INFLAMMASOME-INDEPENDENT INTERLEUKIN-1 BETA DRIVES CD4 T CELL EFFECTOR FUNCTION AND AUTOIMMUNE INFLAMMATION

APPROVED BY SUPERVISORY COMMITTEE

_____

Chandrashekhar Pasare, Ph.D.

_____

Lora V. Hooper, Ph.D.

_____

James S. Malter, Ph.D.

_____

Nicholas K. Conrad, Ph.D.

_____

Anne Satterthwaite, Ph.D.

"Be inspired by the knowledge that exists at the time you enter research, but be irreverent toward this knowledge, for this is the road to true understanding"

Charles A. Janeway, Jr.

ACKNOWLEDGEMENTS

First, I would like to thank my mentor, Dr. Chandrashekhar Pasare. You have trained me well to be an independent scientist. You have taught me how to crystalize abstract ideas while never losing sight of the big picture, to make linear and coherent arguments and to design simple yet powerful experiments. I am thankful for the scientific freedom you have given me as I progressed through graduate school. I must also offer my gratitude for helping me push my boundaries as you had a larger vision for me than I ever did.

To the members of the Pasare lab, please accept my sincere gratitude for your support and invaluable friendships. To Yajing Gao, I feel incredibly fortunate to call you my friend. You have made me a better scientist and helped me persevere through the failures in graduate school and even though we will not be working together anymore, you will continue to inspire me forever. To Garrett Overcast and Ricardo Irizarry Caro, thank you for letting me be a part of your scientific journeys. Training you has been one of the most fulfilling experiences of my life. To, Dr. Ty Troutman and Dr. Wei hu, I appreciate you taking time to help me navigate through graduate school and your continued guidance. To Rustam Bagirzadeh, you have made me a better writer, a better lab citizen and a better friend. To other members of the Pasare lab, both past and present, Margaret McDaniel, Amanpreet Chawla, Lisa Waggoner, Linley Riediger, and Travis Brewer for your assistance, conversations, and support.

To my thesis committee members, Dr. Lora Hooper, Dr. James Malter and Dr. Nicholas Conrad, I sincerely appreciate your guidance throughout my graduate research as well as your support during my transition to Cincinnati.

To my parents, Dr. Suchitra Jain and Dr. Om Prakash Jain, I owe everything to you. You taught me to always strive for excellence and to not let my past define my future. Even though we were a part of a conservative community in India, you did not let societal pressures dictate my journey and for that, I will forever be grateful. It is my honor to be your daughter and I will continue to work hard to make you proud. To my sister, Dr. Arpana Jain, who introduced me to science and instilled within me my curiosity, my work ethic and my tenacity. These attributes will always be key to my accomplishments as I move forward in my scientific career.

To Nilabh Ohol, for your love, support and companionship. You have always believed in me and showed me what is possible. You have helped me enjoy my life as a whole and your constant encouragement is invaluable to my scientific achievements.

INFLAMMASOME INDEPENDENT INTERLEUKIN-1 BETA DRIVES CD4 T CELL EFFECTOR FUNCTION AND AUTOIMMUNE INFLAMMATION

by

AAKANKSHA JAIN

DISSERTATION

Presented to the Faculty of the Graduate School of Biomedical Sciences

The University of Texas Southwestern Medical Center at Dallas

In Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

The University of Texas Southwestern Medical Center at Dallas

Dallas, Texas

May, 2019

ABSTRACT

Successful generation of protective immunity is a result highly orchestrated interactions between the innate and the adaptive arms of the immune system. Innate immune cells sense the infectious threat to the host and convert it into meaningful information that is relayed to T and B cells. This relaying of information is the fundamental principle that forms the basis of all protective immune responses and is referred to as the innate control of adaptive immunity. For naïve CD4 T cell activation, innate control is exerted by the three-signal paradigm which dictates that a productive naïve CD4 T cell response requires TCR activation, co-stimulation and innate cytokine cues. The present work uncovers two consequential yet previously unknown aspects of the innate and adaptive immune, specifically dendritic cell (DC)-T cell, crosstalk. First, I found that in addition to naïve CD4 T cell activation, the three-signal paradigm continue to operate during the reactivation phase of memory CD4 T cells. Memory CD4 T cell reactivation was presumed to be independent of innate cytokine cues however, I found that signaling through interleukin (IL)-1 receptor family is required for effector cytokine production by memory CD4 T cells. Mechanistically, IL-1R signaling provides post-transcriptional stability to T cell cytokine transcripts thereby enabling productive secretion of these cytokines. Second, I discovered that DC-T cell interaction is significantly more bidirectional than previously appreciated. Innate cells such as DCs, have evolved to sense "non-self" or "altered-self" ligands via pattern recognition receptors (PRRs) and get activated only in case of pathogen invasion or tissue damage. Surprisingly, I found that autoreactive T cells can also instruct DCs to become activated independent of PRR activation. During their cognate interaction, T cell engage Tumor necrosis factor superfamily (TNFRSF) signaling in DCs to trigger innate cytokine secretion. I particularly, focused on the mechanism of IL-1β production during DC-T cell interaction as IL-1β is mediator of several autoimmune and

autoinflammatory diseases. While IL-1β production is commonly attributed to inflammasome activation in autoimmune diseases, I show that that autoreactive T cells elicit IL-1β production by DCs in an inflammasome-independent manner via TNFR-Fas signaling pathway. Furthermore, this novel mechanism of IL-1β secretion drives CD4 T cell effector function, systemic leukocyte infiltration as well as autoimmune inflammation. Altogether, the findings in this dissertation provide a conceptual leap in our understanding of innate and adaptive immune cross-talk, and necessitates revisiting the established paradigm of innate signaling requirements for myeloid cell activation as well as CD4 T cell function. In addition, this work has vast implications on human health as it sheds light on the previously poorly understood mechanism of action of IL-1R blockade therapy as well as offers novel targets for therapeutic intervention of IL-1β mediated autoimmune inflammation.

TABLE OF CONTENTS

LIST OF PUBLICATIONS

Jain, A., Irizarry-Caro, R.A., Chawla, A.S., Philip, N.H., Carroll, K.R., Katz, J., Oberst, A., Chervonsky, A.V., Pasare, C. (2018). T cells instruct dendritic cells to produce inflammasome independent IL-1β causing systemic inflammation, bioRxiv preprint doi: http://dx.doi.org/10.1101/475517

Gao, Y., Deason, K., Jain, A., Irizarry-Caro, R.A., Dozmorov, I., Rauch, I., Wakeland, E.K., Pasare, C. (2018). Transcriptome profiling of pathogen-specific CD4 T cells identifies T-cell-intrinsic caspase-1 as an important regulator of Th17 differentiation. bioRxiv preprint doi: https://doi.org/10.1101/452763

Jain, A., Song, R., Wakeland, E.K., and Pasare, C. (2018). T cell-intrinsic IL-1R signaling licenses effector cytokine production by memory CD4 T cells. Nat Commun 9, 3185.

Deason, K., Troutman, T.D., Jain, A., Challa, D.K., Mandraju, R., Brewer, T., Ward, E.S., and Pasare, C. (2018). BCAP links IL-1R to the PI3K-mTOR pathway and regulates pathogenic Th17 cell differentiation. J Exp Med 215, 2413-2428.

Mandraju, R., Jain, A., Gao, Y., Ouyang, Z., Norgard, M.V., and Pasare, C. (2018). MyD88 Signaling in T Cells Is Critical for Effector CD4 T Cell Differentiation following a Transitional T Follicular Helper Cell Stage. Infect Immun 86.

Jain, A., and Pasare, C. (2017). Innate Control of Adaptive Immunity: Beyond the Three-Signal Paradigm. J Immunol 198, 3791-3800.

Hu, W., Jain, A., Gao, Y., Dozmorov, I.M., Mandraju, R., Wakeland, E.K., and Pasare, C. (2015). Differential outcome of TRIF-mediated signaling in TLR4 and TLR3 induced DC maturation. Proc Natl Acad Sci U S A 112, 13994-13999.

LIST OF FIGURES

LIST OF DEFINITIONS

DC, Dendritic cells

EAE, Experimental autoimmune encephalomyelitis

G-MCSF, Granulocyte - macrophage colony stimulating factor

ICOS, Inducible T-cell COStimulator

IFN, Interferon

IL, Interleukin

ILC, Innate Lymphoid Cell

IRF, Interferon Regulatory Factor

JAK, Janus kinase

LPS, Lipopolysaccharide

MAPK, Mitogen-activated protein kinase

MHC, Major histocompatibility complex

MyD88, Myeloid differentiation primary response gene 88

NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells

NLR, Nucleotide Oligomerization Domain (NOD) like receptor

PAMP, Pathogen associated molecular patterns

PMA, Phorbol 12-myristate 13-acetate

PRR, Pattern recognition receptor

RIP, Receptor interacting protein

RLR, retinoic acid-inducible gene-I-like receptors

RORγt, retinoic acid receptor - related orphan receptor C

SI-LP, Small intestinal – lamina propria

STAT, Signal transducer and activator of transcription

TCR, T cell receptor

TIR, Toll-interleuking-1 receptor

TLR, Toll like receptor

TNF, Tumor necrosis factor

TNFRSF, Tumor necrosis factor receptor superfamily

Tregs, Regulatory T cells

CHAPTER ONE

Introduction

Part of the writing presented in this chapter was previously published as a review article in *Journal of Immunology*, 2017; 198:3791-3800 (Jain and Pasare, 2017)

Overview

T cells and B cells are equipped with a diverse repertoire of receptors capable of recognizing a vast array of antigens. This diversity allows for protection against constantly evolving pathogens but also gives rise to substantial self-reactivity. Avoiding self-reactivity while maintaining clonal diversity is an intriguing evolutionary design problem. A seminal leap in our understanding of the activation of the adaptive immune system was due to the late Charles Janeway Jr. He proposed that innate cells should be equipped with germline encoded pattern recognition receptors (PRRs) to recognize "non-self", conserved microbial components also referred to as pathogen associated molecular patterns (PAMPs) (Janeway, 1989a). Activated innate immune cells would then convey the information about the nature and origin of the antigen to the adaptive immune cells in order to mount an appropriate adaptive immune response. This idea formed the basis for the now-established three-signal paradigm of innate control of adaptive immunity (Curtsinger and Mescher, 2010; Curtsinger et al., 1999). The first signal provided by innate cells is the presentation of the antigenic peptide, which is necessary for activation of the T cell receptor (TCR) and clonal expansion of antigen-specific T cells. Since the peptide can be of self or non-self-origin, antigen presentation alone is unable to provide any qualitative information about the source of the antigen

(Bretscher and Cohn, 1970). The second signal is provided via co-stimulatory molecules that are upregulated on antigen presenting cells only when the antigen is associated with a PAMP (Pasare and Medzhitov, 2004; Schnare et al., 2001). Thus, co-stimulation is necessary for self-versus non-self-distinction. It is not clear if PRRs can modulate T cell activation by regulating expression of specific co-stimulatory molecules but detailed information on how various co-stimulatory molecules affect adaptive immunity can be found in these reviews (Lenschow et al., 1996; Watts, 2005).

The third signal consists of innate cytokines that are produced as a result of PRR activation (Curtsinger et al., 1999). The cytokine milieu helps T cells differentiate into protective T cell subsets required for host immunity against a given pathogen (Reiner, 2007; Zhu et al., 2010). The three-signal model described a fundamental link between the innate and adaptive immune systems and defined the requirements for inducing a measurable T cell response referred to as "productive immunity".

The requirement of concurrent presence of all three signals ensures diverse but selective T cell activation and differentiation. However, the three-signal model mentioned above is a vast oversimplification of the innate and adaptive immune cross-talk. The requirement for innate signals for reactivation of previously differentiated T cells remain poorly understood. Moreover, the central role of PRR signaling in innate immune activation does not explain how T cells elicit effector function during sterile inflammation as seems in autoimmune scenarios. The focus of my dissertation research has been to understand innate regulation of memory CD4 T cell effector function. We have discovered a critical role for IL-1R signaling in effector function of

differentiated CD4 T cells. Furthermore, T cells can instruct IL-1β production by DC upon cognate interaction independent of PRR activation. Together this work is consequential extension to the concept of innate control of adaptive immunity and lay the groundwork for future studies pertaining to CD4 T cell response during host defense as well as autoimmunity.

Innate Control of Adaptive Immunity

Dendritic cell activation

The discovery of various classes of PRRs and identification of their microbial ligands has led to a detailed understanding of innate immune recognition (Kawai and Akira, 2010; Rathinam et al., 2012; Wu and Chen, 2014). PRRs are strategically located in subcellular compartments based on the nature of their ligands that activate unique signal transduction program necessary for host defense (Kawai and Akira, 2007). Although each class of PRR induces distinct innate responses, the activation of adaptive immunity depends on their ability to induce DC maturation (Pasare and Medzhitov, 2004; Schnare et al., 2001). Following PRR activation, DCs upregulate MHC and co-stimulatory molecules and migrate to the draining lymph nodes to interact with naive T cells that are specific to the microbial antigen (Iwasaki and Medzhitov, 2004) . DCs process and present extracellular and endosomal peptides on the MHC class II molecule to activate CD4 T cells while peptides derived from cytosolic proteins are presented on MHC class I to activate CD8 T cells (Blum et al., 2013). In addition to antigen presentation and co-stimulation, the third signal that shapes the quality of T cell responses, is the production of innate cytokines. NF-Kb dependent cytokines such as IL-12, IL-6 and TNFα (Kawai and Akira, 2007) drive acute inflammation as well as dictate T cell differentiation. Two other major classes of innate cytokines, IL-1 family and type I interferons, often cooperate with priming cytokines for generation of protective T cell

immunity. Both immune and non-immune cells can produce IL-1 family of cytokines (IL-1α, IL-1β, IL-18 and IL-33) as an outcome of recognition of virulence factors, cellular stress or cell death. Type I IFNs, on the other hand, are produced following activation of nucleic acid sensors in the endosomes (TLR3, TLR7/8 and TLR9) or in the cytosol (RLRs and cGAS) (Wu and Chen, 2014). IL-1 family of cytokines and type I IFNs drive systemic immune responses by inducing an acute phase response and a global anti-viral state, respectively. Dysregulated production of these cytokines can also drive autoinflammatory disorders and immunopathology.

Naïve CD4 T cell differentiation

Distinct differentiation states of CD4 T cells are traditionally defined by the lineage specific transcription factors and their effector cytokines. Unique T cell subsets are largely shaped by the innate cytokines presented during priming. In brief, IL-12 promotes Th1 differentiation by driving T-bet expression (Yang et al., 2007). IL-4 drives Th2 differentiation in both paracrine and autocrine manner (Kopf et al., 1993; Le Gros et al., 1990; Paul and Ohara, 1987; Swain et al., 1990). The combination of IL-6 and TGFβ promotes Th17 differentiation (Ivanov et al., 2007). IL-6 and TGFβ can synergize with IL-23 and IL-1 to further stabilize the Th17 program (El-Behi et al., 2011). In the absence of an infection, TGFβ produced by migratory DCs induces regulatory T cells (iTregs) that are critical for maintaining peripheral tolerance (Coombes et al., 2007). T cell subsets are often studied using *in vitro* systems, through the use of defined priming cytokine cocktails that result in highly polarized T cell populations. During a real infection, however, activated DCs generate a complex milieu of innate cytokines leading to the priming of a heterogeneous CD4 T cell population. Therefore, *in vitro* polarized T cells might only represent extreme cases removed from physiological differentiation process that gives rise to a diverse

effector population *in vivo*. Recent studies using whole pathogens lysates and live microbes have begun to unravel the complexities cytokine requirements for T cell differentiation. Priming naive human CD4 T cells using whole pathogen lysates has revealed qualitatively distinct patterns of cytokine production between Th17 populations induced by *Staphylococcus aureus* and *Candida albican*s (Zielinski et al., 2012). *S. aureus* induced Th17 cells secrete IL-17 as well as IL-10 independent of IL-1β stimulation, while *C. albicans* specific Th17 cells produce IL-17 and IFNγ requiring IL-1β in addition to IL-6 and IL-23. Furthermore, mouse model of *Citrobacter rodentium* infection showed that IL-22 producing T cells, dependent on IL-23 for differentiation, were required for protection (Basu et al., 2012). These cells expressed RORγt, the master transcriptional regulator of Th17 lineage, but failed to produce IL-17. In the context of experimental autoimmune encephalomyelitis (EAE), IL-23 was alternatively shown to induce "pathogenic Th17" cells, which produce IFNγ and GM-CSF in addition to IL-17 (Lee et al., 2012b). In agreement with these studies, transcriptomic analysis has shown variable gene expression profiles within the Th17 population(Gaublomme et al., 2015). Collectively, these findings suggest that depending on the immune challenge, naive T cells get exposed to a unique innate cytokine milieu that drives functionally peculiar T cell sub-populations even though they may belong to the same umbrella lineage. Studying functional T cell subsets based on the innate cytokine stimulation, that drove their differentiation rather than their narrow effector cytokine profile, will likely reveal more valuable information regarding the generation of protective T cell responses against specific pathogens. Analyzing cytokine receptor expression on CD4 T cells during various stages of immune response could reveal the dependence of those T cells on respective cytokines.

Effector and memory CD4 T cell reactivation

Generation of immunological memory is a hallmark of adaptive immunity. Presence of antigen specific memory enables the host to fight off pathogens with significantly greater potency during reinfection (Rogers et al., 2000). Despite the established importance of memory cells, the regulation of memory CD4 T cell reactivation remains poorly understood. Upon immunological challenge, DCs undergo maturation and induce naïve CD4 T cells activation and clonal expansion. This interaction between naïve CD4 T cells and antigen-loaded DCs takes place in secondary lymphoid organs. Clonal expansion increases the frequency of CD4 T cells that are required for effective B cell and CD8 T cell responses (Bevan, 2004; Crotty, 2015). Another consequence of CD4 T cell activation is their differentiation into Th1, Th2 and Th17 lineages that can play important role in host defense. The expansion is followed by a contraction phase towards the end of the immune response. Most antigen specific T cells undergo apoptosis but a small proportion of cells convert into long lived memory (Dooms and Abbas, 2002; Harrington et al., 2008). Memory T cells confer rapid protection in the barrier tissues as well as give rise to another wave of antigen specific T cell response in the secondary lymphoid organs in case of re-challenge. Current model suggests that CD4 T cell memory can exist as two distinct populations (Sallusto et al., 2004). Differentiated CD4 T cells that travel to the site of infection and secrete their signature cytokines to elicit effector function are referred to as effector cells. On the other hand, stem cell-like memory CD4 T cells that readily proliferate and differentiate into a new pool of effector CD4 T cells are known as central memory CD4 T cells (Sallusto et al., 1999). More recently, a distinct population memory T cells was identified that reside in the tissues for long period of time even after the infection is cleared. These are called tissue resident memory T cells (Mueller et al., 2013;

Teijaro et al., 2011). The importance of these cells in immediate host protection has become increasingly clear in recent years.

There is wealth of knowledge about the innate immune cues that regulate naïve CD4 T cell differentiation as also briefly discussed earlier in this chapter. However, innate signals that mediate CD4 T cell reactivation have remained unclear. We know that memory CD4 T cells display lower threshold for TCR activation (Byrne et al., 1988). Using genetic models, it was also shown that memory CD4 T cells require, albeit low levels of, co-stimulation for optimal effector function (Ndejembi et al., 2006). Memory CD4 T cell reactivation, however, is thought to be independent of innate cytokines. A teleological explanation for reduced innate regulation during reactivation phase is that it allows for immediate memory T cell response (MacLeod et al., 2010). Also, since CD4 T cells have already responded to innate cytokine cues during the priming phase, one can argue against the continued dependence on innate cytokines during CD4 T cell reactivation is a redundant regulatory mechanism. In my dissertation research, I revisit this currently established paradigm for memory CD4 T cell reactivation. I found that memory CD4 T cells continue to depend on innate signals during their reactivation. Whether such rigorous innate control of memory CD4 T cell function evolved to prevent self-reactive T cell activation and immunopathology will need further investigation.

