Jeong Ku, Minho Shong, Ki Hwan Lee, and Hyun Jin Kim. 2016. "The Roles of Adipokines, Proinflammatory Cytokines, and Adipose Tissue Macrophages in Obesity-Associated Insulin Resistance in Modest Obesity and Early Metabolic Dysfunction." *PloS One* 11(4):e0154003.

Kearley, Jennifer, Jane E. Barker, Douglas S. Robinson, and Clare M. Lloyd. 2005. "Resolution of Airway Inflammation and Hyperreactivity after in Vivo Transfer of $CD4^+$ $CD25^+$ Regulatory T Cells Is Interleukin 10 Dependent." *The Journal of Experimental Medicine* 202(11):1539–47.

Kearley, Jennifer, Jonathan S. Silver, Caroline Sanden, Zheng Liu, Aaron A. Berlin, Natalie White, Michiko Mori, Tuyet-Hang Pham, Christine K. Ward, Gerard J. Criner, Nathaniel Marchetti, Tomas Mustelin, Jonas S. Erjefalt, Roland Kolbeck, and Alison A. Humbles. 2015. "Cigarette Smoke Silences Innate Lymphoid Cell Function and Facilitates an Exacerbated Type I Interleukin-33-Dependent Response to Infection." *Immunity* 42(3):566–79.

Kershaw, Erin E. and Jeffrey S. Flier. 2004. "Adipose Tissue as an Endocrine Organ." *The Journal of Clinical Endocrinology & Metabolism* 89(6):2548–56.

Kim, B. S., M. C. Siracusa, S. A. Saenz, M. Noti, L. A. Monticelli, G. F. Sonnenberg, M. R. Hepworth, A. S. Van Voorhees, M. R. Comeau, and D. Artis. 2013. "TSLP Elicits IL-33-Independent Innate Lymphoid Cell

Responses to Promote Skin Inflammation." *Science Translational Medicine* 5(170):170ra16-170ra16.

de Kleer, Ismé M., Mirjam Kool, Marjolein J. W. de Bruijn, Monique Willart, Justine van Moorleghem, Martijn J. Schuijs, Maud Plantinga, Rudi Beyaert, Emily Hams, Padraic G. Fallon, Hamida Hammad, Rudi W. Hendriks, and Bart N. Lambrecht. 2016. "Perinatal Activation of the Interleukin-33 Pathway Promotes Type 2 Immunity in the Developing Lung." *Immunity* 45(6):1285–98.

Klein, Ulf, Stefano Casola, Giorgio Cattoretti, Qiong Shen, Marie Lia, Tongwei Mo, Thomas Ludwig, Klaus Rajewsky, and Riccardo Dalla-Favera. 2006. "Transcription Factor IRF4 Controls Plasma Cell Differentiation and Class-Switch Recombination." *Nature Immunology* 7(7):773–82.

Koeda, T., K. Wakaki, F. Koizumi, T. Yokozawa, and H. Oura. 1988. "Early Changes of Proximal Tubules in the Kidney of Adenine-Ingesting Rats, with Special Reference to Biochemical and Electron Microscopic Studies." *Nihon Jinzo Gakkai Shi* 30(3):239–46.

Kohlgruber, Ayano C., Shani T. Gal-Oz, Nelson M. LaMarche, Moto Shimazaki, Danielle Duquette, Hui-Fern Koay, Hung N. Nguyen, Amir I. Mina, Tyler Paras, Ali Tavakkoli, Ulrich von Andrian, Adam P. Uldrich, Dale I. Godfrey, Alexander S. Banks, Tal Shay, Michael B. Brenner, and Lydia Lynch. 2018. "Γδ T Cells Producing Interleukin-17A Regulate Adipose Regulatory T Cell Homeostasis and Thermogenesis." *Nature Immunology* 19(5):464–74.

Kolodin, Dmitriy, Nicolas van Panhuys, Chaoran Li, Angela M. Magnuson, Daniela Cipolletta, Christine M. Miller, Amy Wagers, Ronald N. Germain, Christophe Benoist, and Diane Mathis. 2015. "Antigen- and Cytokine-Driven Accumulation of Regulatory T Cells in Visceral Adipose Tissue of Lean Mice." *Cell Metabolism* 21(4):543–57.

Korn, Thomas, Estelle Bettelli, Wenda Gao, Amit Awasthi, Anneli Jäger, Terry B. Strom, Mohamed Oukka, and Vijay K. Kuchroo. 2007. "IL-21 Initiates an Alternative Pathway to Induce Proinflammatory TH17 Cells." *Nature* 448(7152):484–87.

Kovats, Susan. 2015. "Estrogen Receptors Regulate Innate Immune Cells and

Signaling Pathways." *Cellular Immunology* 294(2):63–69.

Kovesdy, Csaba P., Susan L. Furth, Carmine Zoccali, and on behalf of the World Kidney Day Steering World Kidney Day Steering Committee. 2017. "Obesity and Kidney Disease: Hidden Consequences of the Epidemic." *Canadian Journal of Kidney Health and Disease* 4:2054358117698669.

Kratofil, Rachel M., Paul Kubes, and Justin F. Deniset. 2017. "Monocyte Conversion During Inflammation and Injury." *Arteriosclerosis, Thrombosis, and Vascular Biology* 37(1):35–42.

Kristiansen, Ole P. and Thomas Mandrup-Poulsen. 2005. "Interleukin-6 and Diabetes: The Good, the Bad, or the Indifferent?" *Diabetes* 54 Suppl 2:S114-24.

Kulski, Jerzy K., Takashi Shiina, Tatsuya Anzai, Sakae Kohara, and Hidetoshi Inoko. 2002. "Comparative Genomic Analysis of the MHC: The Evolution of Class I Duplication Blocks, Diversity and Complexity from Shark to Man." *Immunological Reviews* 190:95–122.

Kumar, Durgesh, Sanket Kumar Pandya, Salil Varshney, Kripa Shankar, Sujith Rajan, Ankita Srivastava, Abhishek Gupta, Sanchita Gupta, Achchhe Lal Vishwakarma, Amit Misra, and Anil N. Gaikwad. 2019. "Temporal Immmunometabolic Profiling of Adipose Tissue in HFD-Induced Obesity: Manifestations of Mast Cells in Fibrosis and Senescence." *International Journal of Obesity* 43(6):1281–94.

Kumar, Himanshu, Taro Kawai, and Shizuo Akira. 2011. "Pathogen Recognition by the Innate Immune System." *International Reviews of Immunology* 30(1):16–34.

Kurosaki, Tomohiro, Kohei Kometani, and Wataru Ise. 2015. "Memory B Cells." *Nature Reviews Immunology* 15(3):149–59.

Kurts, Christian, Ulf Panzer, Hans-Joachim Anders, and Andrew J. Rees. 2013. "The Immune System and Kidney Disease: Basic Concepts and Clinical Implications." *Nature Reviews Immunology* 13(10):738–53.

De Larco, J. E., Beverly R. K. Wuertz, and Leo T. Furcht. 2004. "The Potential Role of Neutrophils in Promoting the Metastatic Phenotype of Tumors Releasing Interleukin-8." *Clinical Cancer Research* 10(15):4895–4900.

Larsen, Kristen M., Maydelis Karla Minaya, Vivek Vaish, and Maria Marjorette O. Peña. 2018. “The Role of IL-33/ST2 Pathway in Tumorigenesis.” *International Journal of Molecular Sciences* 19(9).

Latchman, David S. 1997. “Transcription Factors: An Overview.” *The International Journal of Biochemistry & Cell Biology* 29(12):1305–12.

Lauby-Secretan, Béatrice, Chiara Scoccianti, Dana Loomis, Yann Grosse, Franca Bianchini, and Kurt Straif. 2016. “Body Fatness and Cancer — Viewpoint of the IARC Working Group.” *New England Journal of Medicine* 375(8):794–98.

Law, Charity W., Yunshun Chen, Wei Shi, and Gordon K. Smyth. 2014. “Voom: Precision Weights Unlock Linear Model Analysis Tools for RNA-Seq Read Counts.” *Genome Biology* 15(2):R29.

Lee, Hye-Rim, Tae-Hee Kim, and Kyung-Chul Choi. 2012. “Functions and Physiological Roles of Two Types of Estrogen Receptors, ERα and ERβ, Identified by Estrogen Receptor Knockout Mouse.” *Laboratory Animal Research* 28(2):71–76.

Lee, Mi-Jeong and Susan K. Fried. 2009. “Integration of Hormonal and Nutrient Signals That Regulate Leptin Synthesis and Secretion.” *American Journal of Physiology-Endocrinology and Metabolism* 296(6):E1230–38.

Lewkowicz, Przemyslaw, Natalia Lewkowicz, Andrzej Sasiak, and Henryk Tchórzewski. 2006. “Lipopolysaccharide-Activated CD4+CD25+ T Regulatory Cells Inhibit Neutrophil Function and Promote Their Apoptosis and Death.” *Journal of Immunology (Baltimore, Md. : 1950)* 177(10):7155–63.

Ley, Klaus. 2017. “M1 Means Kill; M2 Means Heal.” *Journal of Immunology (Baltimore, Md. : 1950)* 199(7):2191–93.

Li, Dong, Rodrigo Guabiraba, Anne-Gaëlle Besnard, Mousa Komai-Koma, Majid S. Jabir, Li Zhang, Gerard J. Graham, Mariola Kurowska-Stolarska, Foo Y. Liew, Charles McSharry, and Damo Xu. 2014. “IL-33 Promotes ST2-Dependent Lung Fibrosis by the Induction of Alternatively Activated Macrophages and Innate Lymphoid Cells in Mice.” *Journal of Allergy and Clinical Immunology* 134(6):1422-1432.e11.

Li, Jing, Deliang Shen, Junnan Tang, Yunzhe Wang, Bo Wang, Yue Xiao, Chang Cao, Xiaojing Shi, Hong-Min Liu, Wen Zhao, and Jinying Zhang. 2019. “IL33 Attenuates Ventricular Remodeling after Myocardial Infarction through Inducing Alternatively Activated Macrophages Ethical Standards Statement.” *European Journal of Pharmacology* 854:307–19.

Li, Li and Mark D. Okusa. 2006. “Blocking the Immune Response in Ischemic Acute Kidney Injury: The Role of Adenosine 2A Agonists.” *Nature Clinical Practice Nephrology* 2(8):432–44.

Li, Ming O. and Alexander Y. Rudensky. 2016. “T Cell Receptor Signalling in the Control of Regulatory T Cell Differentiation and Function.” *Nature Reviews Immunology* 16(4):220–33.

Li, Peng, Rosanne Spolski, Wei Liao, Lu Wang, Theresa L. Murphy, Kenneth M. Murphy, and Warren J. Leonard. 2012. “BATF–JUN Is Critical for IRF4-Mediated Transcription in T Cells.” *Nature* 490(7421):543–46.