IL-1R signaling in health and disease

IL-1 family of cytokines in T cell immunity

Priming cytokines like IL-6, IL-12 and IL-4 often synergize with IL-1 family of cytokines for generation of protective immunity. Starting originally with just IL-1α and IL-1β, the IL-1 family

has expanded to 11 family members. IL-1α, IL-1β, IL- 18, IL-33, IL-36a, IL-36b, IL-36g and IL-37 have agonist activity whereas IL-1Ra, IL-36Ra, and IL-38 are antagonists (Garlanda et al., 2013). The IL-1 system exhibits a recurrent theme of accelerators (cytokines and signaling receptors) and brakes (receptor antagonists, binding proteins, decoy receptors) suggesting a tight regulation over IL-1R family signaling. IL-1 was originally described as an anti-microbial factor. IL-1 was also found to be an endogenous pyrogen causing fever and acute phase response as well as a hematopoietic factor promoting granulopoesis (Dinarello, 2009). Although the exact role of each IL-1 family member in regulating T cell responses is not clear, there is substantial evidence that IL-1β, IL-18 and IL-33 contribute to Th17, Th1 and Th2 CD4 T cell responses, respectively (Sims and Smith, 2010). A series of studies have verified the importance of IL-1 in Th17 cell biology (Basu et al., 2015; Chung et al., 2009; Hu et al., 2011; Mailer et al., 2015). T cell intrinsic IL-1R signaling has been shown to promote pathogenicity of Th17 cells by synergizing with IL-6 and IL-23 (Chang et al., 2013). IL-18 has been implicated in Th1 and CD8 T cell function. The combination of IL-12 and IL-18 induces IFNγ production by memory CD8 T cells and Th1 cells independent of TCR activation (Berg et al., 2003; Yang et al., 1999). IL-18 can also enhance T-bet expression in human CD4 T cells thus promoting Th1 function (Blom and Poulsen, 2012). IL-33 is produced as an alarmin when cells undergo inflammatory cell death (Cayrol and Girard, 2014). The adaptive function of IL-33R signaling was first discovered in the context of type 2 immunity as this receptor was prominently expressed on Th2 cells (Schmitz et al., 2005). Recently, it was found that colonic (Schiering et al., 2014), adipose tissue resident (Vasanthakumar et al., 2015) and muscle (Kuswanto et al., 2016) Tregs also express high levels of IL-33R (ST2). IL-33R signaling promotes proliferation and maintenance of iTregs, a novel mechanism of IL-33 dependent suppression of inflammation (Schiering et al., 2014; Vasanthakumar et al., 2015). These

studies emphasize the multifaceted nature of IL-1 family of cytokines and show that they can acts as mediators of acute inflammation during host invasion as well as drivers of homeostatic adaptive immunity at steady state. IL-1R family utilizes a MyD88 dependent signaling pathway, analogous to TLR signaling, for downstream signal transduction. Given the evolutionary relationship between IL-1R and TLR signaling, it is tantalizing to propose that our understanding of TLR signaling pathway in innate cells can serve as a blueprint to delineate the role of IL-1 family of receptors in T cell biology.

Regulation of IL-1β production: Inflammasome and beyond

IL-1β is mainly produced by monocytes, macrophages, DCs and neutrophils for anti-microbial immunity (Ainscough et al., 2014; Beuscher et al., 1990; Guma et al., 2009). IL-1 can trigger inflammation via multiple mechanisms including initiation of acute phase response and recruitment inflammatory cells (Biondo et al., 2014; Zheng et al., 1995). More recently, IL-1β has also been implicated in homeostatic processes such as tissue repair and neuronal homeostasis (Liu et al., 2019; Naik et al., 2017). However, dysregulated production of IL-1β can be harmful to the host as it can cause immunopathology (Lopalco et al., 2015). Due to its highly inflammatory nature, IL-1 β is produced under strict regulation. IL-1β production typically utilizes a two-step mechanism. The first step involves transcription and translation of pro- IL-1β which is dependent on NF-Kb activation (Cogswell et al., 1994). In majority of studies, the pro- IL-1β synthesis step is induced by stimulation of myeloid cells using a TLR ligand. Since pro IL-1β is not biologically active, it requires a second step that leads to proteolytic cleavage of pro-IL-1β into its bioactive form.

Inflammasome activation is the most well studied mechanism for production of bioactive IL-1β as it allows for caspase-1 dependent pro-IL-1β processing. However, several other inflammasome independent pathways involving caspase-8, calpains, cathepsins and neutrophil-derived serine proteases have also been reported for IL-1β maturational cleavage (Gurung et al., 2014; Netea et al., 2015). Recently, IL-21 stimulation was also reported to induce transcriptional upregulation of pro-IL-1β in an NF-κb independent STAT3 dependent manner (Wan et al., 2015). The mechanism leading to processing of pro-IL-1β following IL-21 stimulation remains unknown. It is plausible that while the inflammasome dependent IL-1β drives systemic inflammation, inflammasome independent mechanisms of IL-1 production have evolved for much subtler functions such as influencing T cell biology during priming as well as reactivation. In my research, I have worked towards delineating the mechanism of IL-1β production during CD4 T cell reactivation and T cell mediated autoimmunity.

IL-1β mediated autoinflammatory and autoimmune diseases

IL-1β is a major driver of several autoinflammatory and autoimmune diseases. Inhibition of IL-1β activity using monoclonal antibody or anakinra has shown rapid and sustained reduction in disease severity (Dinarello et al., 2012). IL-1β driven inflammatory conditions can be categorized into two subsets based on their etiology. Autoinflammatory diseases occur due to abnormal activation of the macrophages/monocytes in the absence of any conventional microbial/danger signal. On the other hand, autoimmune diseases are caused by break in immunological tolerance resulting in B cell or T cell activation in response to self-antigens.

Recent surge in genome-wide association studies have shown heritable traits of autoinflammatory diseases. A unifying mechanism of inflammation in these diseases is dysregulated activation of inflammasome due to gain-of-function mutations leading to overproduction of IL-1β. The family of IL-1β driven autoinflammatory diseases include familial Mediterranean fever, periodic fever syndrome and pyogenic and granulomatous disorders (Dinarello, 2011). These diseases are characterized by rise in acute phase proteins and systemic amyloidosis. Due to the pivotal role of IL-1β in these diseases blocking IL-1β activity via various approaches has delivered promising results.

Several other diseases such as type 1 diabetes, pericarditis, rheumatoid arthritis, and psoriasis are also responsive to IL-1β neutralization (Lopalco et al., 2015). The autoimmune flares in patients are often associated with presence of cytokine secreting T cells (Raphael et al., 2015). Using genetic models of mice, it has become clear that these autoimmune diseases are primarily caused due to dysregulated activation of autoreactive T cells (Bluestone et al., 2015). In addition to genetic predisposition and environmental factors, several mechanisms have been proposed that can lead to self-T cell reactivation (Rosenblum et al., 2015). Improper clonal deletion such as in case of Autoimmune Polyendocrinopathy Syndrome causes escape of self-reactive T cells and formation of natural Tregs towards self- antigens (Anderson et al., 2002; Liston et al., 2003). Molecular mimicry when foreign antigen shares structural similarity with a host antigen can also cause cross reactivity towards self-peptide (Fujinami and Oldstone, 1985). Furthermore, impaired apoptosis of DCs can lead to inappropriate T cell activation and eventual loss in self-tolerance (Hutcheson et al., 2008). IL-1β can promote T cell mediated autoimmunity by enhancing T cell function as

well as inhibiting Tregs mediated suppression (Jain et al., 2018; Schenten et al., 2014). While the exact mechanism of IL-1β production in T cell mediated autoimmunity is not known, targeting of IL-1β has shown promise in clinical trials.

Adaptive instruction of innate immunity

T cell function during infections and autoimmunity is dependent on complex cross-talk that occurs between the innate and the adaptive immune compartment (Jain and Pasare, 2017). The majority of the studies done to delineate this cross talk have focused on the innate cues are required to mount an appropriate T cell response. The paradigm of innate control of adaptive immunity dictates that antigen presenting myeloid cells are required to be stimulated with microbial ligands for activation. It is established that productive T cells immunity can only be induced upon successful activation of the APCs via PRRs (Pasare and Medzhitov, 2004). However, there are certain scenarios where T cell function is observed even in the absence of microbial presence. For example, in T cell mediated autoimmune diseases there is clear evidence of T cell effector function but no explanation for activation of APCs needed to elicit the T cell function. Moreover, while our data shows that IL-1 is required for memory T cell reactivation (Jain and Pasare, 2017), it has been reported that PRR sensing by DCs is dispensable for memory CD4 T cell responses (Pasare and Medzhitov, 2004).

It is important to note that the interaction between the myeloid cells and T cells is, in fact, a two-way street (Strutt et al., 2011). Memory CD4 T cells were shown to block inflammasome activation in macrophages upon cognate interaction thereby controlling inflammation (Guarda et al., 2009). Similarly, T cell deficient mice exhibited unleashed innate immune response causing cytokine

storm (Kim et al., 2007b). In addition to suppressing innate immune responses, T cell have also been shown to trigger innate immunity in a PRR independent manner (Strutt et al., 2010). Following influenza infection, CD4 memory T cells were found to enhance innate cytokine and chemokine production via an antigen dependent but PRR independent mechanism (Strutt et al., 2010). While these studies suggest that T cells can modulate innate immune responses, the mechanism of such interaction remains unclear. In my dissertation research, I have delineated the molecular mechanism that can lead to PRR independent activation of APCs upon their cognate interaction with T cells. The findings described in the present work provide a mechanistic explanation for innate immune activation that can occur in the absence of microbial recognition.

Summary of the dissertation

This dissertation studies the cross-talk between the dendritic cells and memory CD4 T cells during their antigen-specific reactivation. I found that, analogous naïve CD4 T cell priming, memory CD4 T cells also rely on innate cytokine cues for optimal effector function. In particular, IL-1β signaling is critical for enabling cytokine production by CD4 T cells of all lineages. I demonstrate that IL-1R signaling is required for post-transcriptional stabilization of T cell cytokines transcripts. The present work also reveals a novel mechanism of IL-1β production that is independent of pattern recognition receptor signaling as well as inflammasome activation. I discovered that during cognate interaction, CD4 T cells can instruct DCs to produce mature IL-1β via a TNFR-Fas dependent mechanism. T cell induced IL-1β was found to be is independent of the canonical as well as non-canonical inflammasomes. More importantly, I show that the TNFR-Fas pathway is responsible for inflammation during T cell mediated autoimmune disease. As reported before, inflammasome activation can play a role in in auto-inflammatory diseases (CAPS, Gout, etc.) but

the novel pathway described here warrants revisiting the currently presumed role for inflammasome in T cell driven autoimmune pathology. Finally, I provide evidence that sterile high avidity interaction with T cells can remodel the transcriptional landscape of DCs that resembles PRR signaling.

CHAPTER TWO

Methodology

Mice

C57BL/6 wild-type control mice were obtained from the UT Southwestern Mouse Breeding Core Facility. *Il1a*$^{-/-}$ and *Il1b*$^{-/-}$ mice were provided to us by Fayyaz S. Sutterwala at Cedars Sinai. IL-1α-/- mice were generated by Yochiro Iwakura, Tokyo University of Science. *Il1b*$^{-/-}$ mice were generated by David Chaplin, UA at Birmingham. *Il1r*$^{-/-}$ mice were purchased from The Jackson Laboratory. *Myd88*$^{-/-}$ were generated by Shizou Akira, Osaka University and provided to us by Ruslan Medzhitov, Yale University. *Rip3*$^{-/-}$ and *Rip3*$^{-/-}$*Casp8*$^{-/-}$ KO were provided by Andrew Oberst at the University of Washington. B6.MRL-*Fas*^{lpr}/J (*lpr*), B6.129S-*Tnf*^{tm1Gkl}/J (*Tnfa*$^{-/-}$), B6.129S-*Tnfrsf1a*^{tm1Imx} *Tnfrsf1b*^{tm1Imx}/J (Tnfrab$^{-/-}$) and 2D2 T cell transgenic mice were obtained from Jackson Laboratories. All mice were bred and housed in a specific pathogen-free facility at UT Southwestern Medical Center or Cincinnati Children's Hospital Medical Center. For isolation of steady state CD4 memory T cells, mice were housed in conventional facility for 2-4 weeks before tissue isolation. All mouse experiments were done as per protocols approved by Institutional Animal Care and Use Committee (IACUC) at UT Southwestern Medical Center.

Antibodies

Mouse CD4 BV421 Biolegend 100443 (1:400), Mouse CD4 FITC Biolegend 100405 (1:400), CD44 AF700 Biolegend 103025 (1:400), CD44 pacific blue Biolegend 103020 (1:400), CD62L APC Tonbo Biosciences 20-0621-U100 (1:400), IL-6r Biotin Biolegend 115803 (1:100), IL-12rb1

PE RnD systems FAB1998P-025 (10ul/test), IL-4Ra PE Biolegend 144803 (1:100), IL-1R1 Biotin Biolegend 113503 (1:100), IL-18Ra APC Biolegend 132903 (5ul/test), IL-17A FITC Biolegend 506908 (1:300), IL-22 PE Biolegend 516404 (5ul/test), IFNg PE Biolegend 505807 (1:200), ICOS APC Biolegend 313509 (1:400), CD69 PE BD Biosciences 553237 (1:400), CD25 Biotin BD Biosiences (1:400), Zombie Yellow live dead stain Biolegend 423103 (1:500), Streptavidin BV421 Biolegend 405241 (1:400), CFSE Biolegend 423801 (1:1000), IL-17A purified Biolegend 505807 (1:1000), antiIL-17A biotin Biolegend 507002 (1:1000), Anti IL-22 purified Biolegend 516401 (1:250), Anti IL-22 biotin Biolegend 516407 (1:200), Anti IFNg purified Biolegend 505702 (1:1000), Anti IFNg biotin Biolegend 505804 (1:1000), Anti IL-17F Capture antibody eBioscience 14-7473-68 (1:200), Anti IL-17F Detection antibody eBioscience 13-7474-68 (1:250), Anti IL-13 purified eBioscience 14-7133-85 (1:125), Anti IL-13 biotin eBioscience 13-7135-85 (1:2000), Anti IL-4 purified Biolegend 504102 (1:200), Anti IL-4 biotin Biolegend 504202 (1:2000), Anti IL-5 purified Biolegend 504301 (1:500), Anti IL-5 biotin Biolegend 504402 (1:500), Anti-mouse Pro-IL-1β APC (eBioscience, 17-7114-80; 1:500), Anti-mouse CD11b BV785 (Biolegend, 101243; 1:400), Anti-mouse CD11C FITC (Biolegend; 117306, 1:400), Anti-mouse Ly6G FITC (Biolegend, 127605; 1:400), Ly6C BV711 (Biolegend, 12803; 1:400), Anti-mouse TNFα FITC (Biolegend, 506304, 1:1000), Anti-mouse F4/80 APC-eflour 780 (Invitrogen, 47-4801-80; 1:400), Anti-mouse CD90 Pacific blue (Biolegend, 105324; 1:400), Anti-mouse FasL PE (Biolegend, 106605; 1:100), Anti-mouse CD62L biotin (Biolegend, 104404; 1:200), Anti-mouse CD11c biotin (Biolegend,117304; 1:500)

Reagents

IL-1Ra (R&D Systems; 480-RM, 50ng/ml), rTNFα (Peprotech; 315-01Am, 20ng/ml), αCD3e (Biolegend; 100331 for *in vitro* assays and 100340 for *in vivo assays*), αTNF (Biolegend; 506332, 20μg/ml), αFasL (Biolegend; 106608, 10μg/ml), αCD40L (Biolegend; 310812, 10 μg/ml), IL-1β ELISA (R&D Systems; DY-401), TNFα ELISA(Biolegend; 510802 for capture and 506312 for detection), Naïve T cell Mojosort (Biolegend; 480040), total CD4 T cell Mojosort (Biolegend; 480006), Zombie yellow (Biolegend; 423103), DAPI (Biolegend; 422801), IETD (R&D Systems; FMK007, 10μM), rGMCSF (Biolegend; 576306), Anti-biotin beads (Milteny Biotec; 130-090-485), $OVA_{323-339}$ (Invivogen; vac-isq), LPS (Sigma, 100ng/ml), ATP (Invivogen; tlrl-atpl, 5nM), IL17a ELISA (Biolegend; 506902 for capture and 507002 for detection), EAE immunization kit (Hooke Laboratories, EK-2110) mouse MOG_{35-55} (CSbiologicals, CS0681)

Infections and Immunizations

C. rodentium was cultured in nalidixic acid (30□g ml^{-1}) containing LB broth. *L. monocytogenes* was cultured in BHI. For the oral infection model, WT mice were treated with 200µl 1% $NaHCO_3$ followed by intragastric infection with $1x10^8$ CFU of *C. rodentium*. Mesenteric lymph nodes were excised 9-10 dpi. For the systemic infection model WT mice were infected with 20,000 CFU of *L. monocytogenes* i.p. Spleens were harvested 7 dpi. Infection dose was confirmed via retrospective counting. For antigen-specific reactivation, pathogen extracts were prepared via freeze thawing bacteria followed by total protein quantification. For subcutaneous immunization, 25 µg OVA (Invitrogen) and 2.5 µg LPS (Sigma) was emulsified in IFA and injected subcutaneously into footpad (hind limbs). Popliteal and inguinal lymph nodes were harvested 7 days post immunization.

Isolation of total, naïve and memory CD4 T cells

Single cell suspension was obtained from spleen and peripheral lymph nodes. Total Naïve CD4 T cells were isolated using mouse an isolation kit (Biolegend) following manufacturer's instructions. For isolation of CD4+CD44+CD62L^{lo}, total CD4 T cells were labeled with a biotinylated CD62L antibody followed by anti-biotin microbeads (Miltenyi). CD62L^{lo} cells were isolated using negative selection by AutoMacs. Approximately 95% cells were CD4+CD44+CD62L^{lo}.

Isolation of splenic DCs

CD11C+ cells were magnetically sorted from splenocytes using positive selection by AutoMacs. Approximately 95-99% cells were CD11c+MHCII+.

***In vitro* differentiation of bone marrow derived dendritic cells and retroviral transduction**

Mouse progenitors were isolated from bone marrow (femurs and tibias). Following RBC lysis, cells were plated at $0.75x10^6$ mL^{-1} in BMDC media (5% FCS containing complete RPMI + 1% rGMCSF (Biolegend, 100ng mL^{-1})). Media was replaced on day 2 and day 4 and cells were harvested for experiments on day 5 by gently flushing each well. For retroviral transduction, following RBC lysis cells were plated at $10x10^6$ mL^{-1} in 2mL of retroviral supernatant containing 8μL mL^{-1} polybrene. Cells were spinfected at 2500 rpm, 32ºC, for 90 minutes, then 3mL of BMDC media was added to each well and cells were incubated overnight. The next morning, ~70% of the media was removed from the wells and spinfection was repeated with fresh retroviral supernatant. On day 5 cells were harvested for experiments.

In vitro differentiation of naïve CD4 T cells

Cell culture treated plates were coated with αCD3 (5μg mL^{-1}) and αCD28 (5μg mL^{-1}) for 3-4hrs at 37ºC. $CD4^{+}CD62L^{hi}$ naïve CD4 T cells were isolated from splenocytes using a Mojosort naïve CD4 T cell isolation kit according to manufacturer's protocol. Purified naïve T cells were plated in antibody-coated plates with appropriate polarizing conditions for 5 days. T cell were cultured in complete RPMI supplemented with 10% FCS. Cytokine cocktails for *in vitro* polarization: Th1 – IL-12 (10ng mL^{-1}, peprotech), IL-2 (50U mL^{-1}, peprotech), αIL-4 (10μg mL^{-1}, Biolegend); Th17 – IL-6 (20ng mL^{-1}, peprotech), hTGFβ (5ng mL^{-1}, peprotech), αIL-4 (10μg mL^{-1}, Biolegend), αIFNγ (10μg mL^{-1}, Biolegend), IL-23 (20ng mL^{-1}, Biolegend) and IL-1β (20ng mL^{-1}, peprotech), Th2- IL-4 (4ng mL^{-1}, peprotech), IL-2 (50U mL^{-1}, Biolegend), and αIFNγ (10μg mL^{-1}, Biolegend), Th0 – IL-2 (50U ml^{-1}). For co-culture experiments T cells were rested in 10% RPMI supplemented with IL-2 (10U mL^{-1}) for 36hrs before co-culture.

In vitro reactivation of polyclonal memory CD4 T cells

Memory T cell re-stimulation via TCR ligation in vitro

CD4 T cells were stimulated with either plate bound αCD3 (0.5 μg/ml) + αCD28 (0.5 μg/ml), or B cells (B cell:T cell = 2:1), or DCs (DC:T cell = 1:5) in the presence of 30 ng ml-1 soluble αCD3 for 24-48hrs.

Stimulation of CD4 T cells using PMA and ionomycin

CD4 T cells were treated with PMA (1 mg/ml, Sigma) and ionomycin (100 nM, Sigma) for 4-6hrs. For intracellular staining brefeldin A was also added.

In vivo polyclonal T cell activation

6-8 weeks old mice were treated with 20μg αCD3 or PBS by i.p. injection. Lamina propria cells and splenocytes were isolated at given time points after stimulation followed by surface and intracellular staining. 50μg αCD3 or PBS was injected intravenously in acute experiments as mentioned in the legends.

Flow cytometry and intracellular staining

Cells were stained with relevant antibodies for 30 min on ice and washed. For intracellular staining, Foxp3 staining buffer set (ebioscience) was used according to manufacturer's protocol. The stained cells were analyzed with BD LSRII or Novocyte (ACEA biosciences). For cytokine receptor staining, control refers to fluorescence minus one control. Data were analyzed with FlowJo 10 Software.

Western blot analysis

Cells were lysed in 1X RIPA Buffer and protein was quantified using Pierce™ BCA Protein Assay Kit. Cell lysates where boiled in 1X Laemmli buffer at 95ºC for 10 mins. Cell lysates were separated by SDS–PAGE and transferred onto PVDF membranes. Blots were incubated with anti-IL1β [1:1000] (R&D AF-401-SP), anti-caspase8 [1:1000] (Enzo ALX-804-447-C100), anti-caspase1 [1:1000] (Genentech), anti-βtubulin [1:5000] (CST 2146S). As secondary antibodies, anti-rabbit-IgG-HRP (Biorad) [1:5000], anti-mouse-IgG-HRP and anti-goat-IgG-HRP [1:10000] (Jackson ImmunoResearch Laboratory) were used. Anti-β-actin (C4, Santa Cruz, 1:5000) was used as control. Western blot was develop using SuperSignal™ West Pico PLUS

Chemiluminescent Substrate (Thermo Fisher) and ECL signal was recorded on X-Ray Films using a developer (Kodak).

Assay for quantifying T cell induced IL-1β production by DCs

1 million DCs were cultured with 4 million T cells in 12 well plate. DC-T cell interaction was triggered by adding either αCD3 (αCD3, 200ng mL^{-1}) or $OVA_{323-339}$ as described in the legends. In experiments measuring secreted IL-1β, IL-1R antagonist (50ng mL^{-1}) was added 1hr prior to DC stimulation to block IL-1β consumption. Co-cultures were also pretreated with neutralizing antibodies and inhibitor wherever described. Co-culture experiments were performed in complete RPMI supplemented with 10% FCS. For Western blot analysis of the culture supernatant cells were cultured in 1% FCS containing complete RPMI.

RNA isolation and quantitative PCR

RNA was isolated using Qiagen RNA extraction kit using manufacturer's protocol. cDNA was synthesized using Random primers (Invitrogen) and MMLV reverse transcriptase (Invitrogen). The QuantStudio 7 Flex Real-Time PCR System (ThermoFisher Scientific) was used to measure SGBR green (ThermoFisher Scientific) incorporation. All data is normalized to 18s rRNA. qPCR primers sequences are as follows: IL-1R1F: CAGGAGAAGTCGCAGGAAGT, IL-1R1 R: TGGAACAGAGCCAGTGTCAGIL17A F: TCCAGAAGGCCCTCAGACTA, IL17A R: AGCATCTTCTCGACCCTGAA, IFNg F: TGCCAAGTTTGAGGTCAACAACCCA, IFNg R: CCCACCCCGAATCAGCAGCG, IL17F F: ACCCGTGAAACAGCCATGGTCA, IL17F R: ACCGGTGGGGGTCTCGAGTG, IL22 F: CAATCAGCTCAGCTCCTGTCACAT, IL22 R: TCCCCAATCGCCTTGATCTCTCCA, IL13 F: ACAAGACCAGACTCCCCTGT, IL13 R:

TCTGGGTCCTGTAGATGGCA, 18S F: GTAACCCGTTGAACCCCATT, 18S R: CCATCCAATCGGTAGTAGCG. *Il1b*: Fwd-5'TGTGCTCTGCTTGTGAGGTGCTG 3', Rev 5'CCCTGCAGCTGGAGAGTGTGGA3' | *Hprt1*: Fwd-5' CAGTCCCAGCGTCGTGATTA-3', Rev-5' TGGCCTCCCATCTCCTTCAT-3' | *18S*: Fwd-5' GTAACCCGTTGAACCCCATT, Rev-5' CCATCCAATCGGTAGTAGCG

RNA stability assay

Lymph node (5x106 cells) cells were activated in the presence of soluble αCD3 (200 ng ml-1) for 18hrs. Alternatively, CD4 T cells were isolated from the spleen and LNs and reactivated in vitro with immobilized αCD3 (0.5 μg ml-1) and αCD28 (0.5 μg ml-1). Cells were washed and plated at lower density (1x106 cells ml-1) in the presence of actinomycin D (10 μg ml-1, Sigma). At given time points after addition of actinomycin D cells were washed with cold PBS and lysed with TriZol (Life Technologies) and frozen until further processing.

Transcriptomic profiling

CD45.1 CD11c+ DC were sorted from a heterogenous GMCSF derived BMDC population using Automacs. 99% pure CD11c+ DCs were co-cultured with CD45.2 Th0 polarized T cells in the presence of aCD3 (200ng/ml). After 3 hrs stimulation live CD45.1+CD11c+CD90.2- cells were facs sorted. Cells were immediately lysed in TriZol. RNA was extracted by using the miRNeasy kit (Qiagen). cDNA libraries were performed from purified RNA (performed in the lab of Dr. Edward K. Wakeland) and sequenced, paired end, using Hi-seq Illumina platform. Methods for data normalization and analysis are based on the use of "internal standards" which was slightly modified to the needs of RNA sequencing data analysis (Dozmorov et al., 2010). Dr, Igor Dosmorov, performed the differential gene expression analysis with minimal fold change restriction of 1.5. Yajing Gao performed hierarchical

gene clustering using Gene Pattern software develop by Broad Institute. Gene ontology analysis was performed using DAVID.

Passive EAE induction

9-10 weeks old WT female mice were immunized with MOG_{35-55} emulsion obtained from the Hooke lab EAE immunization kit as per manufacturer's protocol. Mice were also injected with 80ng Pertussis toxin intraperitoneally on day 0 and day 1. Total splenocytes were harvested 11-14 days after immunization and cultured in vitro with MOG (20μg/ml), anti-IFNγ (10μg/ml) and IL-23 (10ng/ml). After 3 days of reactivation, total CD4 T cells isolated using Mojosort Kit (Biolegend). 5-10x10^6 CD4 T cells were transferred intravenously into given genotypes of mice. Recipients also received 80ng Pertussis toxin intraperitoneally on day 0 and day 1 of transfer. Mice were monitored daily and disease severity was scored as follows: 0 = no clinical signs of paralysis, 1 = tail paralysis, 2 = tail paralysis and hind limb weakness, 3 = Complete hind limb weakness, 4 = forelimb paralysis or moribund.

Histology

On day 28 after adoptive transfer, mice were sacrificed and perfused with ice cold PBS. Spinal cords were collected and fixed with 10% neutral-buffered formalin for 36h. Fixed tissues submitted to Cincinnati Children's Hospital Medical Center research pathology core for tissue embedding and Luxol fast blue stain. Slides were imaged at 10X using a Nikon Eclipse Tι.

Quantification and Statistical Analysis

Based on previous and preliminary studies in our lab, we predicted that the reported samples sizes would be sufficient to ensure adequate power. Statistical analyses were performed in Prism (GraphPad) using unpaired or paired *student's t test* or two-way ANOVA as indicated in the figure legends. Data are presented as means ± SEM. Significance was considered at $*p<0.05$, $**p<0.005$, $***p<0.0005$. *n.s.* = not significant.

CHAPTER 3

Role of IL-1 Family of Cytokines in Memory CD4 T Cell Effector Function

Part of the writing presented in this chapter was previously published in *Nature Communications*, **9**, Article Number:3185, 2018 (Jain et al., 2018)

Introduction

Pathogen recognition by dendritic cells (DCs) via activation of pattern recognition receptors (PRRs) results in the upregulation of MHC and co-stimulatory molecules and the secretion of pro-inflammatory cytokines (Iwasaki and Medzhitov, 2015; Jain and Pasare, 2017). The MHC-peptide complex ensures cognate T cell activation while upregulation of co-stimulatory markers reflect the non-self-nature of the antigen (Pasare and Medzhitov, 2005). Although TCR engagement and co-stimulation can lead to activation and proliferation of CD4 T cells, innate cytokines are required for differentiation of naïve T cells into differentially programmed protective subsets tailored to eliminate specific microbial challenges (Curtsinger and Mescher, 2010).

Following pathogen clearance, antigen-specific T cells survive as memory T cells or effector T cells that either recirculate or reside permanently in the tissues (Mueller et al., 2013). In contrast to the three signal requirement for naïve T cell priming (Zhu et al., 2010), it has long been presumed that MHC-TCR interactions alone are sufficient for memory CD4 T cell reactivation and effector function (Bachmann et al., 1999; London et al., 2000). More recent work, however, has shown that co-stimulation via CD80/86 is also critical for reactivation of memory CD4 T cells

(Boesteanu and Katsikis, 2009; Borowski et al., 2007; Ndejembi et al., 2006). While dependence on only one (or two) signal(s) might allow for rapid reactivation of previously primed T cells, insufficient stringency can be inherently dangerous to the host physiology (Janeway, 1992). Using yeast-displayed MHC-peptide libraries, it was found that a single TCR could bind to several related peptides (Birnbaum et al., 2014); later it was shown that pathogenic peptides could cross-react with self-reactive TCRs (Nelson et al., 2015). Thus, pathogen-specific memory T cells are prone to aberrant reactivation by self-antigens. The risk of such undesirable activation is even higher at the barrier surfaces where tissue resident memory T cells are exposed to innocuous tissue restricted self-peptides (Harkiolaki et al., 2009; Poussier et al., 2002). Lack of qualitative information about the origin of the antigen during effector or memory T cell reactivation could thus lead to systemic or local auto-immune and auto-inflammatory responses (Enouz et al., 2012). This inspired us to hypothesize that the innate immune system provides additional cues for memory T cell reactivation beyond antigen presentation and co-stimulation. We propose that cues from the innate immune system regulate T cell responses at stages past differentiation by providing qualitatively distinct signals during naïve T cell priming versus memory T cell reactivation.

Innate cytokines can be categorized based on their dependence on STAT or MyD88 mediated signaling. Priming cytokines such as IL-6, IL-12 and IL-4 signal via the activation of STAT molecules, which induce or stabilize the expression of lineage specific transcription factors (Zhu et al., 2010). On the other hand IL-1 family of cytokines, including IL-1α, IL-1β, IL-18 and IL-33, engage MyD88 dependent signaling to enhance T cell responses that are primarily orchestrated by priming cytokines (Hu et al., 2011; Zhu et al., 2010). Before even the biology of its production and signaling was completely understood, IL-1 was predicted and shown to be a critical signal for

T helper cell growth (Janeway, 1989b). While at the time CD4 T cell subsets were yet to be discovered, IL-1 was thought to be a signal only secondary to antigen presentation during CD4 T cell activation (Janeway, 1989b; Lichtman et al., 1988). In agreement with this hypothesis, it was subsequently shown that T cell intrinsic MyD88 is necessary to block Treg suppression of functional CD4 T cell immunity (Schenten et al., 2014). Over the years, various other unique roles of IL-1 and its related cytokines have been described. More specifically, IL1β was shown to enhance Th17 differentiation *in vitro* (Chung et al., 2009). IL-1R signaling promotes pathogenicity of autoimmune Th17 cells by synergizing with IL-6 and IL-23 (Lee et al., 2012b). IL-1 also amplifies IL-6 induced STAT3 phosphorylation to skew the balance away from iTregs and towards Th17 (Basu et al., 2015). More importantly, IL-1 was required for Th17 immunity irrespective of the tissue microenvironment (Hu et al., 2011). These finding underscores IL-1 as a central signal rather than a secondary cue for Th17 mediated immunity. The role of IL-1 family of cytokines extends to other CD4 T cell lineages as well. IL-18 aids Th1 priming by upregulating T-bet expression (Blom and Poulsen, 2012). IL-18 in conjunction with IL-12 can also induce antigen independent memory CD8 and CD4 T cell effector function (Berg et al., 2003; Yang et al., 1999). The adaptive function of IL-33 was first discovered in the context of Th2 immunity (Schmitz et al., 2005). However, colonic Tregs were recently found to also express the IL-33R and depend on IL-33 stimulation for optimal function (Schiering et al., 2014). In summary, diverse yet unique functions of the IL-1 family of cytokines have been reported in all effector CD4 T cell lineages, giving rise to the following questions. First, is there a common principle that underlines the dependence of all CD4 T cell lineages on the IL-1 family of cytokines for protective immunity? Second, why did T cells adopt a MyD88 dependent pathway, which is analogous to TLR signaling, during the course of evolution of the adaptive immune system?

It is crucial to note that in the aforementioned studies, IL-1 or its related cytokines were either exogenously provided to an ongoing T cell response, which does not reveal the necessity of these cytokines or IL-1R signaling components were genetically ablated in animal models, which makes it challenging to differentiate between the function of IL-1 related cytokines during priming and reactivation. Here, we explored the role of the IL-1 family of cytokines in regulating effector function of memory T cells by specifically ablating signaling downstream of the IL-1 family of receptors during reactivation of already primed effector or memory T cells. This methodology enables exclusion of the previously confounding factors.

Our data demonstrate that T cell intrinsic signaling downstream of IL-1 family of receptors, all of which are MyD88 dependent, is required for "licensing" cytokine production by effector and effector memory CD4 T cells. More importantly, this function is conserved across all effector CD4 T cell lineages. Furthermore, IL-1R signaling in T cells provides post-transcriptional stability to otherwise unstable T cell cytokine transcripts, analogous to TLR mediated stabilization of innate cytokine transcripts in myeloid cells. Together, our data show that T cell intrinsic MyD88 signaling is a critical rather than an accessory signal for productive T cell effector function and provide a previously unrecognized evolutionary basis for adaptation of MyD88 signaling pathway in T cells.

Results

Dendritic cells provide key signals necessary for optimal effector CD4 T cell function

It has long been understood that engagement of TCR and co-stimulatory molecules on antigen-experienced CD4 T cells (effector, effector memory and tissue resident memory cells) is sufficient

to drive rapid production of effector cytokines (Borowski et al., 2007; London et al., 2000). This is based on the conceptual framework that once primed, CD4 T cells need not depend on additional innate immune signals as this would impose a degree of redundancy and might delay the effector response. We formally tested this concept by either strictly providing TCR and co-stimulatory signals (using plate-bound antibodies) to circulating effector memory CD4 T cells ($CD44^{hi}CD62L^{lo}$, here on referred to as memory CD4 T cells) or by activating these cells in the presence of live DCs. Consistent with the existing paradigm, we found that engagement of the T cell receptor and the co-stimulatory molecule CD28 was sufficient for memory CD4 T cells to produce canonical Th1 (IFNγ), Th2 (IL-13) and Th17 (IL-17A) cytokines (Figure 3-1a). However, when compared to the quantities of effector cytokines produced by memory CD4 T cells co-cultured with live DCs, the effector cytokines produced by plate-bound antibodies were significantly lower (Figure 3-1a). This is remarkable since much higher concentrations of antibodies were used for plate bound stimulation compared to soluble αCD3 used to mediate DC-T cell interactions. We further asked if B cells, naïve or previously activated by TLR ligands, would provide adequate signals for optimal functioning of these memory CD4 T cells. Surprisingly, we found that neither naïve nor activated B cells could induce optimal cytokine production comparable to those induced by live DCs (Figure 3-1b). Collectively, this set of experiments demonstrates that TCR and co-stimulatory signals are not sufficient to trigger effector cytokine production by Th1, Th2 and Th17 cells and that additional signals, potentially resulting from a complex cross-talk between memory CD4 T cells and live DCs, could be critical for T cell effector function.

Effector CD4 T cells constitutively express IL-1R

T cell receptor activation and co-stimulatory signaling activate and induce clonal expansion of CD4 T cells. Naïve CD4 T cells, in addition, depend on cytokine signals for differentiation into protective subsets. The role of innate cytokines in CD4 T cell responses can be postulated in part by analyzing the expression of their receptors on memory CD4 T cells. The receptors for priming cytokines (especially IL-6 and IL-4) were expressed constitutively on naïve CD4 T cells but were downregulated on effector memory CD4 T cells (Figure 3-2a, 3-2b). IL-12R was not constitutively expressed on naïve CD4 T cells but was induced following T cell receptor stimulation and then rapidly downregulated (Figure 3-2d). However, when we examined the expression of receptors for the IL-1 family of cytokines, IL-1R, IL-18R and IL-33R, we found that IL-1R and IL-18R were constitutively expressed on effector memory CD4 T cells in the secondary lymphoid organs (Figure 3-2c). Interestingly, circulating effector memory CD4 T cells did not show significant expression of ST2, the receptor for IL-33 (Figure 3-2c). It is well known that *in vivo* generated Th2 cells can respond to IL-33 (Peine et al., 2016) and perhaps IL-33R could be regulated by different mechanisms than those that regulate IL-1 and IL-18 receptors. In agreement with IL-1R staining, transcriptional expression of IL-1R was also higher on effector memory CD4 T cells relative to their naïve counterparts (Figure 3-2e). Collectively, these data led us to consider the possibility that signals from the IL-1 family of cytokines, particularly IL-1, could be critical for regulating effector function of previously differentiated CD4 T cells.

T cell intrinsic IL-1R signaling is critical for effector function of polyclonal as well antigen specific effector CD4 T cells

To test the importance of constitutive expression of IL-1R on memory CD4 T cells, we specifically blocked IL-1R signaling only during the reactivation phase of WT memory CD4 T cells using IL-1R antagonist (IL-1Ra), a potent biological competitive inhibitor of both IL-1α and IL-1β. This approach ensures optimal *in vivo* T cell differentiation under IL-1 sufficient conditions and uncouples effector function from priming, which is not possible when using genetic deletion of the receptor or downstream signaling molecules. Abrogation of IL-1R signaling during memory T cell reactivation led to greatly diminished production of IL-17A, IL-17F and IL-22 (Figure 3-3a). We then decided to test if the requirement of IL-1R signaling for effector function also extends to other CD4 T cell lineages. Indeed, we found significant deficit in IL-13, IL-4 and IL-5 as well as IFNγ production when we abrogated IL-1R signaling (Figure 3-3b, 3-3c). The role of DC intrinsic IL-1R signaling was excluded since we observed no defect in effector cytokine production when CD4 T cells were reactivated with IL-1R deficient DCs (Figure 3-3d). IL-18 has been implicated in driving IFNγ production by CD8 T cells(Okamura et al., 1995; Okamura et al., 1998); however, blocking IL-18R signaling did not affect IFNγ production by memory CD4 T cells (Figure 3-3e). Likewise, IL-33 neutralization did not significantly affect IL-13 production by memory CD4 T cells (Figure 3-3f). Requirement of IL-1R signaling appears to be specific for effector cytokines since IL-2 production was normal (Figure 3-4a). Acute blockade of IL-1R signaling during the reactivation phase did not affect CD25, CD69, ICOS and CD44 expression, suggesting that effector cytokine production was compromised despite normal T cell activation (Figure 3-4b).