Liao, Juan, Sean E. Humphrey, Stacie Poston, and Elizabeth J. Taparowsky. 2011. “Batf Promotes Growth Arrest and Terminal Differentiation of Mouse Myeloid Leukemia Cells.” *Molecular Cancer Research : MCR* 9(3):350–63.

Liao, Y., G. K. Smyth, and W. Shi. 2014. “FeatureCounts: An Efficient General Purpose Program for Assigning Sequence Reads to Genomic Features.” *Bioinformatics* 30(7):923–30.

Liao, Yang, Gordon K. Smyth, and Wei Shi. 2013. “The Subread Aligner: Fast, Accurate and Scalable Read Mapping by Seed-and-Vote.” *Nucleic Acids Research* 41(10):e108–e108.

Liew, Foo Yew, Jean-Philippe Girard, and Heth Roderick Turnquist. 2016. “Interleukin-33 in Health and Disease.” *Nature Reviews Immunology* 16(11):676–89.

Lim, Andy Kh. 2014. “Diabetic Nephropathy - Complications and Treatment.” *International Journal of Nephrology and Renovascular Disease* 7:361–81.

Lin, Shuei Liong, Ana P. Castaño, Brian T. Nowlin, Mark L. Lupher, and Jeremy S. Duffield. 2009. “Bone Marrow Ly6C ^{High} Monocytes Are Selectively Recruited to Injured Kidney and Differentiate into Functionally Distinct Populations.” *The Journal of Immunology* 183(10):6733–43.

Lisovska, Natalya and Nasrulla Shanazarov. 2019. “Tumor Progression Mechanisms: Insights from the Central Immune Regulation of Tissue Homeostasis.” *Oncology Letters* 17(6):5311–18.

Liu, Jianguo, Shanjin Cao, Lisa M. Herman, and Xiaojing Ma. 2003. “Differential Regulation of Interleukin (IL)-12 P35 and P40 Gene Expression and Interferon (IFN)-Gamma-Primed IL-12 Production by IFN Regulatory Factor 1.” *The Journal of Experimental Medicine* 198(8):1265–76.

Lohoff, M., H. W. Mittrucker, S. Prechtl, S. Bischof, F. Sommer, S. Kock, D. A. Ferrick, G. S. Duncan, A. Gessner, and T. W. Mak. 2002. “Dysregulated T Helper Cell Differentiation in the Absence of Interferon Regulatory Factor 4.” *Proceedings of the National Academy of Sciences* 99(18):11808–12.

Lohoff, Michael and Tak W. Mak. 2005. “Roles of Interferon-Regulatory Factors in T-Helper-Cell Differentiation.” *Nature Reviews Immunology* 5(2):125–35.

Lott, J. M., T. L. Sumpter, and H. R. Turnquist. 2015. “New Dog and New Tricks: Evolving Roles for IL-33 in Type 2 Immunity.” *Journal of Leukocyte Biology* 97(6):1037–48.

Lu, Jingli, Junjie Zhao, Haiyang Meng, and Xiaojian Zhang. 2019. “Adipose Tissue-Resident Immune Cells in Obesity and Type 2 Diabetes.” *Frontiers in Immunology* 10:1173.

de Luca, Carl and Jerrold M. Olefsky. 2008. “Inflammation and Insulin Resistance.” *FEBS Letters* 582(1):97–105.

Ludwig-Portugall, Isis, Eva Bartok, Ermanila Dhana, Beatrix D. G. Evers, Michael J. Primiano, J. Perry Hall, Bernardo S. Franklin, Percy A. Knolle, Veit Hornung, Gunther Hartmann, Peter Boor, Eicke Latz, and Christian Kurts. 2016. “An NLRP3-Specific Inflammasome Inhibitor Attenuates Crystal-Induced Kidney Fibrosis in Mice.” *Kidney International* 90(3):525–39.

Lumeng, Carey N., Jennifer L. Bodzin, and Alan R. Saltiel. 2007. “Obesity Induces a Phenotypic Switch in Adipose Tissue Macrophage Polarization.” *The Journal of Clinical Investigation* 117(1):175–84.

Lumeng, Carey N., Jennifer B. DelProposto, Daniel J. Westcott, and Alan R. Saltiel. 2008. “Phenotypic Switching of Adipose Tissue Macrophages With Obesity Is Generated by Spatiotemporal Differences in Macrophage

Subtypes.” *Diabetes* 57(12):3239–46.

Lunderius-Andersson, Carolina, Mattias Enoksson, and Gunnar Nilsson. 2012. “Mast Cells Respond to Cell Injury through the Recognition of IL-33.” *Frontiers in Immunology* 3:82.

Lüthi, Alexander U., Sean P. Cullen, Edel A. McNeela, Patrick J. Duriez, Inna S. Afonina, Clare Sheridan, Gabriela Brumatti, Rebecca C. Taylor, Kristof Kersse, Peter Vandenabeele, Ed C. Lavelle, and Seamus J. Martin. 2009. “Suppression of Interleukin-33 Bioactivity through Proteolysis by Apoptotic Caspases.” *Immunity* 31(1):84–98.

Lynch, Lydia, Michael Nowak, Bindu Varghese, Justice Clark, Andrew E. Hogan, Vasillis Toxavidis, Steven P. Balk, Donal O’Shea, Cliona O’Farrelly, and Mark A. Exley. 2012. “Adipose Tissue Invariant NKT Cells Protect against Diet-Induced Obesity and Metabolic Disorder through Regulatory Cytokine Production.” *Immunity* 37(3):574–87.

Macdougall, Claire E., Elizabeth G. Wood, Jakob Loschko, Valeria Scagliotti, Féaron C. Cassidy, Mark E. Robinson, Niklas Feldhahn, Leandro Castellano, Mathieu-Benoit Voisin, Federica Marelli-Berg, Carles Gaston-Massuet, Marika Charalambous, and M. Paula Longhi. 2018. “Visceral Adipose Tissue Immune Homeostasis Is Regulated by the Crosstalk between Adipocytes and Dendritic Cell Subsets.” *Cell Metabolism* 27(3):588-601.e4.

Macotela, Y., J. Boucher, T. T. Tran, and C. R. Kahn. 2009. “Sex and Depot Differences in Adipocyte Insulin Sensitivity and Glucose Metabolism.” *Diabetes* 58(4):803–12.

Mager, Lukas F., Carsten Riether, Christian M. Schürch, Yara Banz, Marie-Hélène Wasmer, Regula Stuber, Alexandre P. Theocharides, Xiaohong Li, Yu Xia, Hirohisa Saito, Susumu Nakae, Gabriela M. Baerlocher, Markus G. Manz, Kathy D. McCoy, Andrew J. Macpherson, Adrian F. Ochsenbein, Bruce Beutler, and Philippe Krebs. 2015. “IL-33 Signaling Contributes to the Pathogenesis of Myeloproliferative Neoplasms.” *The Journal of Clinical Investigation* 125(7):2579–91.

Mahajan, D., Yiping Wang, Xiahong Qin, Ying Wang, Guoping Zheng, Yuan Min Wang, Stephen I. Alexander, and David C. H. Harris. 2006. “CD4+CD25+

Regulatory T Cells Protect against Injury in an Innate Murine Model of Chronic Kidney Disease." *Journal of the American Society of Nephrology* 17(10):2731–41.

Malavasi, Fabio, Silvia Deaglio, Ada Funaro, Enza Ferrero, Alberto L. Horenstein, Erika Ortolan, Tiziana Vaisitti, and Semra Aydin. 2008. "Evolution and Function of the ADP Ribosyl Cyclase/CD38 Gene Family in Physiology and Pathology." *Physiological Reviews* 88(3):841–86.

Malavasi, Fabio, Ada Funaro, Massimo Alessio, Lucia B. DeMonte, Clara M. Ausiello, Umberto Dianzani, Francesco Lanza, Elisabetta Magrini, Monica Momo, and Stefano Roggero. 1992. "CD38: A Multi-Lineage Cell Activation Molecule with a Split Personality." *International Journal of Clinical & Laboratory Research* 22(1–4):73–80.

Man, Kevin, Sarah S. Gabriel, Yang Liao, Renee Gloury, Simon Preston, Darren C. Henstridge, Marc Pellegrini, Dietmar Zehn, Friederike Berberich-Siebelt, Mark A. Febbraio, Wei Shi, and Axel Kallies. 2017. "Transcription Factor IRF4 Promotes CD8+ T Cell Exhaustion and Limits the Development of Memory-like T Cells during Chronic Infection." *Immunity* 47(6):1129-1141.e5.

Mantegazza, Adriana R., Joao G. Magalhaes, Sebastian Amigorena, and Michael S. Marks. 2013. "Presentation of Phagocytosed Antigens by MHC Class I and II." *Traffic (Copenhagen, Denmark)* 14(2):135–52.

Martín-Fontecha, Alfonso, Antonio Lanzavecchia, and Federica Sallusto. 2009. "Dendritic Cell Migration to Peripheral Lymph Nodes." Pp. 31–49 in *Dendritic Cells*. Berlin, Heidelberg: Springer Berlin Heidelberg.

Martinez-Pomares, Luisa. 2012. "The Mannose Receptor." *Journal of Leukocyte Biology* 92(6):1177–86.

Martinez, Fernando O., Siamon Gordon, Massimo Locati, and Alberto Mantovani. 2006. "Transcriptional Profiling of the Human Monocyte-to-Macrophage Differentiation and Polarization: New Molecules and Patterns of Gene Expression." *Journal of Immunology (Baltimore, Md. : 1950)* 177(10):7303–11.

Maton, Anthea. 1993. *Human Biology and Health*. Prentice Hall.

Matsuyama, T., T. Kimura, M. Kitagawa, K. Pfeffer, T. Kawakami, N. Watanabe, T. M. Kündig, R. Amakawa, K. Kishihara, and A. Wakeham. 1993. “Targeted Disruption of IRF-1 or IRF-2 Results in Abnormal Type I IFN Gene Induction and Aberrant Lymphocyte Development.” *Cell* 75(1):83–97.

Matta, B. M., D. K. Reichenbach, X. Zhang, L. Mathews, B. H. Koehn, G. K. Dwyer, J. M. Lott, F. M. Uhl, D. Pfeifer, C. J. Feser, M. J. Smith, Q. Liu, R. Zeiser, B. R. Blazar, and H. R. Turnquist. 2016. “Peri-AlloHCT IL-33 Administration Expands Recipient T-Regulatory Cells That Protect Mice against Acute GVHD.” *Blood* 128(3):427–39.

Maverakis, Emanual, Kyoungmi Kim, Michiko Shimoda, M. Eric Gershwin, Forum Patel, Reason Wilken, Siba Raychaudhuri, L. Renee Ruhaak, and Carlito B. Lebrilla. 2015. “Glycans in the Immune System and The Altered Glycan Theory of Autoimmunity: A Critical Review.” *Journal of Autoimmunity* 57:1–13.