We, and others have previously shown that priming environments can have significant impact on the quality of CD4 T cells that are generated (Hu et al., 2011; Pepper et al., 2010). Moreover, the nature of the immune challenge can dictate innate cytokine requirements for CD4 T cell function (Havenar-Daughton et al., 2006). Naturally arising memory CD4 T cells used so far in this study are most likely specific to commensals present at steady state (Ivanov et al., 2009). Requirements for the generation and function of acutely activated T cells could be different than the ones primed under homeostasis (Hirota et al., 2013; McGeachy et al., 2009). Therefore, we decided to test if antigen-specific effector CD4 T cells generated following various routes of immune challenge also depend on IL-1R signaling for cytokine production, during secondary challenge. To this end, we transferred OT-II T cells into a WT host and immunized the recipient mice with ovalbumin (OVA) mixed with lipopolysaccharide (LPS) and incomplete Freund's adjuvant for *in vivo* priming of the donor OT-II T cells. We observed that following immunization, there was constitutive IL-1R expression on primed and clonally expanded OT-II T cells (Figure 3-5a). Peptide-specific cytokine production by these OT-II T cells upon secondary stimulation with OVA peptide$_{323-339}$ was dependent on IL-1R signaling (Figure 3-5b). We also generated polyclonal OVA-specific CD4 T cells following subcutaneous immunization. WT CD4 T cells isolated from the draining lymph nodes of the immunized mice were reactivated by live DCs in the presence of titrating doses of OVA. IFNγ and IL-17A production was heavily compromised upon IL-1R blockade during reactivation (Figure 3-5c). In line with secreted cytokines, the frequency of IL-17A+ and IFNγ+ T cells in response to OVA re-stimulation was also significantly reduced when IL-1R signaling was abrogated (Figure 3-5d). Addition of exogenous IL-1β has been shown to promote CD4 T cell proliferation during priming (Ben-Sasson et al., 2009). However, we found no defect in the

frequency of activated CD4 T cells as well as antigen-specific proliferation in the presence of IL-1Ra (Figure 3-6).

Next, we challenged mice with *C. rodentium* using an intra-gastric route of infection. *C. rodentium* infection predominantly leads to Th17 and Th22 cell differentiation(Basu et al., 2012). *C. rodentium* specific WT Th17 cells generated following intra-gastric infection also required IL-1R signaling for effector function as measured by IL-17A and IL-22 production following reactivation with *C. rodentium* lysate (Figure 3-7a). The proportion of IL-17A+ as well as IL-22+ CD4 T cells was also significantly compromised in the presence of IL-1Ra (Figure 3-7b). No cytokine production was detected without antigen discounting the possibility of any constitutive T cell response (Figure 3-7c). Lack of IL-17A production in T cells challenged with unrelated pathogen lysate further demonstrates the specificity of the effector T cell response in this assay (Figure 3-7c). Proportion of activated CD4 T cells was not affected in the absence of IL-1R signaling. (Figure 3-7d).

Since oral and systemic infections have been shown to have different cytokine requirements for priming (Hu et al., 2011) we wanted to test the requirement for IL-1R dependent signaling in systemic infections. We infected WT mice with *L. monocytogenes* intra-peritoneally, which results in Th1-skewed responses (Hsieh et al., 1993; Pepper et al., 2010). CD4 T cells were isolated from the spleens of infected mice and re-stimulated *in vitro* with *L. monocytogenes* lysate for antigen-specific CD4 T cell reactivation. Interestingly, IFNγ production by *L. monocytogenes*-specific CD4 T cells was not affected by the absence of IL-1R signaling (Figure 3-8a). Since there is evidence for IL-18R signaling regulating production of IFNγ by both memory CD8 T cells and

NK cells (Berg et al., 2003; Hu et al., 2011), we tested whether *L. monocytogenes*-specific IFNγ production by effector CD4 T cells was dependent on IL-18R signaling. We found that ablation of IL-18R signaling significantly compromised IFNγ production (Figure 3-8a). Notably, the mean fluorescent intensity (MFI) of IFNγ was also reduced upon blocking IL-18R, suggesting the necessity of IL-18R signaling at the level of every cell (Figure 3-8b). The activation of CD4 T cells upon pathogen-specific reactivation was normal in the absence of IL-18R signaling as determined by the frequency of activated CD4 T cells (Figure 3-8c). Collectively, these data demonstrate a critical role for IL-1R and IL-18R signaling in regulating antigen-specific effector function of CD4 T cells. In addition, these data also highlight how the nature of the pathogen and route of infection can dictate dependence of Th1 lineage cells on IL-1 or IL-18 signals.

Dependence on IL-1R signaling for cytokine production extends to tissue resident memory CD4 T cells

CD4 memory T cells can exist as circulating effector memory T cells or tissue resident memory T cells. Effector memory T cells reside in the spleen and provide defense against systemic infections. In recent years, tissue resident memory T cells, populating the barriers tissues, have been demonstrated as the first line of defense and are found to respond rapidly to pathogens invading via tissues such as the skin and mucosal epithelia (Ariotti et al., 2014; Gebhardt et al., 2009; Jiang et al., 2012; Masopust et al., 2001; Schenkel et al., 2014). Lymphocytes isolated from the tissues express high levels of CD44 and CD69 suggesting that they maintain an activated status either by default or because they are constantly exposed to environmental insult (Shin and Iwasaki, 2013). We therefore deemed it critical that effector functions of these cells are regulated rigorously to avoid auto-immunity. It is known that the breakdown of cell extrinsic regulatory mechanisms such

as the loss of Treg function or the absence of IL-10 leads to colitis, but little is known about cell intrinsic regulation of CD4 T cell effector function at the barrier sites (Kuhn et al., 1993; Paust and Cantor, 2005). We set out to determine if IL-1R controls the function of these highly critical tissue resident memory CD4 T cells. A vast heterogeneity has been described in the myeloid cell populations that line the barrier tissues and play an unique role in T cell activation (Jain and Pasare, 2017). Therefore, we activated tissue resident CD4 T cells in the presence of their respective myeloid cell populations. Consistent with circulating effector memory T cell data, lymphocytes isolated from lung, small intestinal lamina propria as well as skin require IL-1R mediated signaling for optimal IL-17A and IL-13 production upon reactivation (Figure 3-9). Overall, these data provide credence to the idea that the IL-1 family of cytokines licenses the functioning of effector T cells, irrespective of whether they reside in the secondary lymphoid organs or in the tissues.

IL-1β licenses IL-17A, IL-13 and IFNγ production by circulating effector CD4 T cells

So far, we have used IL-1Ra to block IL-1R mediated signaling. Although both IL-1α and IL-1β utilize IL-1R for downstream signal transduction, these cytokines differ in how they are made and might in fact have different biological roles in regulating adaptive immunity (Garlanda et al., 2013). IL-17A production by memory CD4 T cells activated via plate bound αCD3 and αCD28 was enhanced by addition of either IL-1α or IL-1β (Figure 3-10a). However, the minimal cytokine production by memory CD4 T cells induced by TCR and co-stimulation alone was found to be independent of IL-1R signaling (Figure 3-11a). In order to establish which one of these cytokines is necessary for IL-17A production especially when CD4 T cells are interacting with DCs we specifically neutralized IL-1α or IL-1β during CD4 T cell reactivation by live DCs. We found that IL-1β but not IL-1α was required for optimal cytokine production by Th17 cells (Figure 3-10b).

Furthermore, IL-13 and IFNγ production was also significantly compromised upon specific neutralization of IL-1β (Figure 3-10c). Consistently, *Il1 1α*$^{-/-}$ DCs but not *Il1 1β*$^{-/-}$ DCs were able to induce optimal IL-17A, IL-13 and IFNγ production by CD4 T cells (Figure 3-10d). Moreover, exogenous IL-1β restored the ability of B cells to induce IL-17 production by memory T cells to the levels induced by live DCs (Figure 3-10e). Notably, unlike cytokine production by memory CD8 T cells, which can be induced by IL-12 and IL-18 in absence of TCR ligation (Berg et al., 2003), IL-1β driven cytokine production by CD4 T cells was strictly dependent on concurrent TCR activation and co-stimulation (Figure 3-11b). In fact, blocking CD28 interaction with co-stimulatory molecules completely abrogated the ability of memory T cells to produce cytokines suggesting that co-stimulation continues to be a hierarchically superior signal (Figure 3-11c). Collectively, our data has revealed that IL-1β acts as a "licensing cytokine" for effector function of memory CD4 T cells of all lineages thus providing an additional layer of regulation of adaptive immune responses.

IL-1R deficient T cell exhibit normal steady state differentiation but compromised effector function

The majority of studies examining the role of IL-1R in CD4 T cell immunity use cytokine production as a measure of T cell response, making it challenging to ascertain the requirement of IL-1R signaling for lineage commitment versus effector function(Chung et al., 2009; Hu et al., 2011; Schenten et al., 2014; Shaw et al., 2012). Our results showing the requirement of IL-1 for optimal cytokine production by successfully committed CD4 T cells dictated us to examine the status of Th1, Th2 and Th17 lineage cells in the spleen and the lamina propria of cohoused WT and *Il1r*$^{-/-}$ mice. Consistent with previous reports (Atarashi et al., 2008; Ivanov et al., 2008;

Mufazalov et al., 2016; Naik et al., 2012), we found no defect in the proportion of CD4 T cells committed to the Th17 lineage in the absence of IL-1R (Figure 3-12a). This suggests that even though IL-1 is a critical player in CD4 T cell activation and Th17 differentiation following immunizations and infections(Anderson, 2008; Chung et al., 2009; Hu et al., 2011; Schenten et al., 2014), other cues (IL-6, IL-23, etc.) might be able to drive Th17 lineage commitment of commensal-specific CD4 T cells in the absence of IL-1R signaling. We also found no defects in the proportion of cells that were committed to Th1 and Th2 lineage in the absence of IL-1R (Figure 3-12a). We took advantage of this IL-1R independent *in vivo* commitment of naturally arising effector CD4 T cells to ask if TCR-mediated stimulation is sufficient to drive cytokine production. We discovered that CD4 T cells that lacked IL-1R had impaired cytokine production when stimulated via their TCR (Figure 3-12b). This was not a result of impaired secretion since acute stimulation of the same CD4 T cells with phorbol 12-myristate 13-acetate (PMA) and Ionomycin could bypass the requirement of IL-1R signaling for IL-17A, IL-13 and IFNγ production (Figure 3-12c). These data establish that even though CD4 T cells can undergo effector lineage commitment *in vivo* in the absence of IL-1R signaling, they are unable to secrete effector cytokines without a functional IL-1R. PMA+Ionomycin stimulation has long been used to reveal lineage commitment as well as effector function of CD4 T cells, as it provides necessary signals that can drive transcription of accessible loci (Chatila et al., 1989). In addition, PMA+Ionomycin also induces activation of the p38 MAP kinase (DeSilva et al., 1997), thus overriding the physiological dependence on IL-1 stimulus to drive cytokine production from already committed *Il1r*$^{-/-}$ CD4 T cells. We demonstrate that lineage commitment does not ensure optimal cytokine production upon TCR activation since IL-1 remains a critical innate cue for effector function despite successful differentiation. These data provide critical *in vivo* genetic evidence for the physiological

importance of IL-1R signaling in regulating effector function of pre-committed memory CD4 T cells.

Th17 effector function is compromised in mice and humans in the absence of IL-1β mediated signaling

IL-1 has been linked to several auto-inflammatory disorders primarily because of its role in promoting Th17 responses. Naturally arising Th17 cells that are likely to cause auto-inflammation are primed against commensal microbiota and predominantly reside in the small intestine (Esplugues et al., 2011; Harkiolaki et al., 2009). In contrast to CD8 T cells, the tools and experimental techniques for measuring direct effector functions of memory CD4 T cells *in vivo* are limited (Jenkins and Moon, 2012; Merica et al., 2000; Pagan et al., 2013). It has been previously reported that simply ligating the TCR through injection of αCD3 antibody leads to robust production of IL-17 by T cells in the small intestine (Esplugues et al., 2011). Therefore, we adopted this approach to test if *in vivo* IL-17A production by pre-committed Th17 cells in the lamina propria is dependent on IL-1β. In order to investigate *in situ* Th17 effector function, CD4 T cells isolated from the lamina propria of the small intestine, following αCD3 administration, were cultured in the presence of brefeldin A without any further stimulation. In agreement with previous studies, we observed robust enhancement of IL-17A production by T cells upon αCD3 treatment (Figure 3-13a). However, neutralization of IL-1β considerably compromised the *in vivo* IL-17A production by pre-committed CD4 T cells *in vivo* (Figure 3-13a). Interestingly, *in vivo* IL-17A production was compromised at the cellular level consistent with the role of IL-1R signaling in stabilizing IL-17A transcripts (Figure 3-13a). Next, we extended our studies to circulating human Th17 cells. The hIL-1R antagonist, Anakinra, is a widely used drug for auto-immune

ailments(Jesus and Goldbach-Mansky, 2014). Our mouse T cell data points to the possibility that Anakinra might be acting by abrogating Th17 effector function rather than the pathogenic effects of IL-1α or IL-1β. Indeed, we found that activation of circulating memory CD4 T cells with autologous monocyte derived DCs in the presence of hIL-1R antagonist resulted in significantly diminished hIL-17A production (Figure 3-13b and 3-13c) suggesting a conserved role for IL-1R signaling in regulating effector function of Th17 lineage cells in both mice and humans.

IL-1R signaling stabilizes Th17 and Th2 cytokine transcripts

We sought to determine the molecular mechanism by which IL-1R mediated signaling licenses effector functions of CD4 T cells. We found significantly reduced expression of T cell cytokine transcripts when IL-1R signaling was abrogated during reactivation of CD4 T cells (Figure 3-14a). The IL-1R signaling pathway is analogous to TLR signaling as they both rely on homotypic interactions between TIR domain-containing proteins for signal transduction that culminate in activation of NF-κB and MAPK cascade (Kuno and Matsushima, 1994). TLR signaling is known to impose post-transcriptional control on innate cytokine transcripts such as *Tnfa*, *Ccl10* and *Il6* via the p38 MAPK pathway (Anderson, 2008). We hypothesized that MyD88 dependent signaling (downstream of IL-1 and IL-18 receptors) in CD4 T cells might regulate the post-transcriptional stability of effector cytokines, since pre-committed CD4 T cells are likely to induce transcription of effector cytokines soon after TCR activation and co-stimulation. Consistent with the idea that IL-1R signaling could be influencing the stability of cytokine transcripts, we found that IL-1β-mediated enhancement of cytokine transcripts was p38-dependent, as transcriptional expression of Th17 cytokines and *Il13* were reduced in the presence of a p38 MAP kinase inhibitor (Figure 3-14b).

We further examined the sequence of signature effector cytokine mRNAs and found that those of *Il17a* and *Il13* contain conserved AU-rich motifs in their 3' un-translated regions (Figure 3-15). The presence of these motifs implies their susceptibility to RNA binding proteins (Chen and Shyu, 1995) which can destabilize these inflammatory transcripts, as also reported by other groups (Casolaro et al., 2008; Chen et al., 2013; Hardle et al., 2015; Lee et al., 2012a). However, innate cues that can stabilize cytokine transcripts are not well characterized. This prompts the hypothesis that post-transcriptional stabilization is a function likely to be conserved between TLR and IL-1R signaling. Consistent with our hypothesis, abrogation of IL-1R signaling during reactivation led to rapid decay of *Il17a*, *Il17f*, *Il22*, *Il13* and *Il5* transcripts (Figure 3-16a). Conversely, presence of recombinant IL-1β during CD4 T cell reactivation (via TCR and co-stimulation) rescued destabilized cytokine transcripts (Figure 3-16b). We did not observe enhanced transcriptional decay of *Ifng* in the absence of IL-1R signaling (Figure 3-16a). This is in agreement with earlier reports suggesting a more complex epigenetically regulated mechanism of Ifng production (Schoenborn et al., 2007; Schoenborn and Wilson, 2007; Villarino et al., 2011). Since other cell types such as macrophages, NK cells and CD8 T cells also produce IFNγ to exert their effector function, it is possible that the regulatory mechanisms of IFNγ production evolved distinctly from other T cell effector cytokines such as IL-17A and IL-13. These data establish that IL-1R signaling in T cells licenses cytokine production by providing post-transcriptional stability to effector cytokine transcripts. It is, however, possible that other additional mechanisms, such as IL-1-dependent *de novo* transcription and post-translational regulation, exist that need further investigation.

Discussion

IL-1β and IL-18 are "licensing cytokines" for CD4 T cell effector function

T cells are exposed to a diverse array of innate cytokines throughout their lives that determine the quality of their response (Jain and Pasare, 2017; Zhu et al., 2010). Our studies reveal that two distinct sets of innate cytokines control priming versus reactivation of CD4 T cells. It is well established that IL-12, IL-4 and IL-6 drive the differentiation program for Th1, Th2 and Th17 cells priming, respectively (Zhu et al., 2010). Here we show that IL-1 and related cytokines play a critical role during the reactivation of previously primed effector and memory CD4 T cells. Taken together it is tantalizing to postulate that, while the JAK-STAT signaling mediated pathway is primarily engaged during priming, MyD88 dependent signaling is required for effector function during the reactivation phase. It is important to note here that activation of MyD88 dependent signaling in CD4 T cells does not bypass the requirement of TCR engagement or co-stimulation, but provides an additional layer of regulation for CD4 T cell effector function. Furthermore, the presence of a pattern recognition receptor ligands during CD4 T cell reactivation, which would lead to production of a variety of pro-inflammatory cytokines by DCs, does not eliminate the requirement of IL-1R signaling for optimal functioning of CD4 T cell effector lineages. When OVA specific or pathogen specific CD4 T cells are reactivated by DCs in the presence of endotoxin free OVA or specific pathogen lysate, respectively, the central requirement for IL-1R signaling in CD4 T cells does not change. This suggests that IL-1R signaling controls cytokine production by CD4 T cells not only under sterile conditions, but also during pathogenic insults. It will be interesting to test if the dependence on the IL-1 family of cytokines for effector cytokine production is critical to keep autoimmune cells under check and/or to curtail T cell mediated immunopathology during pathogen specific responses.

Our data also suggest that DC-T cell cognate interaction results in biologically relevant quantities of active IL-1β production even during sterile re-activation where OVA specific T cells are stimulated by DCs presenting OVA derived peptides. This is reconcilable with the published work showing that memory CD4 T cell reactivation can occur independently of TLR stimulation (Pasare and Medzhitov, 2005). It will be valuable to determine the underlying mechanisms that lead to IL-1β production in the absence of any apparent TLR or inflammasome ligands. It is possible that the affinity or avidity of the interaction between T cells and DCs dictates the production of IL-1β, although this hypothesis needs to be rigorously tested. It is conceivable that in patients with autoimmunity, high affinity auto-reactive T cells (Zehn and Bevan, 2006) might interact with DCs in tissues or secondary lymphoid organs to extract the signals necessary for inflammatory cytokine production. The fact that Anakinra, an IL-1R antagonist is an effective biological drug used to treat various autoimmune conditions (Hoffman, 2009) certainly supports this line of thought. Identification of the molecular players involved in IL-1β production resulting from DC-T cell interactions might uncover novel targets to treat autoimmunity as well as autoinflammatory diseases.

MyD88 is a central node that drives effector function of antigen experienced CD4 T cells

IL-1 family of cytokines has an established role in synergizing with priming cytokines to promote clonal expansion and differentiation (Basu et al., 2015; Chang et al., 2013; Schmitz et al., 2005; Sims and Smith, 2010; Yang et al., 1999; Zhu et al., 2010). Our data demonstrate that in addition to its role in priming, MyD88 dependent signaling in T cells relays a critical signal for effector function of already committed CD4 T cells. Our findings have revealed a previously unknown function of IL-1β as a licensing molecule for the production of Th2 and Th17 cytokines.

Furthermore, our data demonstrate that IL-1 and IL-18 differentially control IFNγ production by CD4 T cells based on the nature of immune challenge as well as the anatomical location of primed T cells. This is in agreement with the vast amount of recent literature showing that the priming microenvironment determines the necessity for innate cues by CD4 T cells (Hu and Pasare, 2013; Hu et al., 2011; Naik et al., 2012; Pepper et al., 2010).

The expression of IL-1R in Th2 cells was reported three decades ago (Lichtman et al., 1988) followed by several reports suggesting the role of IL-1 in promoting Th2 responses. IL-1α and IL-1β deficient mice exhibited worse protection against nematode infection (Helmby and Grencis, 2004). IL-1R antagonist deficient mice displayed exacerbated Th2 allergic responses (Satoskar et al., 1998). However, these studies did not reveal the exact function of IL-1R signaling in Th2 cells. Our data confer clarity to these previous observations and demonstrate a requirement for IL-1R signaling for Th2 signature cytokine production by pre-committed Th2 cells. Our findings also provide a mechanistic basis for the use of IL-1 blocking agents for Th2 mediated immunopathology. Whether all Th2 responses depend on IL-1R signaling or there are differences based on the nature of the priming stimuli (allergen versus parasite) remains to be examined.

IL-1α and IL-33 can be produced by non-myeloid cells as a consequence of tissue damage (Lukens et al., 2012). While it is possible that these cytokines, coming from stromal cells, contribute to the effector function of CD4 T cells reactivated in the tissues, we do not find them to be critical during reactivation of CD4 T cells by dendritic cells. Nevertheless, the requirement of IL-1 family of cytokines for effector function seems to be a conserved mechanism adopted across effector CD4 T cell lineages. MyD88 dependent signaling via the ST2 receptor has been shown to promote

function of previously differentiated Tregs (Schiering et al., 2014), which further strengthens our conclusion.

Post-transcriptional regulation of T cell cytokine transcripts by IL-1R signaling

One of the hallmarks of memory T cell reactivation is the rapid kinetics of response. Since effector T cells have already undergone differentiation, engagement of TCR during reactivation triggers transcription of poised loci, which results in respective cytokine production (Hermann-Kleiter and Baier, 2010). We have identified conserved AU rich motifs in the 3'UTR of Il17a, Il22, Il17f and Il13 that are likely to render these transcripts highly unstable (Chen and Shyu, 1995). Post-transcriptional stabilization by IL-1R signaling perhaps guarantees rapid kinetics of cytokine response while maintaining inflammatory cytokine transcripts under strict regulation. Such a phenomenon has been previously reported for TLR dependent cytokines (Anderson, 2008). In addition to providing critical signals for productive effector function, dependence on IL-1R signaling might be particularly useful to rapidly turn on and off the effector cytokine production especially since these cytokines can have devastating effects on host tissues (Khabar, 2007). Post-transcriptional stabilization of inflammatory cytokine transcripts is perhaps a conserved function across cell types and receptor families that use MyD88 for signaling. Given the role of IL-1 family of cytokines in activating various innate lymphoid cells (ILCs), it will be interesting to see if ILCs also employ a similar mechanism for regulating their effector cytokine production in response to IL-1β and IL-18 from myeloid cells (Garlanda et al., 2013).

In summary, our data provide compelling evidence for a broadly applicable "licensing function" of IL-1R signaling in enabling optimal effector function of pre-committed CD4 T cells. Even

though Th1, Th2 and Th17 lineages exhibit differential requirements for priming cytokines, we demonstrate that all three lineages depend on the IL-1 family of cytokines to mount effector function, illustrating convergent evolution across all T cell lineages. It is remarkable that the adaptive immune system, despite having sophisticated features to define specificity, co-opted this MyD88 dependent signaling pathway, thus highlighting the importance of evolutionarily conserved ancient pathways for host defense. In addition, this study sheds light on the complex cross talk between innate and adaptive immune compartments that extends beyond priming and differentiation and continues in a long-lasting manner to the reactivation phase of CD4 T immunity.

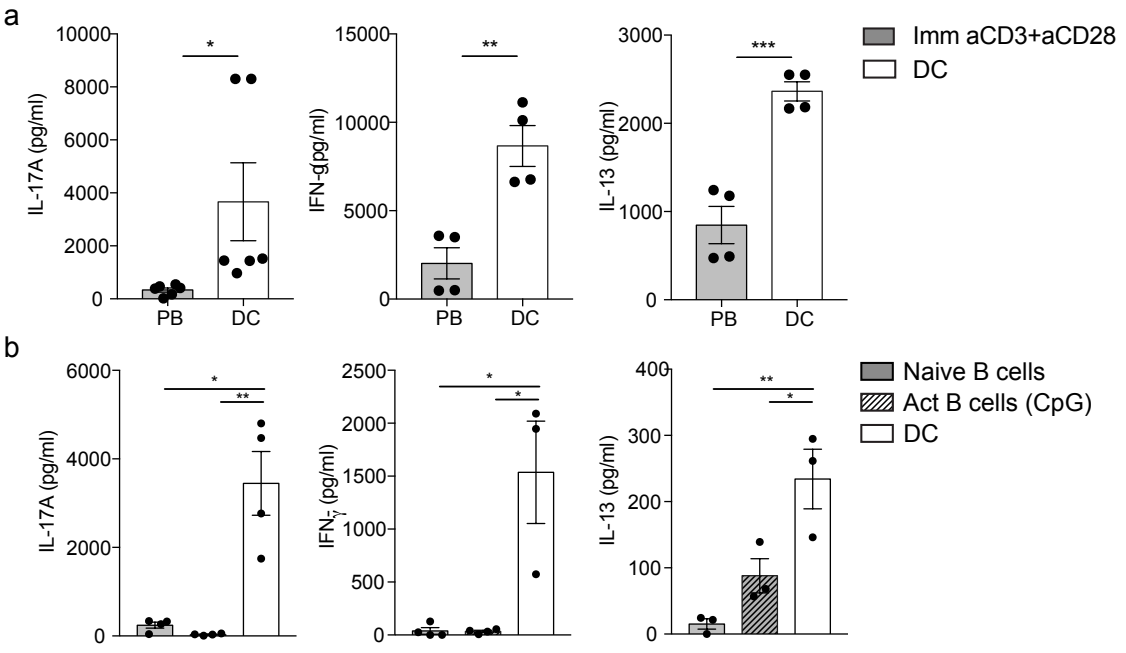

Figure 3-1. Dendritic cells provide key signals necessary for optimal functioning of effector CD4 T cells

(a) $CD44^{hi}$ $CD62L^{lo}$ CD4 T cells (T_{em}) were stimulated with soluble αCD3 (30 ng/ml) in the presence of either live splenic CD11C+ DCs or αCD3 (0.5 μg/ml) and αCD28 (0.5 μg/ml) antibodies immobilized on a plate (b) $CD44^{hi}$ $CD62L^{lo}$ CD4 T cells (T_{em}) were stimulated with soluble αCD3 (30 ng/ml) in the presence of either live DCs, naïve and activated (CpG-stimulated) CD19+ B cells. Cytokine levels in the culture supernatants were determined after 48 hr of activation using paired-antibody ELISAs. Error bars indicate SEM; paired *t* test, n.d. = not detected.

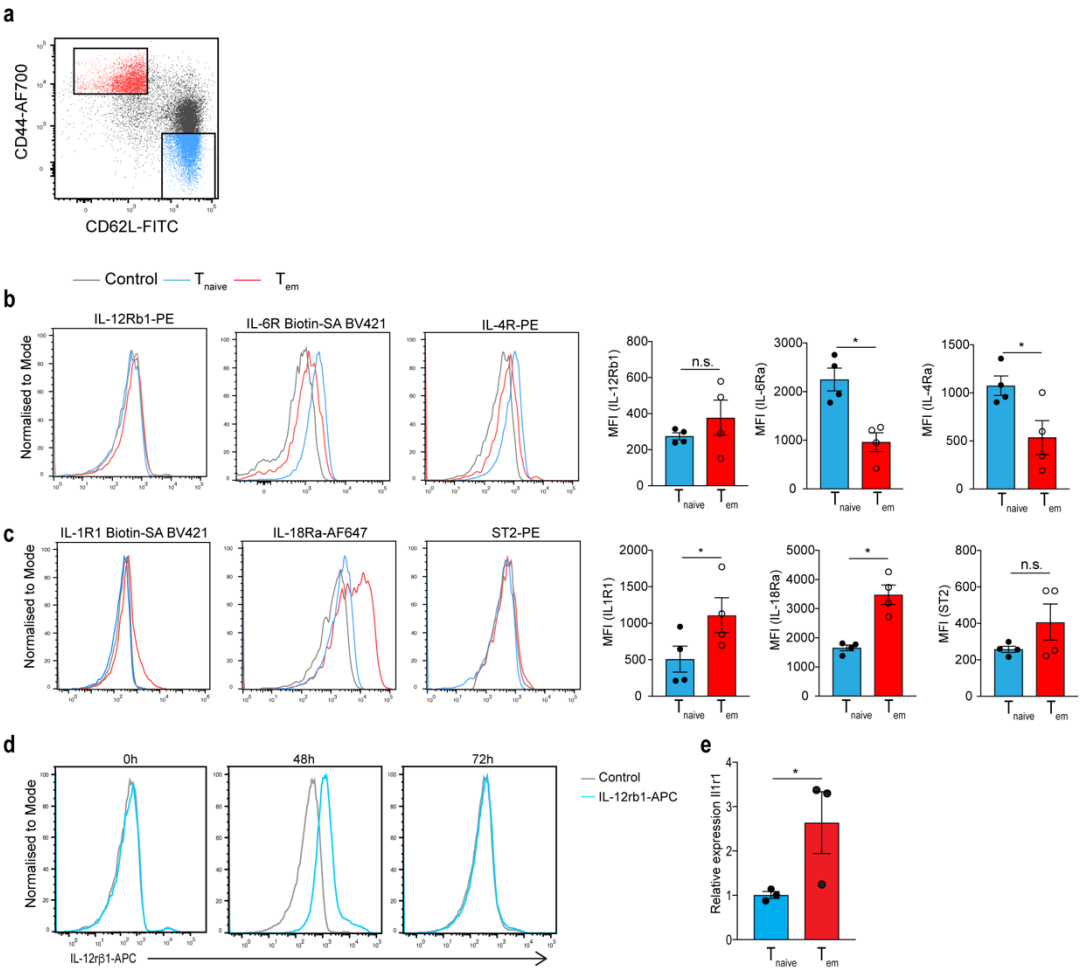

Figure 3-2. Effector CD4 T Cells constitutively express IL-1R

(a) Splenic CD4 T cells were gated on naïve (T_{naive}) and memory (T_{em}) (b) Cells from (a) were stained for (b) IL-6Ra, IL-4Ra and IL-12Rb1 (c) IL-1R1, IL-18Rα and IL-33R (ST2). (d) Naïve CD4 T cells were stimulated with immobilized αCD3 (5µg/ml) and αCD28 (5µg/ml). Cells were stained for IL-12R at given time points following stimulation. (e) Relative expression of *Il1r1* transcripts in T_{naive} and T_{em} cells Data is normalized to *18s*. (a-d) Gated on Live CD4+ cells. Data are representative of 2 independent experiments.

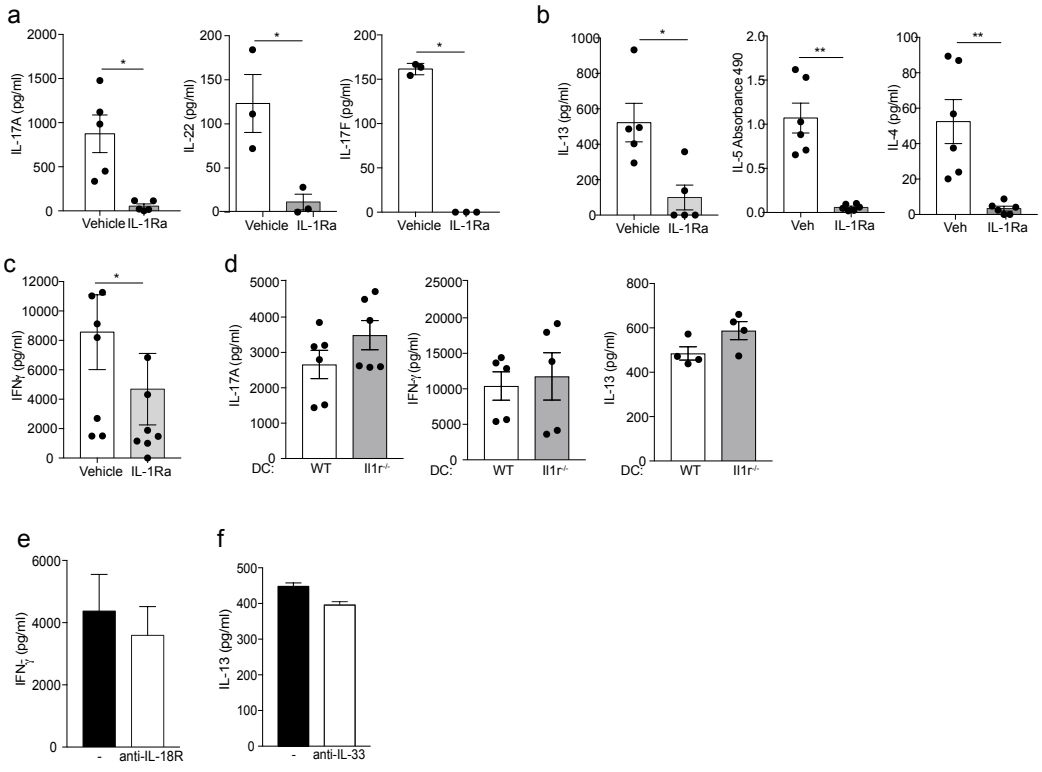

Figure 3-3. T cell intrinsic IL-1R signaling is necessary for their effector cytokine production

(a-c) T_{em} cells were stimulated with splenic DCs and soluble αCD3 (30 ng/ml) in the presence or absence of IL-1R antagonist (IL-1Ra) for 48 hrs (a) IL-17A, IL-17F, IL-22, (b) IL-13, IL-4, IL-5 and (c) IFNγ were measured. (d) IL-17A, IL-13 and IFNγ levels in supernatants obtained from T_{em} cell co-cultures with WT or *Il1r*$^{-/-}$ CD11c+ splenic DCs in the presence of αCD3 (30 ng/ml) after 48 hrs of stimulation. (e and f) T_{em} were stimulated with WT CD11C+ splenic DCs and αCD3 (30 ng/ml) in the presence or absence of (e) αIL-18Ra (0.5 μg/ml) and (f) αIL-33 (100 ng/ml). Secreted cytokines levels were analyzed 48hrs after stimulation. Error bars indicate SEM; paired

t test, (a-c) 4-8 mice were pooled per experiment. (e-f) Data are representative of two independent experiments.

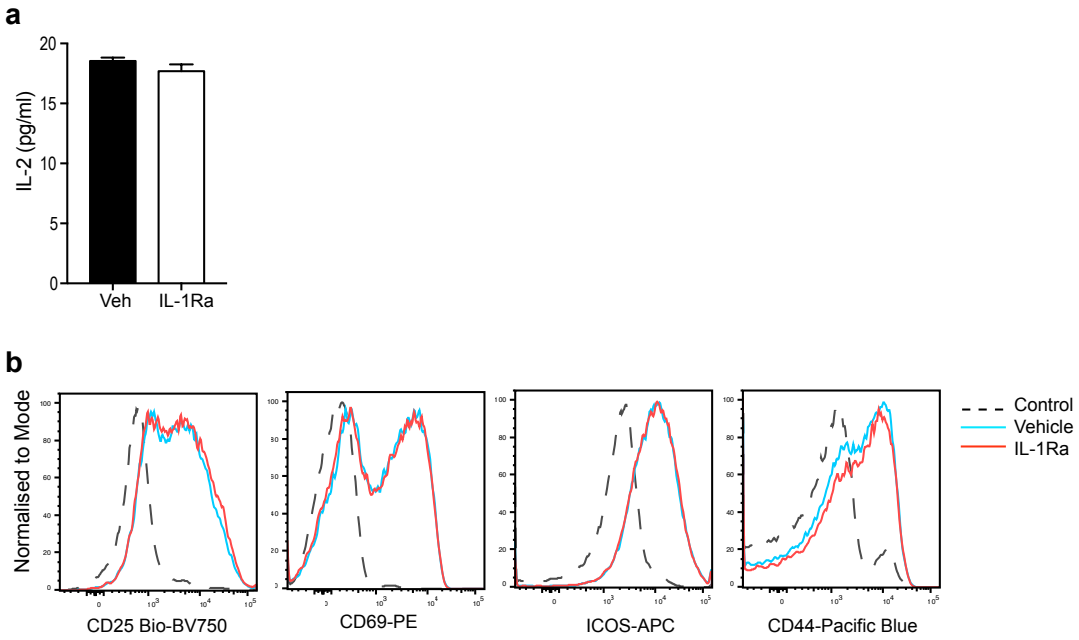

Figure 3-4. Activation of status of CD4 T cell upon in vitro re-stimulation in the absence of IL-1R signaling

(a) T_{em} were stimulated with WT CD11C+ splenic DCs and αCD3 (30 ng/ml) in the presence or absence of IL-1Ra for 48hrs (b) T_{em} cells were stimulated with CD11C+ splenic DCs and αCD3 (30 ng/ml) in the presence or absence of IL-1Ra for 36hrs followed by staining of respective T cell activation markers. Cells are gated on Live CD90.2+CD4+ events. Error bars indicate SEM; Data are representative of 3 independent experiments.

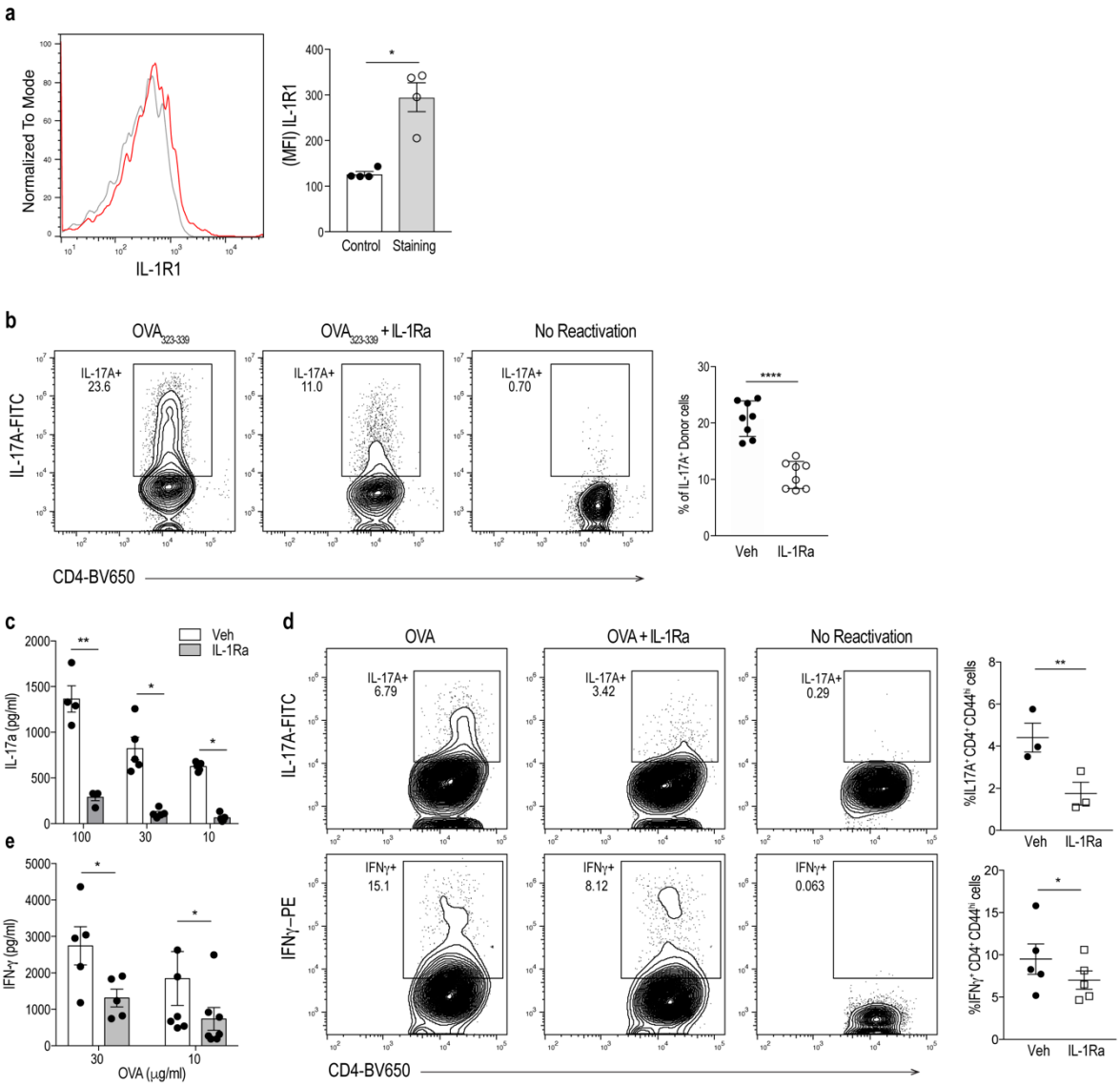

Figure 3-5. IL-1R signaling is necessary for effector cytokine production of newly primed CD4 T cells in the draining lymph nodes following subcutaneous immunization

(a and b) 5x10^5 naïve CD45.2 OT-II T cells were transferred into CD45.1 WT mice intravenously. The next day, recipient mice were immunized with OVA+LPS emulsified in IFA. (a) 7 days post-immunization, IL-1R expression on CD45.2 donor T cells from draining lymph nodes was

examined. “Control” refers to fluorescence minus one control. (b) OT-II T cells from draining lymph nodes were reactivated with $OVA_{323-339}$ for 12 hrs. Brefeldin A was added in the last 6 hrs of stimulation. Intracellular staining for IL-17A was performed following fixation and permeabilization of stimulated cells. Data is gated on live, CD4+, CD45.2+ T cells. (c) Total cells from draining LN of mice immunized with LPS+OVA+IFA were cultured with OVA (100μg ml^{-1}) for 18 hrs and brefeldin A was added during last 6 hrs of culture. Intracellular staining for IL-17A and IFNγ was performed following fixation and permeabilization of stimulated cells. Live $CD4+CD44^{hi}$ cells were gated and analyzed for intracellular expression of IL-17A or IFNγ. Unstimulated control cells serve as baselines for constitutive cytokine production by CD4 T cells. (d) CD4+ T cells from draining lymph nodes of mice immunized with LPS+OVA+IFA were reactivated using WT splenic DCs as antigen presenting cells in the presence of titrating doses of OVA for 48 hrs. IL-17A (top) and IFNγ (bottom) production was measured using paired antibody ELISA. Error bars indicate SEM; paired *t* test.