McCarthy, D. J. and G. K. Smyth. 2009. “Testing Significance Relative to a Fold-Change Threshold Is a TREAT.” *Bioinformatics* 25(6):765–71.

McHeyzer-Williams, Louise J., Nadege Pelletier, Linda Mark, Nicolas Fazilleau, and Michael G. McHeyzer-Williams. 2009. “Follicular Helper T Cells as Cognate Regulators of B Cell Immunity.” *Current Opinion in Immunology* 21(3):266–73.

Meng, Xiao-Ming, David J. Nikolic-Paterson, and Hui Yao Lan. 2014. “Inflammatory Processes in Renal Fibrosis.” *Nature Reviews Nephrology* 10(9):493–503.

Merad, Miriam, Priyanka Sathe, Julie Helft, Jennifer Miller, and Arthur Mortha. 2013. “The Dendritic Cell Lineage: Ontogeny and Function of Dendritic Cells and Their Subsets in the Steady State and the Inflamed Setting.” *Annual Review of Immunology* 31(1):563–604.

van der Merwe, P. Anton, Dale L. Bodian, Susan Daenke, Peter Linsley, and Simon J. Davis. 1997. “CD80 (B7-1) Binds Both CD28 and CTLA-4 with a Low Affinity and Very Fast Kinetics.” *The Journal of Experimental Medicine* 185(3):393–404.

Mildner, Alexander, Goran Marinkovic, and Steffen Jung. 2016. “Murine

Monocytes: Origins, Subsets, Fates, and Functions." *Microbiology Spectrum* 4(5).

Mildner, Alexander, Simon Yona, and Steffen Jung. 2013. "A Close Encounter of the Third Kind." Pp. 69–103 in *Advances in immunology*. Vol. 120.

Millar, Neal L., Derek S. Gilchrist, Moeed Akbar, James H. Reilly, Shauna C. Kerr, Abigail L. Campbell, George A. C. Murrell, Foo Y. Liew, Mariola Kurowska-Stolarska, and Iain B. McInnes. 2015. "MicroRNA29a Regulates IL-33-Mediated Tissue Remodelling in Tendon Disease." *Nature Communications* 6(1):6774.

Miller, Ashley M., Darren L. Asquith, Axel J. Hueber, Lesley A. Anderson, William M. Holmes, Andrew N. McKenzie, Damo Xu, Naveed Sattar, Iain B. McInnes, and Foo Y. Liew. 2010. "Interleukin-33 Induces Protective Effects in Adipose Tissue Inflammation during Obesity in Mice." *Circulation Research* 107(5):650–58.

Miller, J. F. 1975. "Cellular Basis of the Immune Response." *Acta Endocrinologica. Supplementum* 194:55–76.

Mills, C. D., K. Kincaid, J. M. Alt, M. J. Heilman, and A. M. Hill. 2000. "M-1/M-2 Macrophages and the Th1/Th2 Paradigm." *Journal of Immunology (Baltimore, Md. : 1950)* 164(12):6166–73.

Mills, Charles D. 2012. "M1 and M2 Macrophages: Oracles of Health and Disease." *Critical Reviews in Immunology* 32(6):463–88.

Mirchandani, Ananda S., Robert J. Salmond, and Foo Y. Liew. 2012. "Interleukin-33 and the Function of Innate Lymphoid Cells." *Trends in Immunology* 33(8):389–96.

Mitchell, Andrew J., Ben Roediger, and Wolfgang Weninger. 2014. "Monocyte Homeostasis and the Plasticity of Inflammatory Monocytes." *Cellular Immunology* 291(1–2):22–31.

Mittrucker, H. W., T. Matsuyama, A. Grossman, T. M. Kündig, J. Potter, A. Shahinian, A. Wakeham, B. Patterson, P. S. Ohashi, and T. W. Mak. 1997. "Requirement for the Transcription Factor LSIRF/IRF4 for Mature B and T Lymphocyte Function." *Science* 275(5299):540–43.

Miyara, Makoto and Shimon Sakaguchi. 2007. "Natural Regulatory T Cells:

Mechanisms of Suppression." *Trends in Molecular Medicine* 13(3):108–16.

Moller, D. E. 2000. "Potential Role of TNF-Alpha in the Pathogenesis of Insulin Resistance and Type 2 Diabetes." *Trends in Endocrinology and Metabolism: TEM* 11(6):212–17.

Morrow, Edward H. 2015. "The Evolution of Sex Differences in Disease." *Biology of Sex Differences* 6:5.

Mortensen, Elin S., Kristin A. Fenton, and Ole P. Rekvig. 2008. "Lupus Nephritis: The Central Role of Nucleosomes Revealed." *American Journal of Pathology* 172(2):275–83.

Morton, Gregory J. and Michael W. Schwartz. 2011. "Leptin and the Central Nervous System Control of Glucose Metabolism." *Physiological Reviews* 91(2):389–411.

Mosmann, T. R. and R. L. Coffman. 1989. "TH1 and TH2 Cells: Different Patterns of Lymphokine Secretion Lead to Different Functional Properties." *Annual Review of Immunology* 7(1):145–73.

Mosser, David M. and Justin P. Edwards. 2008. "Exploring the Full Spectrum of Macrophage Activation." *Nature Reviews Immunology* 8(12):958–69.

Moussion, Christine, Nathalie Ortega, and Jean-Philippe Girard. 2008. "The IL-1-Like Cytokine IL-33 Is Constitutively Expressed in the Nucleus of Endothelial Cells and Epithelial Cells In Vivo: A Novel 'Alarmin'?" edited by D. Unutmaz. *PLoS ONE* 3(10):e3331.

Mulay, Shrikant R. and Hans-Joachim Anders. 2017. "Crystal Nephropathies: Mechanisms of Crystal-Induced Kidney Injury." *Nature Reviews Nephrology* 13(4):226–40.

Mulder, Willem J. M., Jordi Ochando, Leo A. B. Joosten, Zahi A. Fayad, and Mihai G. Netea. 2019. "Therapeutic Targeting of Trained Immunity." *Nature Reviews Drug Discovery* 18(7):553–66.

Muller, Paul Andrew, Balázs Koscsó, Gaurav Manohar Rajani, Korey Stevanovic, Marie-Luise Berres, Daigo Hashimoto, Arthur Mortha, Marylene Leboeuf, Xiu-Min Li, Daniel Mucida, E. Richard Stanley, Stephanie Dahan, Kara Gross Margolis, Michael David Gershon, Miriam Merad, and Milena Bogunovic. 2014. "Crosstalk between Muscularis Macrophages and Enteric Neurons

Regulates Gastrointestinal Motility." *Cell* 158(2):300–313.

Munro, David A. D. and Jeremy Hughes. 2017. "The Origins and Functions of Tissue-Resident Macrophages in Kidney Development." *Frontiers in Physiology* 8:837.

Murphy, Kenneth and Casey Weaver. 2017. *Janeway's Immunobiology*. Innate Imm.

Murphy, Theresa L., Roxane Tussiwand, and Kenneth M. Murphy. 2013. "Specificity through Cooperation: BATF-IRF Interactions Control Immune-Regulatory Networks." *Nat Rev Immunol* 13(7):499–509.

Nam, Sorim and Jong-Seok Lim. 2016. "Essential Role of Interferon Regulatory Factor 4 (IRF4) in Immune Cell Development." *Archives of Pharmacal Research* 39(11):1548–55.

Nawaz, Allah, Aminuddin Aminuddin, Tomonobu Kado, Akiko Takikawa, Seiji Yamamoto, Koichi Tsuneyama, Yoshiko Igarashi, Masashi Ikutani, Yasuhiro Nishida, Yoshinori Nagai, Kiyoshi Takatsu, Johji Imura, Masakiyo Sasahara, Yukiko Okazaki, Kohjiro Ueki, Tadashi Okamura, Kumpei Tokuyama, Akira Ando, Michihiro Matsumoto, Hisashi Mori, Takashi Nakagawa, Norihiko Kobayashi, Kumiko Saeki, Isao Usui, Shiho Fujisaka, and Kazuyuki Tobe. 2017. "CD206+ M2-like Macrophages Regulate Systemic Glucose Metabolism by Inhibiting Proliferation of Adipocyte Progenitors." *Nature Communications* 8(1):286.

Nedvídková, J., K. Smitka, V. Kopský, and V. Hainer. 2005. "Adiponectin, an Adipocyte-Derived Protein." *Physiological Research* 54(2):133–40.

Negishi, Hideo, Yasuyuki Fujita, Hideyuki Yanai, Shinya Sakaguchi, Xinshou Ouyang, Masahiro Shinohara, Hiroshi Takayanagi, Yusuke Ohba, Tadatsugu Taniguchi, and Kenya Honda. 2006. "Evidence for Licensing of IFN-Gamma-Induced IFN Regulatory Factor 1 Transcription Factor by MyD88 in Toll-like Receptor-Dependent Gene Induction Program." *Proceedings of the National Academy of Sciences of the United States of America* 103(41):15136–41.

Neill, Daniel R., See Heng Wong, Agustin Bellosi, Robin J. Flynn, Maria Daly, Theresa K. A. Langford, Christine Bucks, Colleen M. Kane, Padraic G.

Fallon, Richard Pannell, Helen E. Jolin, and Andrew N. J. McKenzie. 2010. “Nuocytes Represent a New Innate Effector Leukocyte That Mediates Type-2 Immunity.” *Nature* 464(7293):1367–70.

Neugarten, Joel, Ladan Golestaneh, and Nitin V. Kolhe. 2018. “Sex Differences in Acute Kidney Injury Requiring Dialysis.” *BMC Nephrology* 19(1):131.

Ngo, S. T., F. J. Steyn, and P. A. McCombe. 2014. “Gender Differences in Autoimmune Disease.” *Frontiers in Neuroendocrinology* 35(3):347–69.

Nishimura, Satoshi, Ichiro Manabe, Mika Nagasaki, Koji Eto, Hiroshi Yamashita, Mitsuru Ohsugi, Makoto Otsu, Kazuo Hara, Kohjiro Ueki, Seiryo Sugiura, Kotaro Yoshimura, Takashi Kadowaki, and Ryozo Nagai. 2009. “CD8+ Effector T Cells Contribute to Macrophage Recruitment and Adipose Tissue Inflammation in Obesity.” *Nature Medicine* 15(8):914–20.

Nishizaki, Tomoyuki. 2018. “IL-33 Suppresses GSK-3β Activation through an ST2-Independent MyD88/TRAF6/RIP/PI3K/Akt Pathway.” *Heliyon* 4(11):e00971.

Nutt, Stephen L., Philip D. Hodgkin, David M. Tarlinton, and Lynn M. Corcoran. 2015. “The Generation of Antibody-Secreting Plasma Cells.” *Nature Reviews Immunology* 15(3):160–71.