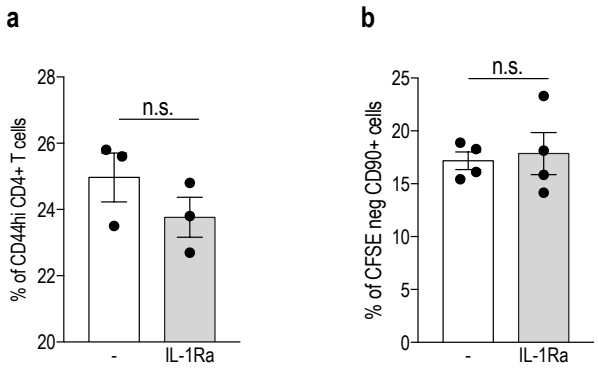

Figure 3-6. Absence IL-1R signaling does not affect antigen specific activation and proliferation

(a) Frequency of CD44hi CD4+ T cells upon OVA specific reactivation of CD4 T cells in the presence or absence of IL-1Ra. (b) Proportion of proliferating cells from during OVA specific reactivation of CD4 T cells isolated from OVA immunized mice.

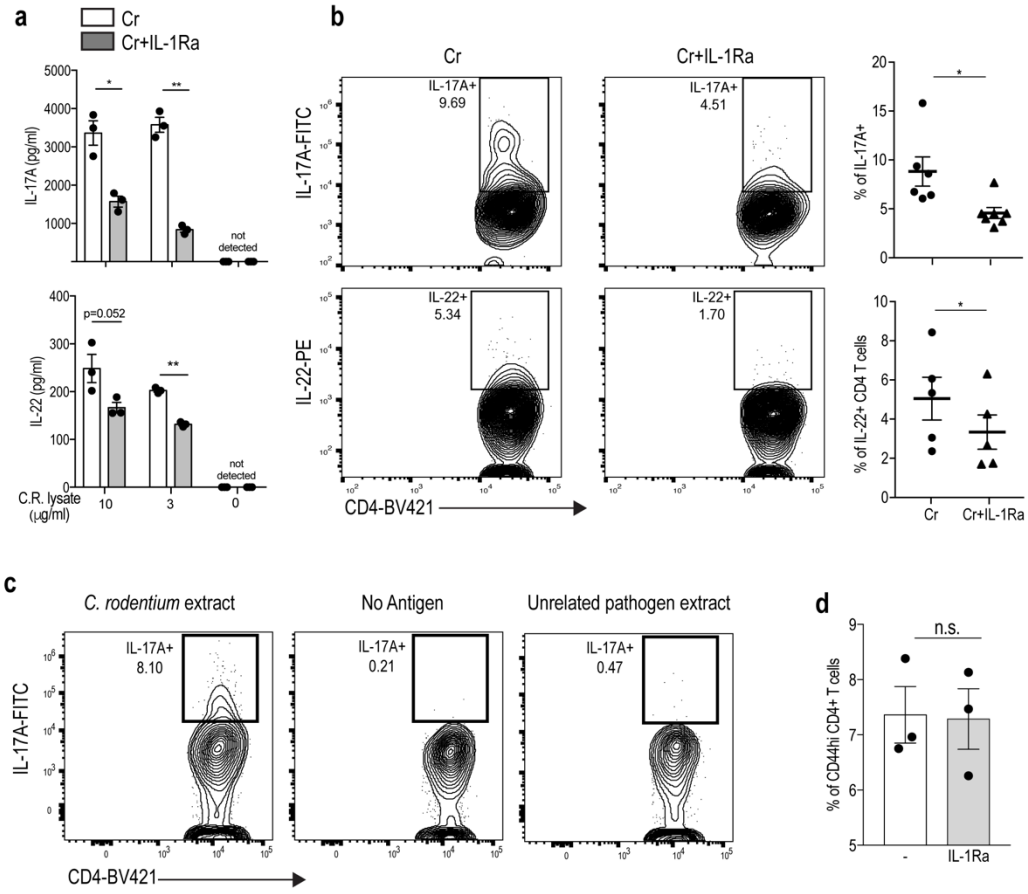

Figure 3-7. Antigen specific effector function of CD4 T Cells primed following oral infection is dependent on IL-1R

(a) CD4+ T cells from mesenteric lymph nodes of *C. rodentium* infected mice were re-stimulated using WT splenic DCs as antigen presenting cells in the presence of titrating concentrations of *C. rodentium* lysate for 48 hrs. IL-17A and IL-22 production was measured using paired antibody ELISA. (b) Intracellular staining for IL-17A (top) and IL-22 (bottom) following pathogen-specific re-stimulation in the presence of brefeldin A. Live CD4+ CD44hi cells were gated to examine intracellular expression of IL-17A and IL-22. (c) Frequency of IL-17A producing cells from

mesenteric lymph nodes of *C. Rodentium* infected WT mice in the presence of given stimulation. Unrelated pathogen is *L. monocytogenes*. (d) Frequency of CD44hi CD4+ T cells upon *C. rodentium* specific reactivation of CD4 T cells in the presence or absence of IL-1Ra. Unstimulated control cells serve as baselines for constitutive cytokine production by CD4 T cells. Error bars indicate SEM; unpaired *t* test.

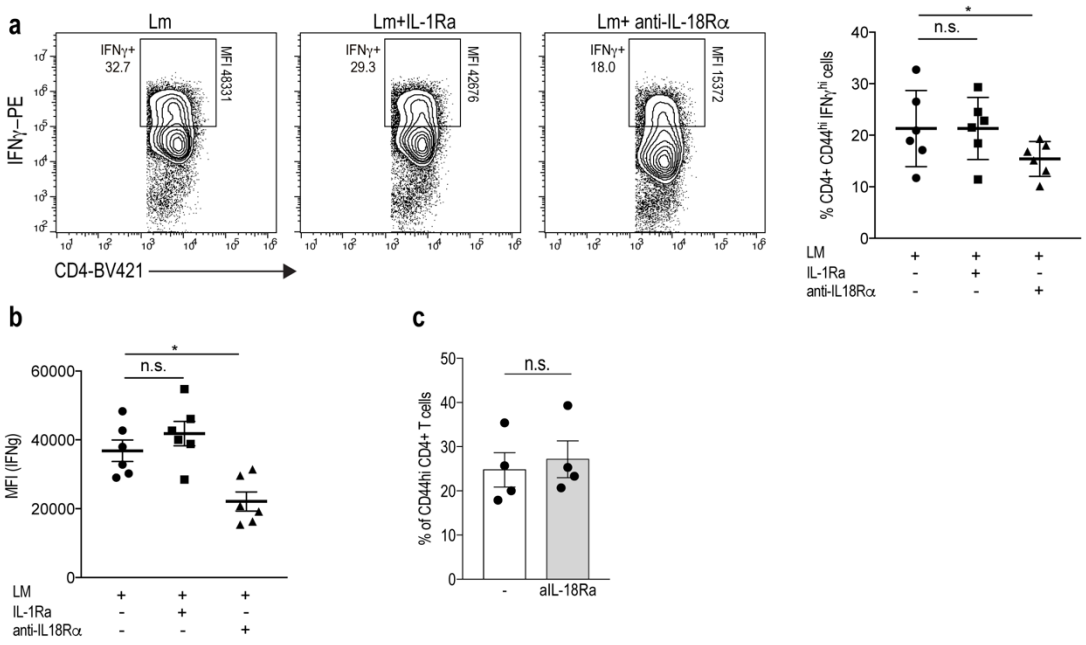

Figure 3-8. Antigen specific effector function of CD4 T Cells primed following systemic infection is dependent on IL-18R signaling

(a) CD4 T cells from spleens of *L. monocytogenes* infected mice were cultured in the presence of live DCs and *L. monocytogenes* lysate. Intracellular staining for IFNγ following *L. monocytogenes* specific re-stimulation in the presence of brefeldin A. Live CD4+CD44hi cells were gated to examine intracellular expression of IFNγ under various conditions. (b) MFI quantification of IFNγ on CD4+CD44hi cells following *L. monocytogenes*-specific re-stimulation under various conditions. (c) Frequency of CD44hiCD4+ T cells upon *L. monocytogenes* specific reactivation of CD4 T cells in the presence or absence of anti-IL-18Rα. Unstimulated control cells serve as baselines for constitutive cytokine production by CD4 T cells. Error bars indicate SEM; unpaired *t* test.

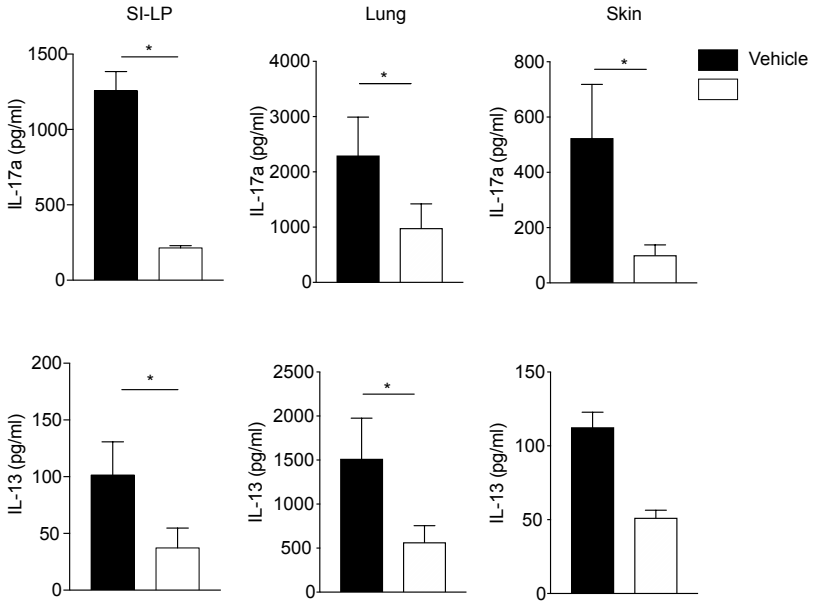

Figure 3-9. IL-1R signaling is critical for tissue resident CD4 T cell effector function

CD4 T cells from given tissues were stimulated in the presence of myeloid cells from respective tissues using αCD3 in the presence or absence of IL-1Ra. Cytokine production was analyzed 48hrs post stimulation using ELISA. Error bars indicate SEM; unpaired *t* test**.**

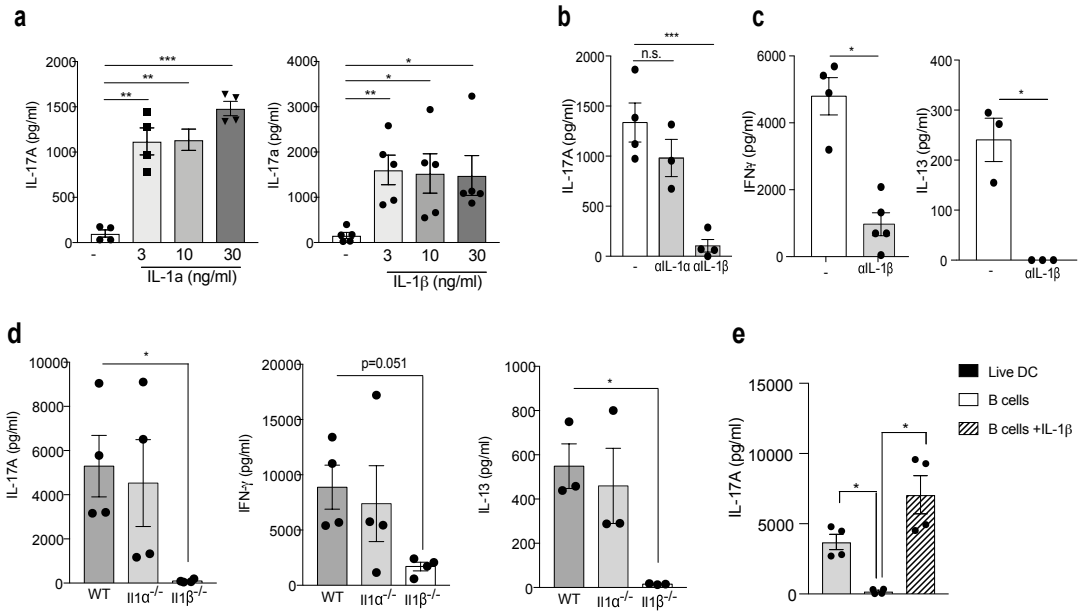

Fig 3-10. IL-1β licenses IL-17A, IFNγ and IL-13 production by circulating effector CD4 T cells

(a) $CD44^{hi}$ $CD62L^{lo}$ T_{em} cells were stimulated with immobilized αCD3 (0.5 μg/ml) and αCD28 (0.5 μg/ml) in the presence of indicated concentrations of recombinant IL-1α (left) and IL-1β (right). (b and c) T_{em} cells were co-cultured with splenic DCs and soluble αCD3 (30 ng/ml) in the presence or absence of neutralizing antibodies against IL-1α or IL-1β (10 μg/ml) (d) T_{em} cells from WT mice were stimulated with soluble αCD3 (30 ng/ml) in the presence of splenic DCs from indicated genotypes. (e) T_{em} cells were stimulated with αCD3 (30 ng/ml) using either splenic DCs or naïve B cells as APCs in the presence or absence of recombinant IL-1β (10 ng/ml). Cytokine concentrations in the culture supernatants were determined using paired-antibody ELISA. Error bars indicate SEM; paired *t* test.

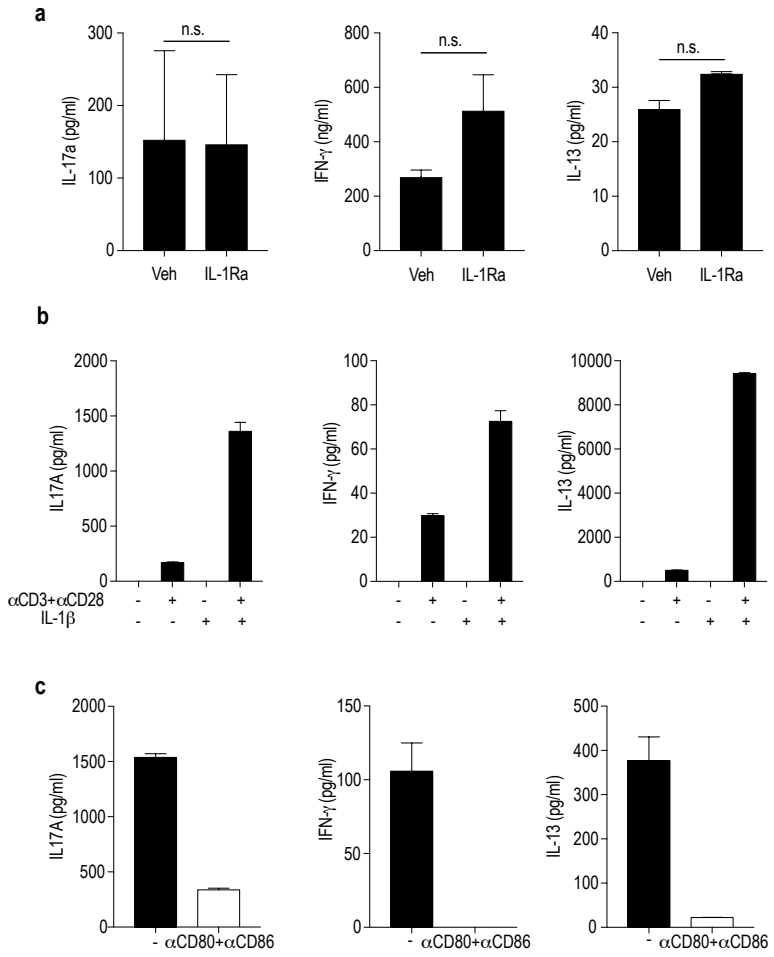

Figure 3-11. IL-1R signaling does not bypass the requirement of co-stimulation

(a) T_{em} cells were stimulated with immobilized αCD3 (0.5μg/ml) and αCD28 (0.5μg/ml) in the presence or absence of IL-1Ra for 24 hrs. (b) T_{em} cells were stimulated with or without immobilized αCD3 (0.5μg/ml) and αCD28 (0.5μg/ml) and IL-1β (ng/ml) for 48hrs. (c) T_{em} cells were co-cultured with WT splenic DCs and αCD3 (30ng/m) in the presence of CD80 (10μg/ml) and CD86 (10μg/ml) neutralizing antibodies. DCs were incubated with antibodies for 30min before addition of T cells. Error bars indicate SEM; (a) n.s.= not significant. (b and c) Data are representative of 2 independent experiments.

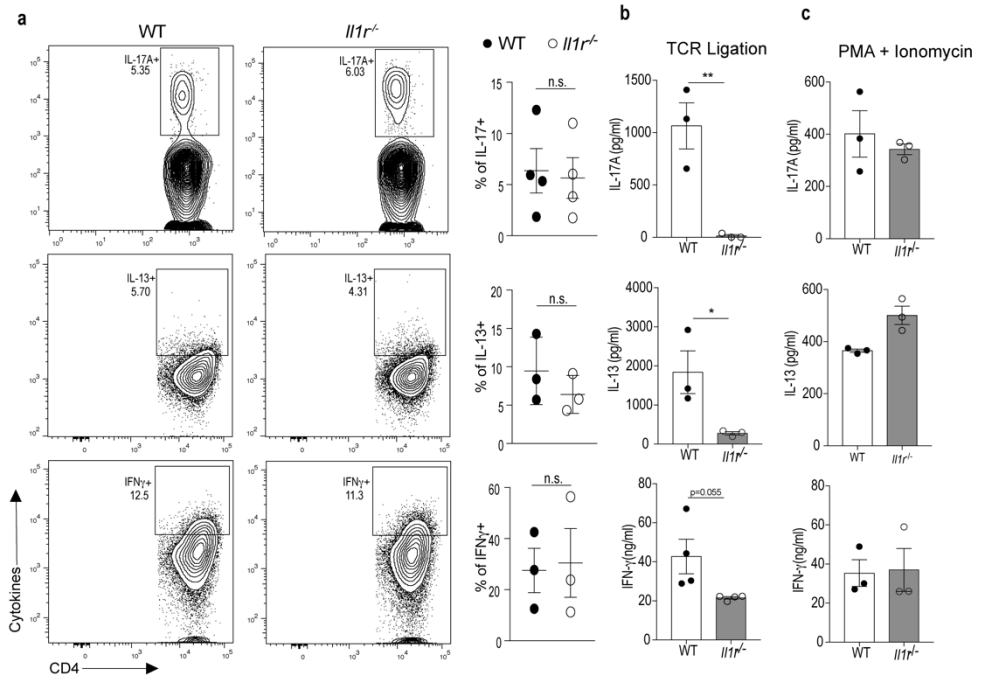

Figure 3-12. IL-1R signaling is dispensable for CD4 T cell priming but critical for effector function

(a-c) WT and *Il1r*$^{-/-}$ mice were cohoused for at least 3 weeks. (a) Intracellular staining following activation of T_{em} with PMA + Ionomycin. (b) Secreted cytokines were measured 24 hours following activation of splenic T_{em} with CD11c+ DCs in the presence of soluble αCD3 (30 ng/ml). (c) Secreted cytokines were measured 6 hours following activation of splenic T_{em} cells with PMA and Ionomycin. Error bars indicate SEM; paired *t* test.