O’Sullivan, Timothy E., Moritz Rapp, Xiying Fan, Orr-El Weizman, Priya Bhardwaj, Nicholas M. Adams, Thierry Walzer, Andrew J. Dannenberg, and Joseph C. Sun. 2016. “Adipose-Resident Group 1 Innate Lymphoid Cells Promote Obesity-Associated Insulin Resistance.” *Immunity* 45(2):428–41.

Oboki, Keisuke, Tatsukuni Ohno, Naoki Kajiwara, Ken Arae, Hideaki Morita, Akina Ishii, Aya Nambu, Takaya Abe, Hiroshi Kiyonari, Kenji Matsumoto, Katsuko Sudo, Ko Okumura, Hirohisa Saito, and Susumu Nakae. 2010. “IL-33 Is a Crucial Amplifier of Innate Rather than Acquired Immunity.” *Proceedings of the National Academy of Sciences of the United States of America* 107(43):18581–86.

Ochel, Aaron, Gisa Tiegs, and Katrin Neumann. 2019. “Type 2 Innate Lymphoid Cells in Liver and Gut: From Current Knowledge to Future Perspectives.” *International Journal of Molecular Sciences* 20(8):1896.

Odegaard, Justin I. and Ajay Chawla. 2015. “Type 2 Responses at the Interface

between Immunity and Fat Metabolism." *Current Opinion in Immunology* 36:67–72.

Oh, Da Young, Hidetaka Morinaga, Saswata Talukdar, Eun Ju Bae, and Jerrold M. Olefsky. 2012. "Increased Macrophage Migration into Adipose Tissue in Obese Mice." *Diabetes* 61(2):346–54.

Okabe, Yasutaka and Ruslan Medzhitov. 2014. "Tissue-Specific Signals Control Reversible Program of Localization and Functional Polarization of Macrophages." *Cell* 157(4):832–44.

Okusa, Mark D., Joel Linden, Liping Huang, Jayson M. Rieger, Timothy L. Macdonald, and Long P. Huynh. 2000. "A $_{2A}$ Adenosine Receptor-Mediated Inhibition of Renal Injury and Neutrophil Adhesion." *American Journal of Physiology-Renal Physiology* 279(5):F809–18.

Olefsky, Jerrold M. and Christopher K. Glass. 2010. "Macrophages, Inflammation, and Insulin Resistance." *Annual Review of Physiology* 72(1):219–46.

Olingy, Claire E., Huy Q. Dinh, and Catherine C. Hedrick. 2019. "Monocyte Heterogeneity and Functions in Cancer." *Journal of Leukocyte Biology* 106(2):309–22.

Ost, Michael, Anurag Singh, Andreas Peschel, Roman Mehling, Nikolaus Rieber, and Dominik Hartl. 2016. "Myeloid-Derived Suppressor Cells in Bacterial Infections." *Frontiers in Cellular and Infection Microbiology* 6:37.

Owens, Evan P., David A. Vesey, Andrew J. Kassianos, Helen Healy, Wendy E. Hoy, and Glenda C. Gobe. 2019. "Biomarkers and the Role of Mast Cells as Facilitators of Inflammation and Fibrosis in Chronic Kidney Disease." *Translational Andrology and Urology* 8(Suppl 2):S175–83.

Palm, Noah W. and Ruslan Medzhitov. 2009. "Pattern Recognition Receptors and Control of Adaptive Immunity." *Immunological Reviews* 227(1):221–33.

Palm, Noah W., Rachel K. Rosenstein, and Ruslan Medzhitov. 2012. "Allergic Host Defences." *Nature* 484(7395):465–72.

Palma, Alessandro, Abdul Salam Jarrah, Paolo Tieri, Gianni Cesareni, and Filippo Castiglione. 2018. "Gene Regulatory Network Modeling of Macrophage Differentiation Corroborates the Continuum Hypothesis of

Polarization States." *Frontiers in Physiology* 9:1659.

Palmer, Biff F. and Deborah J. Clegg. 2015. "The Sexual Dimorphism of Obesity." *Molecular and Cellular Endocrinology* 402:113–19.

Pan, Haitao, Jiao Guo, and Zhengquan Su. 2014. "Advances in Understanding the Interrelations between Leptin Resistance and Obesity." *Physiology & Behavior* 130:157–69.

Panduro, Marisella, Christophe Benoist, and Diane Mathis. 2016. "Tissue Tregs." *Annual Review of Immunology* 34(1):609–33.

Park, Geun Ho, Helen Ki Shinn, Ju-Hee Kang, Won Ju Na, Young Hyo Kim, and Chang-Shin Park. 2016. "Anti-Interleukin-33 Reduces Ovalbumin-Induced Nephrotoxicity and Expression of Kidney Injury Molecule-1." *International Neurourology Journal* 20(2):114–21.

Pascual-Figal, Domingo A. and James L. Januzzi. 2015. "The Biology of ST2: The International ST2 Consensus Panel." *The American Journal of Cardiology* 115(7 Suppl):3B-7B.

Patsouris, David, Ping-Ping Li, Divya Thapar, Justin Chapman, Jerrold M. Olefsky, and Jaap G. Neels. 2008. "Ablation of CD11c-Positive Cells Normalizes Insulin Sensitivity in Obese Insulin Resistant Animals." *Cell Metabolism* 8(4):301–9.

Paul, William E. and Robert A. Seder. 1994. "Lymphocyte Responses and Cytokines." *Cell* 76(2):241–51.

Persson, Emma K., Heli Uronen-Hansson, Monika Semmrich, Aymeric Rivollier, Karin Hägerbrand, Jan Marsal, Sigurdur Gudjonsson, Ulf Håkansson, Boris Reizis, Knut Kotarsky, and William W. Agace. 2013. "IRF4 Transcription-Factor-Dependent CD103+CD11b+ Dendritic Cells Drive Mucosal T Helper 17 Cell Differentiation." *Immunity* 38(5):958–69.

Pesce, John T., Thirumalai R. Ramalingam, Margaret M. Mentink-Kane, Mark S. Wilson, Karim C. E. Kasmi, Amber M. Smith, Robert W. Thompson, Allen W. Cheever, Peter J. Murray, and Thomas A. Wynn. 2009. "Arginase-1-Expressing Macrophages Suppress Th2 Cytokine-Driven Inflammation and Fibrosis." *PLoS Pathogens* 5(4).

Pichery, Mélanie, Emilie Mirey, Pascale Mercier, Emma Lefrancais, Arnaud

Dujardin, Nathalie Ortega, and Jean-Philippe Girard. 2012. “Endogenous IL-33 Is Highly Expressed in Mouse Epithelial Barrier Tissues, Lymphoid Organs, Brain, Embryos, and Inflamed Tissues: In Situ Analysis Using a Novel Il-33-LacZ Gene Trap Reporter Strain.” *Journal of Immunology (Baltimore, Md. : 1950)* 188(7):3488–95.

Pickup, J. C. and M. A. Crook. 1998. “Is Type II Diabetes Mellitus a Disease of the Innate Immune System?” *Diabetologia* 41(10):1241–48.

Platanitis, Ekaterini and Thomas Decker. 2018. “Regulatory Networks Involving STATs, IRFs, and NFκB in Inflammation.” *Frontiers in Immunology* 9:2542.

Plotkin, Stanley A., Walter A. Orenstein, and Paul A. Offit. 2008. *Vaccines*. Saunders/Elsevier.

Popov, Alexey and Joachim L. Schultze. 2008. “IDO-Expressing Regulatory Dendritic Cells in Cancer and Chronic Infection.” *Journal of Molecular Medicine* 86(2):145–60.

Rana, Batika M. J., Eric Jou, Jillian L. Barlow, Noe Rodriguez-Rodriguez, Jennifer A. Walker, Claire Knox, Helen E. Jolin, Clare S. Hardman, Meera Sivasubramaniam, Aydan Szeto, E. Suzanne Cohen, Ian C. Scott, Matthew A. Sleeman, Chiamaka I. Chidomere, Sara Cruz Migoni, Jorge Caamano, Helle F. Jorgensen, Stefania Carobbio, Antonio Vidal-Puig, and Andrew N. J. McKenzie. 2019. “A Stromal Cell Niche Sustains ILC2-Mediated Type-2 Conditioning in Adipose Tissue.” *The Journal of Experimental Medicine* jem.20190689.

Ranjit, Srijana, Emilie Boutet, Pallavi Gandhi, Matthieu Prot, Yoshikazu Tamori, Anil Chawla, Andrew S. Greenberg, Vishwajeet Puri, and Michael P. Czech. 2011. “Regulation of Fat Specific Protein 27 by Isoproterenol and TNF-α to Control Lipolysis in Murine Adipocytes.” *Journal of Lipid Research* 52(2):221–36.

Ratajczak, Weronika, Paulina Niedźwiedzka-Rystwej, Beata Tokarz-Deptuła, and Wiesław Deptuła. 2018. “Immunological Memory Cells.” *Central-European Journal of Immunology* 43(2):194–203.

Reaven, Gerald M. 2011. “Insulin Resistance: The Link between Obesity and Cardiovascular Disease.” *The Medical Clinics of North America* 95(5):875–

92.

Renaldi, Olly, Bowo Pramono, Hemi Sinorita, Luthfan Budi Purnomo, Rizka Humardewayanti Asdie, and Ahmad H. Asdie. 2009. “Hypoadiponectinemia: A Risk Factor for Metabolic Syndrome.” *Acta Medica Indonesiana* 41(1):20–24.

Rengarajan, Jyothi, Kerri A. Mowen, Kathryn D. McBride, Erica D. Smith, Harinder Singh, and Laurie H. Glimcher. 2002. “Interferon Regulatory Factor 4 (IRF4) Interacts with NFATc2 to Modulate Interleukin 4 Gene Expression.” *The Journal of Experimental Medicine* 195(8):1003–12.

Richelsen, B. 1986. “Increased α 2 - but Similar β-Adrenergic Receptor Activities in Subcutaneous Gluteal Adipocytes from Females Compared with Males.” *European Journal of Clinical Investigation* 16(4):302–9.

Riedel, Jan-Hendrik, Martina Becker, Kerstin Kopp, Mathis Düster, Silke R. Brix, Catherine Meyer-Schwesinger, Luis A. Kluth, Ann-Christin Gnirck, Madena Attar, Sonja Krohn, Boris Fehse, Rolf A. K. Stahl, Ulf Panzer, and Jan-Eric Turner. 2017. “IL-33–Mediated Expansion of Type 2 Innate Lymphoid Cells Protects from Progressive Glomerulosclerosis.” *Journal of the American Society of Nephrology* 28(7):2068–80.

del Rio, Maria-Luisa, Günter Bernhardt, Jose-Ignacio Rodriguez-Barbosa, and Reinhold Förster. 2010. “Development and Functional Specialization of CD103 $^{+}$ Dendritic Cells.” *Immunological Reviews* 234(1):268–81.

Ritchie, Matthew E., Belinda Phipson, Di Wu, Yifang Hu, Charity W. Law, Wei Shi, and Gordon K. Smyth. 2015. “Limma Powers Differential Expression Analyses for RNA-Sequencing and Microarray Studies.” *Nucleic Acids Research* 43(7):e47–e47.

Roberto, Christina A., Boyd Swinburn, Corinna Hawkes, Terry T. K. Huang, Sergio A. Costa, Marice Ashe, Lindsey Zwicker, John H. Cawley, and Kelly D. Brownell. 2015. “Patchy Progress on Obesity Prevention: Emerging Examples, Entrenched Barriers, and New Thinking.” *Lancet (London, England)* 385(9985):2400–2409.

Roediger, Ben, Ryan Kyle, Kwok Ho Yip, Nital Sumaria, Thomas V Guy, Brian S. Kim, Andrew J. Mitchell, Szun S. Tay, Rohit Jain, Elizabeth Forbes-Blom, Xi

Chen, Philip L. Tong, Holly A. Bolton, David Artis, William E. Paul, Barbara Fazekas de St Groth, Michele A. Grimbaldeston, Graham Le Gros, and Wolfgang Weninger. 2013. “Cutaneous Immunosurveillance and Regulation of Inflammation by Group 2 Innate Lymphoid Cells.” *Nature Immunology* 14(6):564–73.

Rogers, Natasha M., David A. Ferenbach, Jeffrey S. Isenberg, Angus W. Thomson, and Jeremy Hughes. 2014. “Dendritic Cells and Macrophages in the Kidney: A Spectrum of Good and Evil.” *Nature Reviews. Nephrology* 10(11):625–43.

Rogers, Nicole H., James W. Perfield, Katherine J. Strissel, Martin S. Obin, Andrew S. Greenberg, and Andrew S. Greenberg. 2009. “Reduced Energy Expenditure and Increased Inflammation Are Early Events in the Development of Ovariectomy-Induced Obesity.” *Endocrinology* 150(5):2161–68.

Ruan, Hong and Harvey F. Lodish. 2003. “Insulin Resistance in Adipose Tissue: Direct and Indirect Effects of Tumor Necrosis Factor-Alpha.” *Cytokine & Growth Factor Reviews* 14(5):447–55.

Saghizadeh, M., J. M. Ong, W. T. Garvey, R. R. Henry, and P. A. Kern. 1996. “The Expression of TNF Alpha by Human Muscle. Relationship to Insulin Resistance.” *Journal of Clinical Investigation* 97(4):1111–16.

Sallusto, Federica and Antonio Lanzavecchia. 2002. “The Instructive Role of Dendritic Cells on T-Cell Responses.” *Arthritis Research* 4 Suppl 3(Suppl 3):S127-32.

Sanada, Shoji, Daihiko Hakuno, Luke J. Higgins, Eric R. Schreiter, Andrew N. J. McKenzie, and Richard T. Lee. 2007. “IL-33 and ST2 Comprise a Critical Biomechanically Induced and Cardioprotective Signaling System.” *The Journal of Clinical Investigation* 117(6):1538–49.

Satoh, Takashi, Osamu Takeuchi, Alexis Vandenbon, Koubun Yasuda, Yoshiaki Tanaka, Yutaro Kumagai, Tohru Miyake, Kazufumi Matsushita, Toshihiko Okazaki, Tatsuya Saitoh, Kiri Honma, Toshifumi Matsuyama, Katsuyuki Yui, Tohru Tsujimura, Daron M. Standley, Kenji Nakanishi, Kenta Nakai, and Shizuo Akira. 2010. “The Jmjd3-Irf4 Axis Regulates M2 Macrophage

Polarization and Host Responses against Helminth Infection." *Nature Immunology* 11(10):936–44.

Sawada, Shinichiro, John D. Scarborough, Nigel Killeen, and Dan R. Littman. 1994. "A Lineage-Specific Transcriptional Silencer Regulates CD4 Gene Expression during T Lymphocyte Development." *Cell* 77(6):917–29.

Schiavoni, Giovanna, Fabrizio Mattei, Paola Sestili, Paola Borghi, Massimo Venditti, Herbert C. Morse, Filippo Belardelli, and Lucia Gabriele. 2002. "ICSBP Is Essential for the Development of Mouse Type I Interferon-Producing Cells and for the Generation and Activation of CD8alpha(+) Dendritic Cells." *The Journal of Experimental Medicine* 196(11):1415–25.

Schiering, Chris, Thomas Krausgruber, Agnieszka Chomka, Anja Fröhlich, Krista Adelmann, Elizabeth A. Wohlfert, Johanna Pott, Thibault Griseri, Julia Bollrath, Ahmed N. Hegazy, Oliver J. Harrison, Benjamin M. J. Owens, Max Löhning, Yasmine Belkaid, Padraic G. Fallon, and Fiona Powrie. 2014. "The Alarmin IL-33 Promotes Regulatory T-Cell Function in the Intestine." *Nature* 513(7519):564–68.

Schlitzer, Andreas and Florent Ginhoux. 2014. "Organization of the Mouse and Human DC Network." *Current Opinion in Immunology* 26:90–99.

Schlitzer, Andreas, Naomi McGovern, and Florent Ginhoux. 2015. "Dendritic Cells and Monocyte-Derived Cells: Two Complementary and Integrated Functional Systems." *Seminars in Cell & Developmental Biology* 41:9–22.

Schlitzer, Andreas, Naomi McGovern, Pearline Teo, Teresa Zelante, Koji Atarashi, Donovan Low, Adrian W. S. Ho, Peter See, Amanda Shin, Pavandip Singh Wasan, Guillaume Hoeffel, Benoit Malleret, Alexander Heiseke, Samantha Chew, Laura Jardine, Harriet A. Purvis, Catharien M. U. Hilkens, John Tam, Michael Poidinger, E. Richard Stanley, Anne B. Krug, Laurent Renia, Baalasubramanian Sivasankar, Lai Guan Ng, Matthew Collin, Paola Ricciardi-Castagnoli, Kenya Honda, Muzlifah Haniffa, and Florent Ginhoux. 2013. "IRF4 Transcription Factor-Dependent CD11b+ Dendritic Cells in Human and Mouse Control Mucosal IL-17 Cytokine Responses." *Immunity* 38(5):970–83.

Schmidt, Susanne V., Andrea C. Nino-Castro, and Joachim L. Schultze. 2012.

"Regulatory Dendritic Cells: There Is More than Just Immune Activation." *Frontiers in Immunology* 3:274.

Schmitz, Jochen, Alexander Owyang, Elizabeth Oldham, Yaoli Song, Erin Murphy, Terril K. McClanahan, Gerard Zurawski, Mehrdad Moshrefi, Jinzhong Qin, Xiaoxia Li, Daniel M. Gorman, J. Fernando Bazan, and Robert A. Kastelein. 2005. "IL-33, an Interleukin-1-like Cytokine That Signals via the IL-1 Receptor-Related Protein ST2 and Induces T Helper Type 2-Associated Cytokines." *Immunity* 23(5):479–90.

Schraml, Barbara U., Kai Hildner, Wataru Ise, Wan-Ling Lee, Whitney A. E. Smith, Ben Solomon, Gurmukh Sahota, Julia Sim, Ryuta Mukasa, Saso Cemerski, Robin D. Hatton, Gary D. Stormo, Casey T. Weaver, John H. Russell, Theresa L. Murphy, and Kenneth M. Murphy. 2009. "The AP-1 Transcription Factor Batf Controls TH17 Differentiation." *Nature* 460(7253):405–9.

Schultz, Michael B. and David A. Sinclair. 2016. "Why NAD+ Declines during Aging: It's Destroyed." *Cell Metabolism* 23(6):965–66.

Schulz, Christian, Elisa Gomez Perdiguero, Laurent Chorro, Heather Szabo-Rogers, Nicolas Cagnard, Katrin Kierdorf, Marco Prinz, Bishan Wu, Sten Eirik W. Jacobsen, Jeffrey W. Pollard, Jon Frampton, Karen J. Liu, and Frederic Geissmann. 2012. "A Lineage of Myeloid Cells Independent of Myb and Hematopoietic Stem Cells." *Science* 336(6077):86–90.

Scott, Charlotte L., Aude M. Aumeunier, and Allan McI. Mowat. 2011. "Intestinal CD103+ Dendritic Cells: Master Regulators of Tolerance?" *Trends in Immunology* 32(9):412–19.

Sedhom, Mamdouh A. K., Mélanie Pichery, Jenna R. Murdoch, Benoit Foligné, Nathalie Ortega, Sylvain Normand, Kirsten Mertz, Devika Sanmugalingam, Lea Brault, Teddy Grandjean, Emma Lefrancais, Padraic G. Fallon, Valérie Quesniaux, Laurent Peyrin-Biroulet, Gieri Cathomas, Tobias Junt, Mathias Chamaillard, Jean-Philippe Girard, and Bernhard Ryffel. 2013. "Neutralisation of the Interleukin-33/ST2 Pathway Ameliorates Experimental Colitis through Enhancement of Mucosal Healing in Mice." *Gut* 62(12):1714–23.

See, Peter, Charles-Antoine Dutertre, Jinmiao Chen, Patrick Günther, Naomi McGovern, Sergio Erdal Irac, Merry Gunawan, Marc Beyer, Kristian Händler, Kaibo Duan, Hermi Rizal Bin Sumatoh, Nicolas Ruffin, Mabel Jouve, Ester Gea-Mallorquí, Raoul C. M. Hennekam, Tony Lim, Chan Chung Yip, Ming Wen, Benoit Malleret, Ivy Low, Nurhidaya Binte Shadan, Charlene Foong Shu Fen, Alicia Tay, Josephine Lum, Francesca Zolezzi, Anis Larbi, Michael Poidinger, Jerry K. Y. Chan, Qingfeng Chen, Laurent Rénia, Muzlifah Haniffa, Philippe Benaroch, Andreas Schlitzer, Joachim L. Schultze, Evan W. Newell, and Florent Ginhoux. 2017. “Mapping the Human DC Lineage through the Integration of High-Dimensional Techniques.” *Science (New York, N.Y.)* 356(6342):eaag3009.

Senovilla, Laura, Lorenzo Galluzzi, Laurence Zitvogel, and Guido Kroemer. 2013. “Immunosurveillance as a Regulator of Tissue Homeostasis.” *Trends in Immunology* 34(10):471–81.

Shedlock, D. J. and Hao Shen. 2003. “Requirement for CD4 T Cell Help in Generating Functional CD8 T Cell Memory.” *Science* 300(5617):337–39.

Sheng, Jianpeng, Christiane Ruedl, and Klaus Karjalainen. 2015. “Most Tissue-Resident Macrophages Except Microglia Are Derived from Fetal Hematopoietic Stem Cells.” *Immunity* 43(2):382–93.

Shi, Chao and Eric G. Pamer. 2011. “Monocyte Recruitment during Infection and Inflammation.” *Nature Reviews Immunology* 11(11):762–74.