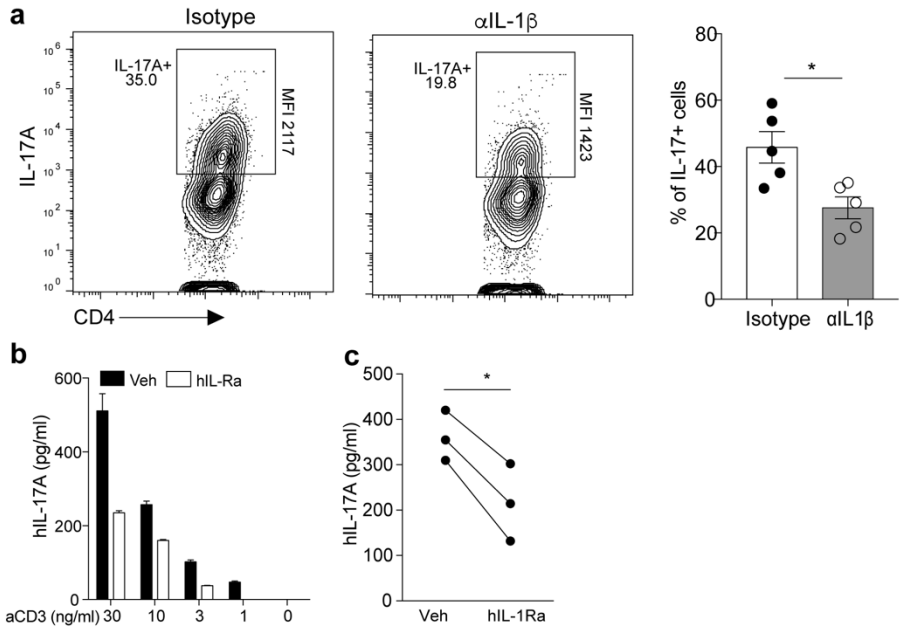

Figure 3-13. IL-1R signaling is critical for cytokine production by primed CD4 T cells in m ice and in humans

(a) *In situ* IL-17A production by CD3+CD4+CD44hi T cells in LPLs from WT mice treated with αIL-1β or isotype control following *in vivo* T cell stimulation via intraperitoneal injection of αCD3 (20 μg). IL-17A MFI on CD4+CD44hi cells is shown. Unpaired *t*-test. (b) IL-17A in the culture medium of human memory (CD4+CD45RO+) T cells reactivated with autologous MDDCs in the presence of αCD3 (OKT3, 30 ng ml^{-1}) and hIL-1Ra (200 ng/ml) for 24 hrs. Memory CD4 T cells and monocytes were isolated from peripheral blood of a healthy donor. Mean +/- SEM of technical duplicates are presented. (c) IL-17A in the culture medium of human memory T cells reactivated with autologous MDDCs from several donors in the presence of given concentrations of αCD3 (OKT3, 30 ng/ml) and hIL-1Ra (200 ng/ml) for 24 hrs. Each line represents an independent donor. Untreated mice serve as baselines for constitutive cytokine production by CD4 T cells. Error bars indicate SEM. Paired *t*-test.

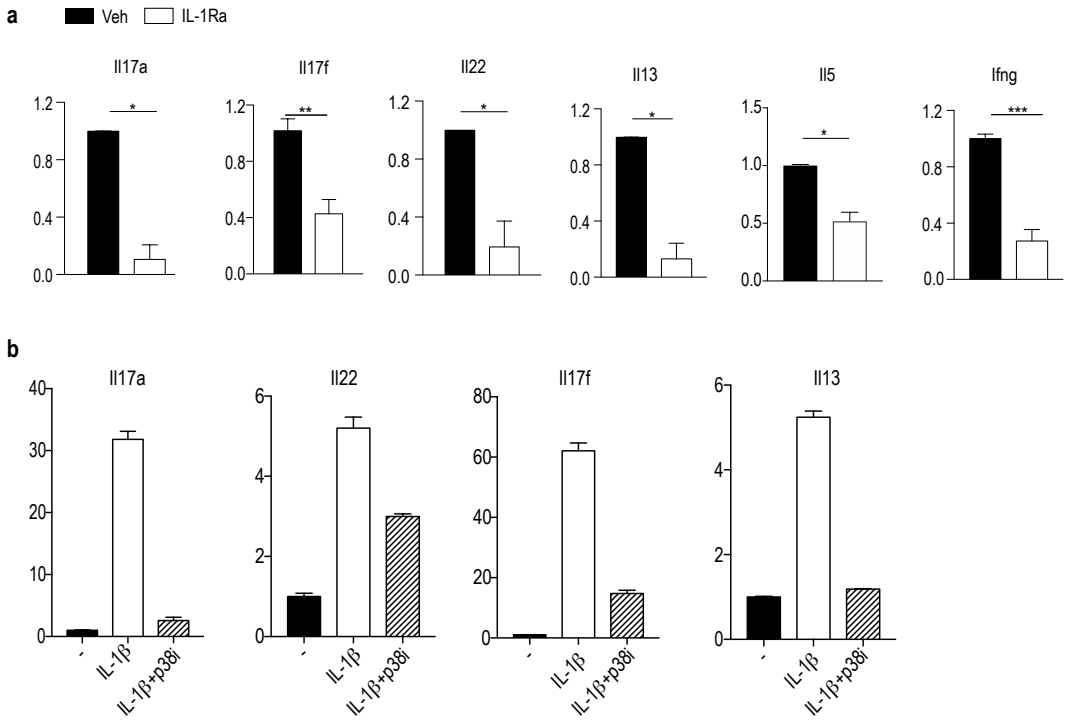

Fig 3-14. IL-1R enhances transcriptional expression of T cell cytokines in a p38 dependent manner

(a) $CD44^{hi}$ $CD62L^{lo}$ T_{em} cells were stimulation by splenic DCs and soluble αCD3 (30 ng/ml) in the presence or absence of IL-1Ra for 48hrs. Respective cytokine transcripts were quantified in DC-T cell co-culture cell lysate. (b) Quantification of cytokine transcripts in activated purified CD4 T cells in the presence of IL-1β (20ng/ml) and p38i (5μM). All data is normalized to *18s* and untreated control. Error bars indicate SEM. Paired *t*-test. (b) Data are representative of two independent experiments.

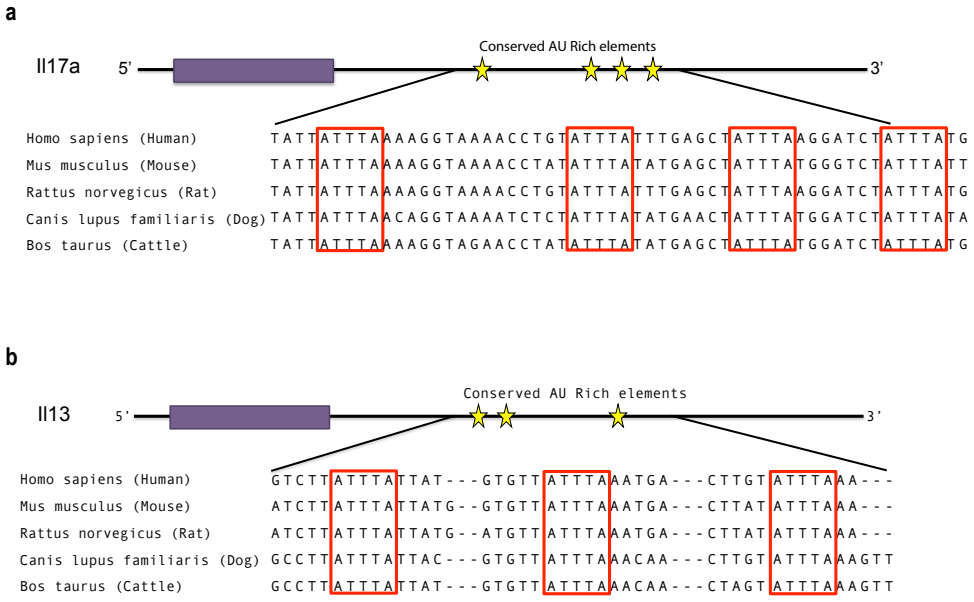

Figure 3-15. Multiple sequence alignment of 3'UTR of Il17a and Il13 from various species

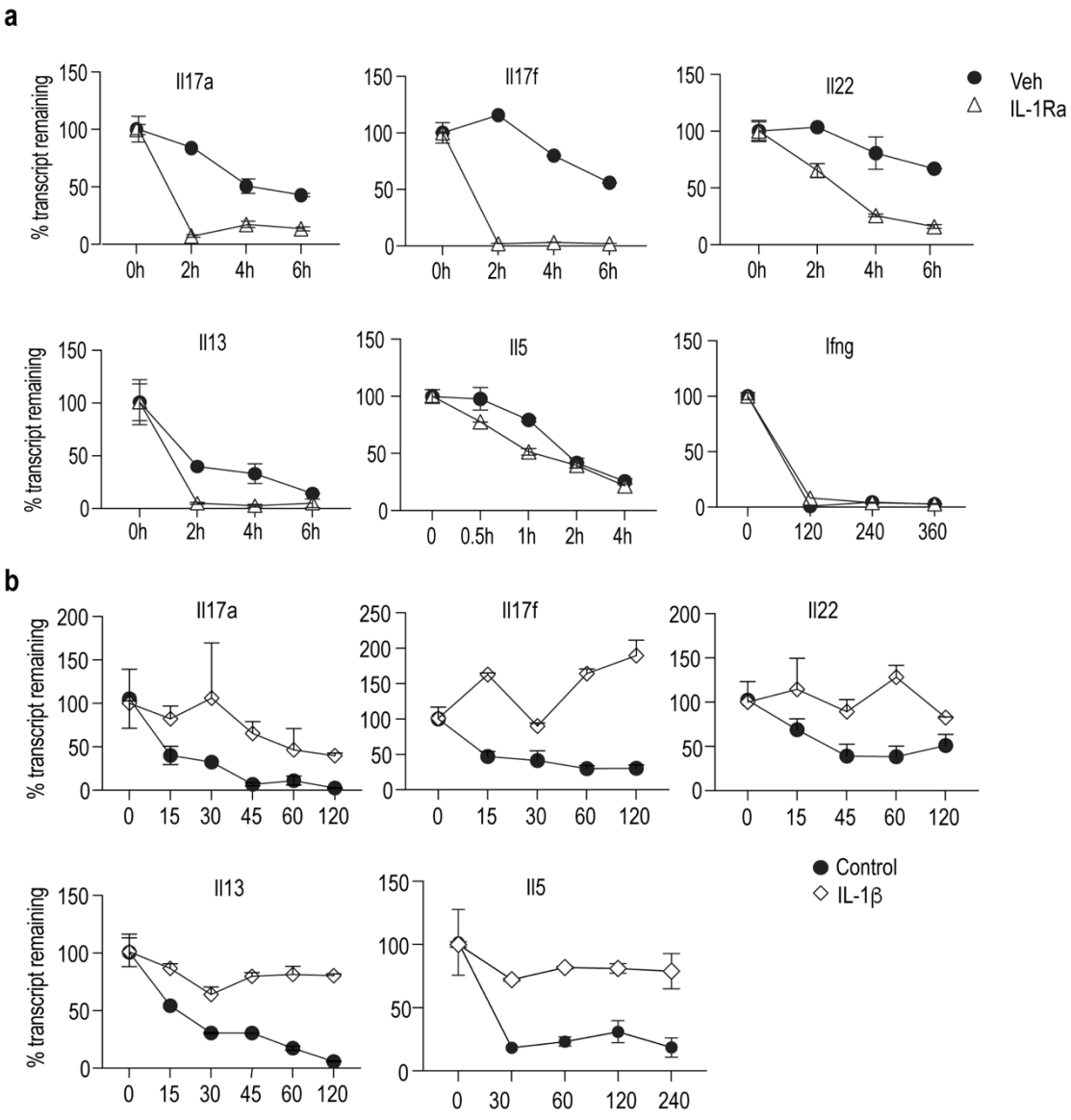

Fig 3-16. IL-1R signaling stabilizes Th17 and Th2 cytokine transcripts

(a) CD4 T cells were stimulated with CD11c+ DCs and soluble αCD3. (b) CD4 T cells were stimulated using immobilized αCD3 (0.5 μg/ml) and αCD28 (0.5 μg/ml). (a and b) Actinomycin D was added 18hr post stimulation and lysates were collected at indicated time points post-actinomycin D treatment. RNA was isolated post-stimulation and cDNA was synthesized for qRT

PCR. All data is normalized to *18S* rRNA. Error bars indicate SEM; paired *t*-test. Data are representative of three to four independent experiments.

CHAPTER 4

Part of the writing presented in this chapter was previously published on the preprint server Biorxiv, doi: https://doi.org/10.1101/475517

T Cells Instruct Dendritic Cells to Produce Inflammasome-Independent Il-1β Causing Systemic Inflammation and Autoimmunity

Introduction

IL-1β mediates host immunity through its ability to influence both innate and adaptive immune responses. It promotes innate immunity by inducing the acute phase response and recruiting inflammatory cells (Biondo et al., 2014; Miller et al., 2006; Rider et al., 2011; Zheng et al., 1995). In the adaptive immune system, IL-1β enhances T cell priming and differentiation (Santarlasci et al., 2013), and more importantly acts as a licensing cytokine to enable memory CD4 T cell effector function (Jain et al., 2018). However, aberrant production of IL-1β in the absence of pathogenic insult can result in immunopathology associated with several auto-immune and auto-inflammatory diseases (Dinarello, 2011). In fact, IL-1β blockade has been proven to be a successful treatment for several autoimmune conditions including psoriasis, rheumatoid arthritis (RA), and uveitis (Lopalco et al., 2015). Autoinflammatory disorders can be a consequence of gain-of-function mutations in inflammasome machinery which results in spontaneous IL-1β production (Hoffman et al., 2001; Hugot et al., 2001; Levandowski et al., 2013; Martinon et al., 2006; Ogura et al., 2001). Due to this association, IL-1β production in these disorders is often attributed to inflammasome activation (Shaw et al., 2011). In the case of T cell driven auto-immunity, where

IL-1β is implicated as a major driver of pathology, the role of inflammasome in IL-1β production remains obscure (Ippagunta et al., 2010; Joosten et al., 2009; Shaw et al., 2011; Stehlik, 2009). GWAS studies have also failed to report significant genetic association between inflammasome proteins and T cell dependent autoimmunity (Shaw et al., 2011). Additionally, disease progression in mouse models of RA was found to be independent of the inflammasome components NLRP3 and caspase-1 (Ippagunta et al., 2010). Similarly, caspase-1 deficiency did not mitigate diabetes in NOD mice(Schott et al., 2004). Together, this prompted us to investigate how bioactive IL-1β is produced during T cell-driven autoimmune diseases in the absence of overt infection or injury.

Here, we have discovered an inflammasome-independent pathway of IL-1β production that is triggered upon cognate interactions between dendritic cells and effector CD4 T cells. Analogous to inflammasome activation, this "T cell-instructed IL-1β" also relies on two independent signaling events. TNFα produced by activated CD4 T cells engages TNFR signaling on DCs leading to pro-IL-1β synthesis. Subsequently, FasL, also expressed by effector CD4 T cells, engages Fas on DCs leading to caspase-8 dependent pro-IL-1β cleavage. Remarkably, this two-step mechanism is completely independent of pattern recognition receptor activation. IL-1β produced upon cognate DC-effector CD4 T cell interaction causes wide spread leukocyte infiltration, a hallmark of systemic inflammation as well as autoimmune pathology. This study has uncovered a novel feature of DC-T cell cross-talk that allows for active IL-1β secretion independent of innate sensing pathways and provides a mechanistic explanation for IL-1β production and its downstream consequences in CD4 T cell driven autoimmune pathology.

Results

Cognate interaction between DCs and effector CD4 T cells leads to inflammasome independent production of cleaved IL-1β by DCs

We have previously demonstrated that T cell intrinsic IL-1R signaling is critical for optimal cytokine production by effector and memory CD4 T cells following their reactivation using DCs(Jain et al., 2018). The DCs used in these experiments were not stimulated by any microbial ligands, thus leading us to hypothesize that cognate interaction between DCs and effector CD4 T cells might elicit the production of bioactive IL-1β independent of pattern recognition receptor (PRR) signaling. Indeed, cognate interaction of DCs with effector CD4 T cells led to secretion of IL-1β in the absence of innate immune sensing (Figure 4-1a). Furthermore, we found that DC interaction with effector CD4 T cells of Th1, Th2 and Th17 lineages all led to secretion of IL-1β, pointing to a broadly conserved pathway of IL-1β production (Figure 4-1b). Since all CD4 T cell lineages were found to instruct IL-1β production by DCs, we henceforth use Th0 cells to represent effector CD4 T cells, unless otherwise noted. These initial experiments were performed using anti-TCR antibody stimulation that activates T cells regardless of their specificity. We therefore examined if this IL-1β production was in fact dependent on presentation of cognate peptide by DCs. When OVA peptide-restricted OT-II TCR transgenic effector T cells were reactivated using DCs, IL-1β production was strictly dependent on the presence of the cognate $OVA_{323-339}$ peptide (Figure 4-2). More interestingly, the quantity of IL-1β found in the supernatants directly correlated with the concentration of the stimulating peptide (Figure 4-2), indicating that the avidity of MHC-TCR interaction is a major determinant of the amount of IL-1β being secreted. While it has been

reported that CD4 T cells can produce IL-1β (Martin et al., 2016), we found that DCs were the only source of IL-1β during their interaction with effector CD4 T cells (Figure 4-1c).

Production of IL-1β relies on two independent signals: a priming signal necessary for transcription and synthesis of proIL-1β, followed by a cleavage signal required for its biological activity (Afonina et al., 2015). We observed that DC-T cell interaction led to rapid transcriptional upregulation of *Il1b* (Figure 4-3a). This experiment was performed using *Il1b*$^{-/-}$ T cells to ensure analysis of *Il1b* transcript specifically in DCs. The transcriptional induction translated into accumulation of intracellular pro-IL-1β (Figure 4-3b). In addition to proIL-1β synthesis, interaction with effector CD4 T cells also induced cleavage of pro-IL-1β into its bioactive 17kDa fragment (Figure 4-3c), which could be detected in the supernatant. These data demonstrate that interaction of DCs with effector CD4 T cells can trigger transcriptional induction of pro-IL-1β as well as its bioactive cleavage.

T cell derived TNFα is critical for induction of pro-IL-1β in DCs

Next, we decided to characterize the signaling events that enable this IL-1β production. Inflammasome activation is a major mechanism by which IL-1β is produced (Martinon et al., 2002). The priming signal during inflammasome activation is provided by sensing of microbial ligands by pattern recognition receptors (PRRs) such as TLRs that culminates into NF-κB activation and transcriptional upregulation of pro-IL-1β (Cogswell et al., 1994). Since DCs used in these experiments were not exposed to any microbial ligands, we posited that a PRR-independent signal is likely to be involved in proIL-1β synthesis. A significant difference in the

magnitude of pro-IL-1β synthesis following TLR stimulation as compared to T cell interaction also suggested a TLR independent mechanism (Figure 4-4a). Nevertheless, to rule out the contribution of inadvertent endotoxin contamination in these *in vitro* cultures, we analyzed induction of proIL-1β in *Tlr2/4*$^{-/-}$ as well as *Myd88*$^{-/-}$ DCs. We found no defect in effector CD4 T cell driven IL-1β induction in DCs deficient for TLR signaling (Figure 4-4b, 4-4c).