Shlomchik, Mark J. and Florian Weisel. 2012. “Germinal Center Selection and the Development of Memory B and Plasma Cells.” *Immunological Reviews* 247(1):52–63.

Shortman, Ken and Shalin H. Naik. 2007. “Steady-State and Inflammatory Dendritic-Cell Development.” *Nature Reviews Immunology* 7(1):19–30.

Shu, Chengyi Jenny, Christophe Benoist, and Diane Mathis. 2012. “The Immune System’s Involvement in Obesity-Driven Type 2 Diabetes.” *Seminars in Immunology* 24(6):436–42.

Sica, Antonio and Alberto Mantovani. 2012. “Macrophage Plasticity and Polarization: In Vivo Veritas.” *The Journal of Clinical Investigation* 122(3):787–95.

Sichien, Dorine, Charlotte L Scott, Liesbet Martens, Matthias Vanderkerken, Sofie Van Gassen, Maud Plantinga, Thorsten Joeris, Sofie De Prijck, Leen Vanhoutte, Manon Vanheerswynghels, Gert Van Isterdael, Wendy Toussaint, Filipe Branco Madeira, Karl Vergote, William W. Agace, Björn E. Clausen, Hamida Hammad, Marc Dalod, Yvan Saeys, Bart N. Lambrecht, and Martin Guilliams. 2016. “IRF8 Transcription Factor Controls Survival and Function of Terminally Differentiated Conventional and Plasmacytoid Dendritic Cells, Respectively.” *Immunity* 45(3):626–40.

Sichien, Dorine, Charlotte L. Scott, Liesbet Martens, Matthias Vanderkerken, Sofie Van Gassen, Maud Plantinga, Thorsten Joeris, Sofie De Prijck, Leen Vanhoutte, Manon Vanheerswynghels, Gert Van Isterdael, Wendy Toussaint, Filipe Branco Madeira, Karl Vergote, William W. Agace, Björn E. Clausen, Hamida Hammad, Marc Dalod, Yvan Saeys, Bart N. Lambrecht, and Martin Guilliams. 2016. “IRF8 Transcription Factor Controls Survival and Function of Terminally Differentiated Conventional and Plasmacytoid Dendritic Cells, Respectively.” *Immunity* 45(3):626–40.

Silhavy, T. J., D. Kahne, and S. Walker. 2010. “The Bacterial Cell Envelope.” *Cold Spring Harbor Perspectives in Biology* 2(5):a000414–a000414.

Singh-Jasuja, Harpreet, Allan Thiolat, Matthieu Ribon, Marie-Christophe Boissier, Natacha Bessis, Hans-Georg Rammensee, and Patrice Decker. 2013. “The Mouse Dendritic Cell Marker CD11c Is Down-Regulated upon Cell Activation through Toll-like Receptor Triggering.” *Immunobiology* 218(1):28–39.

Singh, Gitanjali M., Goodarz Danaei, Farshad Farzadfar, Gretchen A. Stevens, Mark Woodward, David Wormser, Stephen Kaptoge, Gary Whitlock, Qing Qiao, Sarah Lewington, Emanuele Di Angelantonio, Stephen vander Hoorn, Carlene M. M. Lawes, Mohammed K. Ali, Dariush Mozaffarian, Majid Ezzati, Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group; Asia-Pacific Cohort Studies Collaboration (APCSC), Diabetes Epidemiology: Collaborative analysis of Diagnostic criteria in Europe (DECODE), Emerging Risk Factor Collaboration (ERFC), and Prospective Studies Collaboration (PSC). 2013. “The Age-Specific Quantitative Effects

of Metabolic Risk Factors on Cardiovascular Diseases and Diabetes: A Pooled Analysis" edited by G. Wang. *PLoS ONE* 8(7):e65174.

Slifka, M. K., R. Antia, J. K. Whitmire, and R. Ahmed. 1998. "Humoral Immunity Due to Long-Lived Plasma Cells." *Immunity* 8(3):363–72.

Smigiel, Kate S., Elizabeth Richards, Shivani Srivastava, Kerri R. Thomas, Jan C. Dudda, Kimberly D. Klonowski, and Daniel J. Campbell. 2014. "CCR7 Provides Localized Access to IL-2 and Defines Homeostatically Distinct Regulatory T Cell Subsets." *The Journal of Experimental Medicine* 211(1):121–36.

Smith, P. C., A. Hobisch, D. L. Lin, Z. Culig, and E. T. Keller. 2001. "Interleukin-6 and Prostate Cancer Progression." *Cytokine & Growth Factor Reviews* 12(1):33–40.

De Souza, Cláudio T., Eliana P. Araujo, Silvana Bordin, Rika Ashimine, Ricardo L. Zollner, Antonio C. Boschero, Mário J. A. Saad, and Lício A. Velloso. 2005. "Consumption of a Fat-Rich Diet Activates a Proinflammatory Response and Induces Insulin Resistance in the Hypothalamus." *Endocrinology* 146(10):4192–99.

Spallanzani, Raul German, David Zemmour, Tianli Xiao, Teshika Jayewickreme, Chaoran Li, Paul J. Bryce, Christophe Benoist, and Diane Mathis. 2019. "Distinct Immunocyte-Promoting and Adipocyte-Generating Stromal Components Coordinate Adipose Tissue Immune and Metabolic Tenors." *Science Immunology* 4(35):eaaw3658.

Spits, Hergen, David Artis, Marco Colonna, Andreas Diefenbach, James P. Di Santo, Gerard Eberl, Shigeo Koyasu, Richard M. Locksley, Andrew N. J. McKenzie, Reina E. Mebius, Fiona Powrie, and Eric Vivier. 2013. "Innate Lymphoid Cells — a Proposal for Uniform Nomenclature." *Nature Reviews Immunology* 13(2):145–49.

Spits, Hergen and Tom Cupedo. 2012. "Innate Lymphoid Cells: Emerging Insights in Development, Lineage Relationships, and Function." *Annual Review of Immunology* 30(1):647–75.

Sprent, J. and D. Tough. 1994. "Lymphocyte Life-Span and Memory." *Science* 265(5177):1395–1400.

Staudt, Valérie, Evita Bothur, Matthias Klein, Karen Lingnau, Sebastian Reuter, Nadine Grebe, Bastian Gerlitzki, Markus Hoffmann, Alexander Ulges, Christian Taube, Nina Dehzad, Marc Becker, Michael Stassen, Andrea Steinborn, Michael Lohoff, Hansjörg Schild, Edgar Schmitt, and Tobias Bopp. 2010. “Interferon-Regulatory Factor 4 Is Essential for the Developmental Program of T Helper 9 Cells.” *Immunity* 33(2):192–202.

Steer, Catherine A., Itziar Martinez-Gonzalez, Maryam Ghaedi, Patricia Allinger, Laura Mathä, and Fumio Takei. 2017. “Group 2 Innate Lymphoid Cell Activation in the Neonatal Lung Drives Type 2 Immunity and Allergen Sensitization.” *The Journal of Allergy and Clinical Immunology* 140(2):593-595.e3.

Stegmaier, Philip, Alexander E. Kel, and Edgar Wingender. 2004. “Systematic DNA-Binding Domain Classification of Transcription Factors.” *Genome Informatics. International Conference on Genome Informatics* 15(2):276–86.

Stephens, Jacqueline M., Jongsoon Lee, and Paul F. Pilch. 1997. “Tumor Necrosis Factor-α-Induced Insulin Resistance in 3T3-L1 Adipocytes Is Accompanied by a Loss of Insulin Receptor Substrate-1 and GLUT4 Expression without a Loss of Insulin Receptor-Mediated Signal Transduction.” *Journal of Biological Chemistry* 272(2):971–76.

Stier, Matthew T., Jian Zhang, Kasia Goleniewska, Jacqueline Y. Cephus, Mark Rusznak, Lan Wu, Luc Van Kaer, Baohua Zhou, Dawn C. Newcomb, and R. Stokes Peebles. 2018. “IL-33 Promotes the Egress of Group 2 Innate Lymphoid Cells from the Bone Marrow.” *The Journal of Experimental Medicine* 215(1):263–81.

Still, Katherine M., Samantha J. Batista, Jeremy A. Thompson, Nikolas W. Hayes, Carleigh O’Brien, and Tajie H. Harris. 2018. “The Damage Signal IL-33 Promotes a Focal Protective Myeloid Cell Response to Toxoplasma Gondii in the Brain.” *BioRxiv* 338400.

Stremska, Marta E., Sheethal Jose, Vikram Sabapathy, Liping Huang, Amandeep Bajwa, Gilbert R. Kinsey, Poonam R. Sharma, Saleh Mohammad, Diane L. Rosin, Mark D. Okusa, and Rahul Sharma. 2017. “IL233, A Novel IL-2 and IL-33 Hybrid Cytokine, Ameliorates Renal Injury.” *Journal of the American*

Society of Nephrology 28(9):2681–93.

Swardfager, Walter, Krista Lanctôt, Lana Rothenburg, Amy Wong, Jaclyn Cappell, and Nathan Herrmann. 2010. “A Meta-Analysis of Cytokines in Alzheimer’s Disease.” *Biological Psychiatry* 68(10):930–41.

Swirski, F. K., M. Nahrendorf, M. Etzrodt, M. Wildgruber, V. Cortez-Retamozo, P. Panizzi, J. L. Figueiredo, R. H. Kohler, A. Chudnovskiy, P. Waterman, E. Aikawa, T. R. Mempel, P. Libby, R. Weissleder, and M. J. Pittet. 2009. “Identification of Splenic Reservoir Monocytes and Their Deployment to Inflammatory Sites.” *Science* 325(5940):612–16.

Tackey, E., P. E. Lipsky, and G. G. Illei. 2004. “Rationale for Interleukin-6 Blockade in Systemic Lupus Erythematosus.” *Lupus* 13(5):339–43.

Talabot-Ayer, Dominique, Nicolas Calo, Solenne Vigne, Céline Lamacchia, Cem Gabay, and Gaby Palmer. 2012. “The Mouse *Interleukin (Il)33* Gene Is Expressed in a Cell Type- and Stimulus-Dependent Manner from Two Alternative Promoters.” *Journal of Leukocyte Biology* 91(1):119–25.

Talukdar, Saswata, Da Young Oh, Gautam Bandyopadhyay, Dongmei Li, Jianfeng Xu, Joanne McNelis, Min Lu, Pingping Li, Qingyun Yan, Yimin Zhu, Jachelle Ofrecio, Michael Lin, Martin B. Brenner, and Jerrold M. Olefsky. 2012. “Neutrophils Mediate Insulin Resistance in Mice Fed a High-Fat Diet through Secreted Elastase.” *Nature Medicine* 18(9):1407–12.

Tamiya, Taiga, Kenji Ichiyama, Hitoshi Kotani, Tomohiro Fukaya, Takashi Sekiya, Takashi Shichita, Kiri Honma, Katsuyuki Yui, Toshifumi Matsuyama, Takako Nakao, Satoru Fukuyama, Hiromasa Inoue, Masatoshi Nomura, and Akihiko Yoshimura. 2013. “Smad2/3 and IRF4 Play a Cooperative Role in IL-9–Producing T Cell Induction.” *The Journal of Immunology* 191(5):2360–71.

Tamura, Tomohiko, Prafullakumar Tailor, Kunihiro Yamaoka, Hee Jeong Kong, Hideki Tsujimura, John J. O’Shea, Harinder Singh, and Keiko Ozato. 2005. “IFN Regulatory Factor-4 and -8 Govern Dendritic Cell Subset Development and Their Functional Diversity.” *The Journal of Immunology* 174(5):2573–81.

Tamura, Tomohiko, Hideyuki Yanai, David Savitsky, and Tadatsugu Taniguchi. 2008. “The IRF Family Transcription Factors in Immunity and Oncogenesis.” *Annual Review of Immunology* 26(1):535–84.

Tan, Zhongming, Qianghui Liu, Runqiu Jiang, Long Lv, Siamak S. Shoto, Isabelle Maillet, Valerie Quesniaux, Junwei Tang, Wenjie Zhang, Beicheng Sun, and Bernhard Ryffel. 2018. “Interleukin-33 Drives Hepatic Fibrosis through Activation of Hepatic Stellate Cells.” *Cellular & Molecular Immunology* 15(4):388–98.

Taneja, Veena. 2018. “Sex Hormones Determine Immune Response.” *Frontiers in Immunology* 9:1931.

Taniguchi, Tadatsugu, Kouetsu Ogasawara, Akinori Takaoka, and Nobuyuki Tanaka. 2001. “IRF F AMILY OF T RANSCRIPTION F ACTORS AS R EGULATORS OF H OST D EFENSE.” *Annual Review of Immunology* 19(1):623–55.

Teh, Peggy P., Ajithkumar Vasanthakumar, and Axel Kallies. 2015. “Development and Function of Effector Regulatory T Cells.” Pp. 155–74 in *Progress in Molecular Biology and Translational Science*.

Testa, U., E. Stellacci, E. Pelosi, P. Sestili, M. Venditti, R. Orsatti, A. Fragale, E. Petrucci, L. Pasquini, F. Belardelli, L. Gabriele, and A. Battistini. 2004. “Impaired Myelopoiesis in Mice Devoid of Interferon Regulatory Factor 1.” *Leukemia* 18(11):1864–71.

Tete, S., A. Saggini, G. Maccauro, M. Rosati, F. Conti, E. Cianchetti, D. Tripodi, E. Toniato, M. Fulcheri, V. Salini, A. Caraffa, P. Antinolfi, S. Frydas, F. Pandolfi, P. Conti, G. Potalivo, M. Nicoletti, and T. C. Theoharides. 2012. “Interleukin-9 and Mast Cells.” *Journal of Biological Regulators and Homeostatic Agents* 26(3):319–26.

Thau, Lauren and Kunal Mahajan. 2019. *Physiology, Opsonization*. StatPearls Publishing.

The Emerging Risk Factors Collaboration. 2011. “Separate and Combined Associations of Body-Mass Index and Abdominal Adiposity with Cardiovascular Disease: Collaborative Analysis of 58 Prospective Studies.” *The Lancet* 377(9771):1085–95.

Thomas, Marshall P., Jennifer Whangbo, Geoffrey McCrossan, Aaron J. Deutsch, Kimberly Martinod, Michael Walch, and Judy Lieberman. 2014. “Leukocyte Protease Binding to Nucleic Acids Promotes Nuclear

Localization and Cleavage of Nucleic Acid Binding Proteins." *The Journal of Immunology* 192(11):5390–97.

Thompson, Matthew G., Daniel S. Peiffer, Michelle Larson, Flor Navarro, and Stephanie K. Watkins. 2017. "FOXO3, Estrogen Receptor Alpha, and Androgen Receptor Impact Tumor Growth Rate and Infiltration of Dendritic Cell Subsets Differentially between Male and Female Mice." *Cancer Immunology, Immunotherapy* 66(5):615–25.

Tittel, André P., Christoph Heuser, Christina Ohliger, Percy A. Knolle, Daniel R. Engel, and Christian Kurts. 2011. "Kidney Dendritic Cells Induce Innate Immunity against Bacterial Pyelonephritis." *Journal of the American Society of Nephrology : JASN* 22(8):1435–41.

Tominaga, N., Kozo Ohkusu-Tsukada, Heiichiro Udono, Ryo Abe, Toshifumi Matsuyama, and Katsuyuki Yui. 2003. "Development of Th1 and Not Th2 Immune Responses in Mice Lacking IFN-Regulatory Factor-4." *International Immunology* 15(1):1–10.

Townsend, M. J., P. G. Fallon, D. J. Matthews, H. E. Jolin, and A. N. McKenzie. 2000. "T1/ST2-Deficient Mice Demonstrate the Importance of T1/ST2 in Developing Primary T Helper Cell Type 2 Responses." *The Journal of Experimental Medicine* 191(6):1069–76.

Tu, Lei, Jie Chen, Dandan Xu, Zhongming Xie, Bing Yu, Ying Tao, Guixiu Shi, and Lihua Duan. 2017. "IL-33-Induced Alternatively Activated Macrophage Attenuates the Development of TNBS-Induced Colitis." *Oncotarget* 8(17):27704–14.

Turnquist, Heth R., Zhenlin Zhao, Brian R. Rosborough, Quan Liu, Antonino Castellaneta, Kumiko Isse, Zhiliang Wang, Megan Lang, Donna Beer Stolz, Xin Xiao Zheng, A. Jake Demetris, Foo Y. Liew, Kathryn J. Wood, and Angus W. Thomson. 2011. "IL-33 Expands Suppressive CD11b+ Gr-1(Int) and Regulatory T Cells, Including ST2L+ Foxp3+ Cells, and Mediates Regulatory T Cell-Dependent Promotion of Cardiac Allograft Survival." *Journal of Immunology (Baltimore, Md. : 1950)* 187(9):4598–4610.

Tussiwand, Roxane, Wan-Ling Lee, Theresa L. Murphy, Mona Mashayekhi, Wumesh KC, Jörn C. Albring, Ansuman T. Satpathy, Jeffrey A. Rotondo,

Brian T. Edelson, Nicole M. Kretzer, Xiaodi Wu, Leslie A. Weiss, Elke Glasmacher, Peng Li, Wei Liao, Michael Behnke, Samuel S. K. Lam, Cora T. Aurthur, Warren J. Leonard, Harinder Singh, Christina L. Stallings, L. David Sibley, Robert D. Schreiber, and Kenneth M. Murphy. 2012. "Compensatory Dendritic Cell Development Mediated by BATF–IRF Interactions." *Nature* 490(7421):502–7.

Uderhardt, Stefan, Andrew J. Martins, John S. Tsang, Tim Lämmermann, and Ronald N. Germain. 2019. "Resident Macrophages Cloak Tissue Microlesions to Prevent Neutrophil-Driven Inflammatory Damage." *Cell* 177(3):541-555.e17.

Ukkola, Olavi and Merja Santaniemi. 2002. "Adiponectin: A Link between Excess Adiposity and Associated Comorbidities?" *Journal of Molecular Medicine* 80(11):696–702.

Vasanthakumar, Ajithkumar, Kazuyo Moro, Annie Xin, Yang Liao, Renee Gloury, Shimpei Kawamoto, Sidonia Fagarasan, Lisa A. Mielke, Shoukat Afshar-Sterle, Seth L. Masters, Susumu Nakae, Hirohisa Saito, John M. Wentworth, Peng Li, Wei Liao, Warren J. Leonard, Gordon K. Smyth, Wei Shi, Stephen L. Nutt, Shigeo Koyasu, and Axel Kallies. 2015. "The Transcriptional Regulators IRF4, BATF and IL-33 Orchestrate Development and Maintenance of Adipose Tissue–Resident Regulatory T Cells." *Nature Immunology* 16(3):276–85.

Verma, Mukesh, Sucai Liu, Lidia Michalec, Anand Sripada, Magdalena M. Gorska, and Rafeul Alam. 2018. "Experimental Asthma Persists in IL-33 Receptor Knockout Mice Because of the Emergence of Thymic Stromal Lymphopoietin-Driven IL-9+ and IL-13+ Type 2 Innate Lymphoid Cell Subpopulations." *The Journal of Allergy and Clinical Immunology* 142(3):793-803.e8.

Vignali, Dario A. A., Lauren W. Collison, and Creg J. Workman. 2008. "How Regulatory T Cells Work." *Nature Reviews Immunology* 8(7):523–32.

Villadangos, José A. and Louise Young. 2008. "Antigen-Presentation Properties of Plasmacytoid Dendritic Cells." *Immunity* 29(3):352–61.

Villani, Alexandra-Chloé, Rahul Satija, Gary Reynolds, Siranush Sarkizova,

Karthik Shekhar, James Fletcher, Morgane Griesbeck, Andrew Butler, Shiwei Zheng, Suzan Lazo, Laura Jardine, David Dixon, Emily Stephenson, Emil Nilsson, Ida Grundberg, David McDonald, Andrew Filby, Weibo Li, Philip L. De Jager, Orit Rozenblatt-Rosen, Andrew A. Lane, Muzlifah Haniffa, Aviv Regev, and Nir Hacohen. 2017. “Single-Cell RNA-Seq Reveals New Types of Human Blood Dendritic Cells, Monocytes, and Progenitors.” *Science* 356(6335):eaah4573.

Villarroya, Francesc, Rubén Cereijo, Joan Villarroya, Aleix Gavaldà-Navarro, and Marta Giralt. 2018. “Toward an Understanding of How Immune Cells Control Brown and Beige Adipobiology.” *Cell Metabolism* 27(5):954–61.

Vinson, Charles, Max Myakishev, Asha Acharya, Alain A. Mir, Jonathan R. Moll, and Maria Bonovich. 2002. “Classification of Human B-ZIP Proteins Based on Dimerization Properties.” *Molecular and Cellular Biology* 22(18):6321–35.

Vinué, Ángela and Herminia González-Navarro. 2015. “Glucose and Insulin Tolerance Tests in the Mouse.” Pp. 247–54 in. Humana Press, New York, NY.

Walker, Lucy S. K. and David M. Sansom. 2015. “Confusing Signals: Recent Progress in CTLA-4 Biology.” *Trends in Immunology* 36(2):63–70.

Wan, Yisong Y. and Richard A. Flavell. 2009. “How Diverse--CD4 Effector T Cells and Their Functions.” *Journal of Molecular Cell Biology* 1(1):20–36.

Wang, Hongsheng, Ming Yan, Jiafang Sun, Shweta Jain, Ryusuke Yoshimi, Sanaz Momben Abolfath, Keiko Ozato, William G. Coleman, Ashley P. Ng, Donald Metcalf, Ladina DiRago, Stephen L. Nutt, Herbert C. Morse, Herbert C. Morse, and III. 2014. “A Reporter Mouse Reveals Lineage-Specific and Heterogeneous Expression of IRF8 during Lymphoid and Myeloid Cell Differentiation.” *Journal of Immunology (Baltimore, Md. : 1950)* 193(4):1766–77.

Wang, Yiping and David C. H. Harris. 2011. “Macrophages in Renal Disease.” *Journal of the American Society of Nephrology* 22(1):21–27.

Watanabe, Hiroyuki, Kousuke Numata, Takaaki Ito, Katsumasa Takagi, and Akihiro Matsukawa. 2004. “Innate Immune Response in Th1- and Th2-Dominant Mouse Strains.” *Shock (Augusta, Ga.)* 22(5):460–66.

Waugh, D. J. J. and C. Wilson. 2008. “The Interleukin-8 Pathway in Cancer.” *Clinical Cancer Research* 14(21):6735–41.

Weisberg, Stuart P., Daniel McCann, Manisha Desai, Michael Rosenbaum, Rudolph L. Leibel, and Anthony W. Ferrante. 2003. “Obesity Is Associated with Macrophage Accumulation in Adipose Tissue.” *Journal of Clinical Investigation* 112(12):1796–1808.

Weisser, Shelley B., Keith W. McLarren, Etsushi Kuroda, and Laura M. Sly. 2013. “Generation and Characterization of Murine Alternatively Activated Macrophages.” Pp. 225–39 in *Methods in molecular biology (Clifton, N.J.)*. Vol. 946.

Wentworth, John M., Gaetano Naselli, Wendy A. Brown, Lisa Doyle, Belinda Phipson, Gordon K. Smyth, Martin Wabitsch, Paul E. O’Brien, and Leonard C. Harrison. 2010. “Pro-Inflammatory CD11c+CD206+ Adipose Tissue Macrophages Are Associated With Insulin Resistance in Human Obesity.” *Diabetes* 59(7):1648–56.

Wintrob, Zachary A. P., Emmanuel K. Oppong, Matthew Foster, Michael Martorana, Yu C. Tse, Li Zhong, Jeffery M. Welt, Hans R. Boateng, Ioana M. Drumea, John Irlam, Charles M. Levea, Silviu L. Faitar, and Alice C. Ceacareanu. 2014. “Sexual Dimorphism, Weight Gain and Glucose Intolerance in a B- and T-Cell Deficient Mouse Model.” *Cytokine* 67(2):102–6.

Wong, See Heng, Jennifer A. Walker, Helen E. Jolin, Lesley F. Drynan, Emily Hams, Ana Camelo, Jillian L. Barlow, Daniel R. Neill, Veera Panova, Ute Koch, Freddy Radtke, Clare S. Hardman, You Yi Hwang, Padraic G. Fallon, and Andrew N. J. McKenzie. 2012. “Transcription Factor RORα Is Critical for Nuocyte Development.” *Nature Immunology* 13(3):229–36.

Wu, Liyan, Zujin Luo, Jinxu Zheng, Peng Yao, Zhenyan Yuan, Xiaohong Lv, Jing Zhao, and Min Wang. 2018. “IL-33 Can Promote the Process of Pulmonary Fibrosis by Inducing the Imbalance Between MMP-9 and TIMP-1.” *Inflammation* 41(3):878–85.

Wueest, Stephan, Eugen J. Schoenle, and Daniel Konrad. 2012. “Depot-Specific Differences in Adipocyte Insulin Sensitivity in Mice Are Diet- and Function-

Dependent.” *Adipocyte* 1(3):153–56.

Wynn, Thomas A. and Kevin M. Vannella. 2016. “Macrophages in Tissue Repair, Regeneration, and Fibrosis.” *Immunity* 44(3):450–62.

XIE, CHANGLI, CUIYING LIU, BITAO WU, YAN LIN, TINGTING MA, HAIYU XIONG, QIN WANG, ZIWEI LI, CHENYU MA, and ZHIGUANG TU. 2016. “Effects of IRF1 and IFN-β Interaction on the M1 Polarization of Macrophages and Its Antitumor Function.” *International Journal of Molecular Medicine* 38(1):148–60.

Xu, Haiyan, Glenn T. Barnes, Qing Yang, Guo Tan, Daseng Yang, Chieh J. Chou, Jason Sole, Andrew Nichols, Jeffrey S. Ross, Louis A. Tartaglia, and Hong Chen. 2003. “Chronic Inflammation in Fat Plays a Crucial Role in the Development of Obesity-Related Insulin Resistance.” *Journal of Clinical Investigation* 112(12):1821.

Xu, Yuekang, Yifan Zhan, Andrew M. Lew, Shalin H. Naik, and Michael H. Kershaw. 2007. “Differential Development of Murine Dendritic Cells by GM-CSF versus Flt3 Ligand Has Implications for Inflammation and Trafficking.” *The Journal of Immunology* 179(11):7577–84.

Yamamoto, Michio, Takayuki Kato, Chie Hotta, Akira Nishiyama, Daisuke Kurotaki, Masahiro Yoshinari, Masamichi Takami, Motohide Ichino, Masatoshi Nakazawa, Toshifumi Matsuyama, Ryutaro Kamijo, Seiichi Kitagawa, Keiko Ozato, and Tomohiko Tamura. 2011. “Shared and Distinct Functions of the Transcription Factors IRF4 and IRF8 in Myeloid Cell Development” edited by K. D. Bunting. *PLoS ONE* 6(10):e25812.

Yanagawa, Yoshiki, Machiko Matsumoto, and Hiroko Togashi. 2011. “Adrenoceptor-Mediated Enhancement of Interleukin-33 Production by Dendritic Cells.” *Brain, Behavior, and Immunity* 25(7):1427–33.

Yanai, Hideyuki, Hideo Negishi, and Tadatsugu Taniguchi. 2012. “The IRF Family of Transcription Factors: Inception, Impact and Implications in Oncogenesis.” *Oncoimmunology* 1(8):1376–86.

Yang, Yuanyuan, Huan Liu, Haiyue Zhang, Qing Ye, Jianyi Wang, Boyu Yang, Leilei Mao, Wen Zhu, Rehana K. Leak, Bo Xiao, Binfeng Lu, Jun Chen, and Xiaoming Hu. 2017. “ST2/IL-33-Dependent Microglial Response Limits

Acute Ischemic Brain Injury." *Journal of Neuroscience* 37(18):4692–4704.

Yasuda, K., T. Muto, T. Kawagoe, M. Matsumoto, Y. Sasaki, K. Matsushita, Y. Taki, S. Futatsugi-Yumikura, H. Tsutsui, K. J. Ishii, T. Yoshimoto, S. Akira, and K. Nakanishi. 2012. "Contribution of IL-33-Activated Type II Innate Lymphoid Cells to Pulmonary Eosinophilia in Intestinal Nematode-Infected Mice." *Proceedings of the National Academy of Sciences* 109(9):3451–56.

Yona, Simon, Ki-Wook Kim, Yochai Wolf, Alexander Mildner, Diana Varol, Michal Breker, Dalit Strauss-Ayali, Sergey Viukov, Martin Guilliams, Alexander Misharin, David A. Hume, Harris Perlman, Bernard Malissen, Elazar Zelzer, and Steffen Jung. 2013. "Fate Mapping Reveals Origins and Dynamics of Monocytes and Tissue Macrophages under Homeostasis." *Immunity* 38(1):79–91.

Yuseff, Maria-Isabel, Paolo Pierobon, Anne Reversat, and Ana-Maria Lennon-Duménil. 2013. "How B Cells Capture, Process and Present Antigens: A Crucial Role for Cell Polarity." *Nature Reviews Immunology* 13(7):475–86.

Zeyda, Maximilian and Thomas M. Stulnig. 2009. "Obesity, Inflammation, and Insulin Resistance &Ndash; A Mini-Review." *Gerontology* 55(4):379–86.

Zhang, Lisheng, Jiao-Hui Wu, James C. Otto, Susan B. Gurley, Elizabeth R. Hauser, Sudha K. Shenoy, Karim Nagi, Leigh Brian, Virginia Wertman, Natalie Mattocks, Jeffrey H. Lawson, and Neil J. Freedman. 2017. "Interleukin-9 Mediates Chronic Kidney Disease-Dependent Vein Graft Disease: A Role for Mast Cells." *Cardiovascular Research* 113(13):1551–59.

Zhang, X. and D. M. Mosser. 2008. "Macrophage Activation by Endogenous Danger Signals." *The Journal of Pathology* 214(2):161–78.

Zhang, Yiying, Ricardo Proenca, Margherita Maffei, Marisa Barone, Lori Leopold, and Jeffrey M. Friedman. 1994. "Positional Cloning of the Mouse Obese Gene and Its Human Homologue." *Nature* 372(6505):425–32.

Zhao, Dong-Mei, Angela M. Thornton, Richard J. DiPaolo, and Ethan M. Shevach. 2006. "Activated CD4+CD25+ T Cells Selectively Kill B Lymphocytes." *Blood* 107(10):3925–32.

Zhao, Guang-Nian, Ding-Sheng Jiang, and Hongliang Li. 2015. "Interferon

Regulatory Factors: At the Crossroads of Immunity, Metabolism, and Disease." *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 1852(2):365–78.

Zheng, C., Q. Yang, J. Cao, N. Xie, K. Liu, P. Shou, F. Qian, Y. Wang, and Y. Shi. 2016. "Local Proliferation Initiates Macrophage Accumulation in Adipose Tissue during Obesity." *Cell Death & Disease* 7(3):e2167–e2167.

Zheng, Ye, Ashutosh Chaudhry, Arnold Kas, Paul deRoos, Jeong M. Kim, Tin-Tin Chu, Lynn Corcoran, Piper Treuting, Ulf Klein, and Alexander Y. Rudensky. 2009. "Regulatory T-Cell Suppressor Program Co-Opts Transcription Factor IRF4 to Control T(H)2 Responses." *Nature* 458(7236):351–56.

Zhu, Bing, Yoshio Bando, Sheng Xiao, Kaiyong Yang, Ana C. Anderson, Vijay K. Kuchroo, and Samia J. Khoury. 2007. "CD11b+Ly-6C(Hi) Suppressive Monocytes in Experimental Autoimmune Encephalomyelitis." *Journal of Immunology (Baltimore, Md. : 1950)* 179(8):5228–37.

Zoltowska Nilsson, A. M., Y. Lei, M. Adner, and G. P. Nilsson. 2018. "Mast Cell-Dependent IL-33/ST2 Signaling Is Protective against the Development of Airway Hyperresponsiveness in a House Dust Mite Mouse Model of Asthma." *American Journal of Physiology-Lung Cellular and Molecular Physiology* 314(3):L484–92.