It is well known that pro-IL-1β transcription is mediated by NF-κB (Cogswell et al., 1994) and AP-1 (Baldassare et al., 1999), which can also be activated downstream of receptors for the TNF superfamily. This prompted us to examine a potential role for these receptors in pro-IL-1β synthesis in the absence of microbial recognition by DCs. We directly tested the role of TNFR superfamily signaling in IL-1β production by neutralizing individual ligands during DC-T cell interaction. We found that neutralization of TNFα and FasL, but not CD40L, significantly compromised IL-1β production (Figure 4-5a). This suggested that TNFR as well as Fas signaling participate in PRR independent IL-1β production by DCs. Upon careful examination, we observed that the pro-IL-1β levels were compromised only in the absence of TNFα (Figure 4-5b), while Fas signaling was dispensable for pro-IL-1β synthesis (Figure 4-5b). This result suggests that while Fas-FasL interaction is critical for bioactive IL-1β production, it is not required for the transcriptional induction of pro-IL-1β. Consistently, intracellular pro-IL-1β was also significantly reduced upon neutralization of TNFα (Figure 4-5c). Since TNFα neutralization does not result in complete abrogation of pro-IL-1β, additional pathways could contribute to pro-IL-1β synthesis that require further investigation. The existence of diversified mechanisms of T cell induced pro-IL1β, is parallel to the ability of several innate recognition receptors to upregulate IL-1β (Maeda

et al., 2005; Martinon et al., 2002; Shenderov et al., 2013). We found that effector CD4 T cells of all lineages (Th1, Th2 and Th17) rapidly upregulate TNFα following their interaction with DCs (Figure 4-6b). While TNFα is primarily known to be an effector cytokine of the Th17 lineage(Li et al., 2014), we found that primed Th1 and Th2 lineage cells are also capable of producing TNFα (Figure 4-6b), suggesting that activated CD4 T cells of all lineages are poised to engage TNFR on DCs. It is important to note that while TNFα is known to be largely of myeloid origin in PAMP driven inflammation, activated T cells seem to be the predominant TNFα producers during their interactions with DCs (Figure 4-6a, 4-6c). Consistent with this finding, TNFα deficient T cells induced significantly diminished pro-IL-1β (Figure 4-6d) production by DCs. Finally, combined deficiency of TNFR1 and TNFR2 on DCs showed that DC intrinsic TNFR signaling was required for optimal IL-1β production (Figure 4-5d).

Fas-FasL interaction between effector CD4 T cells and DCs leads to Caspase8 dependent cleavage of pro-IL-1β

We then proceeded to identify the molecular mechanism responsible for proteolytic cleavage of pro-IL-1β. Caspase-1, the effector protease of all inflammasomes (Franchi et al., 2009), is largely responsible for bioactive cleavage of pro-IL-1β (Shaw et al., 2011). Indeed, we found that the aspartate residue in pro-IL-1β, where caspase-1 cleaves, is critical for IL-1β production following DC-T cell interaction (Figure 4-7a). Surprisingly, we did not detect active caspase-1 following DC-T cell interaction (Figure 4-7b). Furthermore, we found that genetic ablation of caspase-1 in DCs did not compromise IL-1β release (Figure 4-7c, 4-7d). These data point to the existence of an

inflammasome independent mechanism of IL-1β production that is employed by DCs during their interaction with effector CD4 T cells.

In the experiments above, we found that blocking Fas-FasL interaction led to a complete loss of detectable IL-1β in the supernatants, without affecting the synthesis of pro-IL-1β (Figure 4-5a, 4-5b). Additionally, effector CD4 T cells were found to constitutively express FasL that was further upregulated upon their interaction with DCs (Figure 4-8a). These observations suggested the possibility that Fas signaling in DCs could be involved in the cleavage of pro-IL-1β. Indeed Fas deficient DCs from *lpr* mice (Watanabe-Fukunaga et al., 1992) were unable to secrete cleaved IL-1β underlining the necessity of DC intrinsic Fas signaling in T cell induced IL-1β production (Figure 4-8b, 4-8c). It has been previously shown that Fas signaling in macrophages can trigger cleavage of pro-IL-1β in a caspase-8 dependent manner (Bossaller et al., 2012), however the physiological importance of such IL-1β cleavage is not known. We found that DC-T cell interaction led to maturational cleavage of caspase-8 Figure 4-9a). While TNFR signaling has been reported to cause caspase-8 activation (Wang et al., 2008), we discovered that only Fas, but not TNFR, signaling is critical for caspase-8 cleavage during cognate DC-T cell interaction (Figure 4-9a). Inhibition of caspase-8 enzymatic activity by IETD resulted in abrogation of IL-1β cleavage implicating caspase-8 as the protease involved in mature IL-1β production by DCs (Figure 4-9b). Caspase-8 has been shown to be important for induction NF-κB dependent genes upon TLR activation, including pro-IL-1β (Lemmers et al., 2007; Philip et al., 2016). However, during DC-T cell interactions, caspase-8 inhibition did not affect pro-IL-1β synthesis (Figure 4-9b). Furthermore, genetic deletion of caspase-8, along with RIPK3 to prevent necrosis (Kaiser et al., 2011), in DCs led to a complete loss of secreted IL-1β (Figure 4-9c). Although caspase-8 has been

previously implicated in proteolytic cleavage of pro-IL-1β upon TLR4 activation (Moriwaki et al., 2015), our experiments demonstrate a crucial role for caspase-8 in IL-1β production by DCs in the absence of any PRR activation. Together, these data show that effector CD4 T cells can instruct IL-1β production by DCs thereby revealing a novel PRR-independent mechanism of innate immune activation.

IL-1β produced upon DC-effector CD4 T cell cognate interaction in vivo leads to systemic inflammation marked by inflammatory monocyte and granulocyte recruitment

Although cytokines made by self-reactive T cells contribute to autoimmune inflammation, innate immune activation is known to be responsible for the precipitation of autoimmunity (Bachmann and Kopf, 2001). The pathology of these diseases is associated with infiltration of neutrophils and inflammatory monocytes into the affected tissues. Since the majority of existing mouse models of T cell driven autoimmunity still rely on initial PRR activation, usually in the form of *Mycobacterium tuberculosis*, to break tolerance (Janeway, 1989a), we employed two new PRR independent approaches to mimic cognate DC-T cell interaction likely to occur during auto-immune flares. In the first approach, we employed *in vivo* administration of αCD3 speculating that systemic TCR activation would result in widespread T cell reactivation by myeloid cells. Intravenous administration of αCD3 led to rapid transcriptional upregulation of *Il1b* in the splenocytes (Figure 4-10a). Upregulation of pro-IL-1β protein expression in CD11c+ DCs was also observed upon αCD3 stimulation (Figure 4-10b). Moreover, we saw significant recruitment of neutrophils to the spleen as well as to the small intestinal lamina propria (SI-LP) within 18 hours of stimulation (Figure 4-10c, 4-10d). More importantly, the inflammatory leukocyte recruitment

in both the spleen and the SI-LP was significantly dependent on IL-1β (Figure 4-11) establishing that *in vivo* interactions between T cells and myeloid cells leads to acute IL-1β production, which then promotes inflammatory leukocyte infiltration. In the second approach, we adoptively transferred OT-II TCR Tg Th17 T cells and induced their activation by intravenous administration of the MHCII-restricted OVA peptide ($OVA_{323-339}$) (Figure 4-12). Based on our *in vitro* data, we hypothesized that interaction of activated CD4 T cells with DCs bearing cognate peptides *in vivo* would lead to IL-1β production and result in IL-1β dependent inflammation as measured by expansion of circulating neutrophil populations. In agreement with our hypothesis, we observed that recruitment of neutrophils to the spleens of OT-II T cell recipient mice was dependent on IL-1β (Figure 4-12).

Next, we tested the role of TNFR and Fas signaling in IL-1β production *in vivo*. TNFα deficient mice exhibited significantly compromised transcriptional upregulation of proIL-1β in response to αCD3 administration (Figure 4-13a). Moreover, the expansion of neutrophils in the spleen was also reduced in the absence of TNFα (Figure 4-13b). In order to test the requirement of Fas signaling for T cell induced inflammation *in vivo*, we specifically ablated Fas in CD11c expressing cells (Figure 4-13c) and investigated its impact on IL-1β mediated leukocyte recruitment following DC-T cell interaction. Fas deletion in DCs significantly inhibited neutrophil infiltration in the SI-LP and spleen (Figure 4-13d, 4-13e) thus providing *in vivo* corroboration of our *in vitro* findings.

DC intrinsic TNFR and Fas signaling but not Caspase-1 is critical for memory CD4 T cell function in the absence of PRR activation

We have previously shown that IL-1β is a critical "licensing signal" for IL-17 production(Jain et al., 2018). As we have found an important role for TNFα and Fas in IL-1β production by DCs, we reasoned that TNFR and Fas signaling in DCs may be necessary for cytokine production by Th17 cells during their reactivation. In agreement with our hypothesis, DCs lacking TNFR also had diminished capacity to trigger IL-17 production by effector CD4 T cells (Figure 4-14a). Similarly, we discovered that blocking Fas signaling during T cell reactivation significantly compromised IL-17a production (Figure 4-14b). More importantly, caspase-1 deficient DCs induced normal levels of IL-17a production by memory CD4 T cells which was dependent on IL-1β (Figure 4-14c). We propose that this novel pathway of IL-1β production, mediated by TNFR and Fas signaling, evolved to enable optimal CD4 T cell effector function. However, cognate interactions of self-reactive effector T cells with antigen presenting myeloid cells are also likely to result in production of bioactive IL-1β leading to detrimental immunopathology.

Autoreactive CD4 T cells induce IL-1β production and autoimmune inflammation via TNFR and Fas signaling pathways

Pathogen sensing by PRRs leads to inflammation primarily through secretion of pro-inflammatory cytokines and chemokines from myeloid cells (Yang and Chiang, 2015). Our data suggests that effector CD4 T cells can also contribute to inflammation by eliciting IL-1β production from interacting DCs. Self-reactive CD4 T cells are key players in several IL-1β mediated autoimmune diseases such as multiple sclerosis, RA and type 1 diabetes (Fletcher et al., 2010; Pugliese, 2017;

VanderBorght et al., 2001). IL-1R signaling is known to be critical for autoimmune inflammation seen in EAE, however the mechanism responsible for IL-1β production during this disease has not been clear (Lin and Edelson, 2017; Mannie et al., 1987). To test if *bona fide* self-reactive CD4 T cells can elicit IL-1β production in EAE, we utilized 2D2 TCR Tg cells that are specific for the Myelin oligodendrocyte glycoprotein (MOG_{35-55}) peptide (Bettelli et al., 2003). We stimulated these CD4 T cells with WT DCs in the presence of MOG peptide (Figure 4-15a). Similar to polyclonal effector CD4 T cells, myelin-reactive T cells also induced IL-1β production by DCs in a FasL and TNFα dependent manner (Figure 4-15a). While a role for caspase-1 in EAE induction has been reported (Furlan et al., 1999; Lalor et al., 2011), it is important to note that in these studies mice were immunized with heat killed *Mycobacterium tuberculosis* in the MOG emulsion (Janeway, 1989a). The microbial ligands present in this emulsion can activate PRRs and therefore lead to priming of auto-reactive T cells. To directly investigate the mechanism of T cell induced sterile autoimmune inflammation, we instead took advantage of the passive EAE model where primed MOG-specific CD4 T cells are transferred into naive mice to induce disease (Stromnes and Goverman, 2006b). Given our finding that TNFR and Fas signaling are necessary of T cell induced IL-1β production we asked if EAE caused by adoptive transfer of pathogenic T cells is also dependent on this mechanism. Indeed, we observed protection from T cell induced neurological autoimmunity when the recipients lacked TNFR and Fas signaling pathways (Figure 4-15b, 4-15c). Additionally, demyelination was also significantly reduced in TNFR and Fas deficient hosts (Figure 4-15d). Together, these data demonstrate that autoreactive T cells engage TNFR and Fas to cause IL-1β mediated autoimmunity. It remains to be examined if this pathway is also responsible for inflammation observed in other IL-1β mediated auto-immune diseases.

Discussion

Our study thus establishes a previously unknown feature of DC-T cell cross-talk where effector CD4 T cells can instruct the innate immune system to produce IL-1β independent of inflammasome activation. Of note, the signaling requirements of the "T cell instruction", as described here, are parallel to the inflammasome pathway, in that it also relies on two independent signals that govern pro-IL-1β synthesis and its activation by proteolytic cleavage. The distinction between the two mechanisms of IL-1β production can be appreciated with regard to their physiological ramifications. The "TLR-NLR" inflammasome pathway is primarily employed by macrophages, resulting in production of IL-1β that is necessary for clearance of virulent pathogens (Figure 4-16). In contrast, the T cell instructed IL-1β made by DCs as a result of TNFR-Fas signaling, is likely to be responsible for auto-immune flares in the absence of any overt pathogenic insult (Figure 4-16). Also, since the quantities of IL-1β produced by DCs are directly dictated by the concentration of the peptide, it is unlikely that bystander or low avidity interaction between DCs and T cells will trigger IL-1β production by DCs. Self-reactive CD4 T cells that escaped thymic selection and underwent effector differentiation perhaps employ this inflammasome-independent pathway of IL-1β production during cognate interaction with DCs, thereby causing innate inflammation associated with autoimmunity. Whether self-reactive T cells in fact induce IL-1β *in vivo* using the "TNFR-Fas" pathway to elicit autoimmunity, requires further testing in T cell dependent autoimmune models.

Aberrant inflammasome activation caused by the gain-of-function mutations in NLRP3 and Pyrin drive IL-1β dependent auto-inflammatory conditions that are also initiated independently of TLRs and other PRRs (Shaw et al., 2011). The role of inflammasome in T cell driven autoimmune

diseases, however, is neither discernible nor established. Strikingly, several lines of evidence support the involvement of TNFR and Fas signaling in autoimmune inflammation. Blocking of TNFα has been shown to effectively inhibit production of IL-1β by human synovial cells (Brennan et al., 1989). T cell intrinsic deletion of TNFα as well as Fas deficiency attenuates EAE disease severity (Kruglov et al., 2011; Waldner et al., 1997). Disruption of *Fas* as well as TNFR also prevents autoimmune β cell destruction in non-obese diabetic mouse model (Itoh et al., 1997; Varanasi et al., 2012). Moreover, single nucleotide polymorphisms have been reported in the Tnfrsf1a, Faslg and Fas loci in strong association with IL-1β mediated autoimmune diseases (De Jager et al., 2009; Ferreira et al., 2017; Hinks et al., 2013; International Multiple Sclerosis Genetics et al., 2013; Jin et al., 2016; Tsoi et al., 2017), thus providing credence to the argument that IL-1β in chronic autoimmune conditions could be dependent on "T cell instruction", rather than inflammasome activation. Our data establishes a novel mechanism of IL-1β production that is initiated by effector CD4 T cells and thus presents an explanation for the presence of IL-1β seen in T cell mediated autoimmunity.

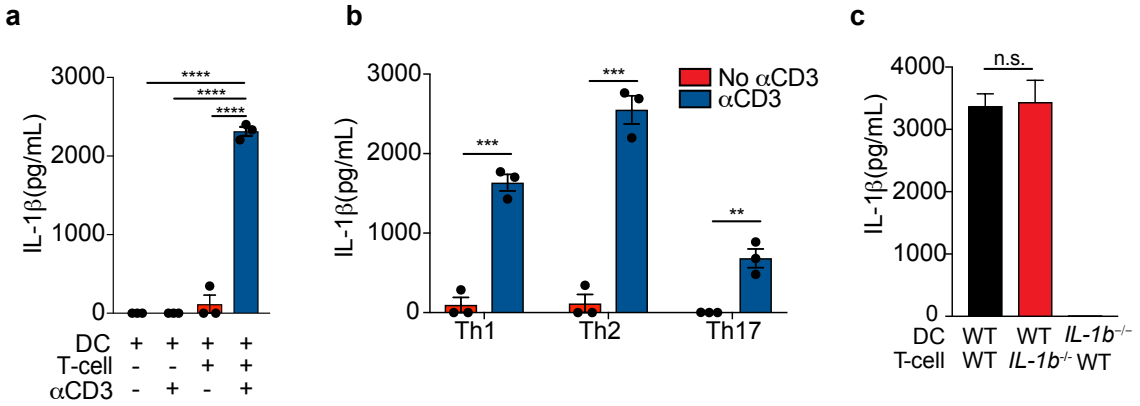

Figure 4-1. DCs secrete IL-1β during interaction with effector CD4 T cells

(a) WT effector CD4 (Th0) cells were stimulated with WT DCs using αCD3 for a period of 18h and lL-1β was quantified in the supernatants. (b) Effector CD4 T cells polarized to Th1, Th2 and Th17 lineage were stimulated with WT DCs using αCD3 for 18h followed by IL-1β ELISA (c) Effector CD4 (Th0) cells of given genotypes were stimulated with αCD3 using DCs of indicated genotypes and IL-1β was measured following 18h of culture. Error bars indicate SEM; paired *t*-test.

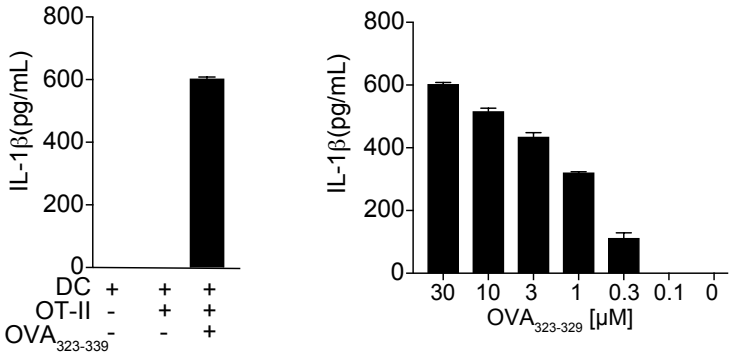

Figure 4-2. T cell induced IL-1β production by DCs is dependent on cognate interaction and proportional to the avidity of interaction

IL-1β was quantified in the supernatants following stimulation of Th17 polarized OT-II T cells (18h) using (left) fixed (100μM) or (right) titrating concentrations of OT-II$_{323\text{-}339}$ peptide presented by DCs. Error bars indicate SEM; Data are representative of three independent experiments.

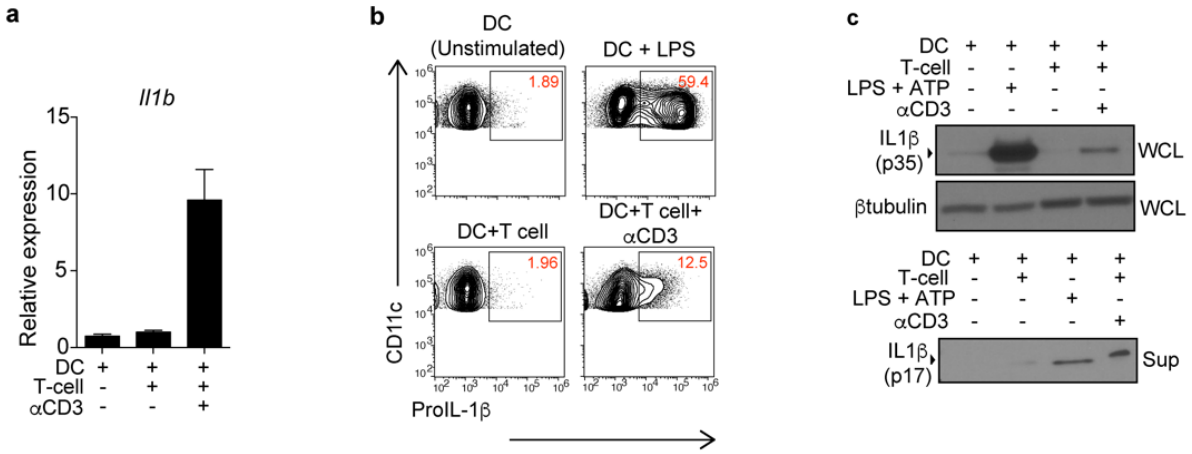

Figure 4-3. CD4 T cell can induce synthesis as well as proteolytic cleavage of IL-1β in interacting DCs

(a) WT DCs and αCD3 were used to stimulate *Il1b*$^{-/-}$ Th17 cells for 3h and lysates from co-cultures were used to make cDNA to assess *Il1b* mRNA by qPCR. Data are normalized to 18s rRNA. (b) WT DCs were stimulated with LPS (100ng ml^{-1}) or cultured with Th17 cells in the presence of αCD3 and brefeldin A for 6h. Intracellular pro-IL-1β expression in live CD90-veCD11c+ve cells was measured using flow cytometry. (c) WT DCs were used to stimulate effector CD4 T cells (Th0) with αCD3 for 18h and pro-IL-1β (p35) and cleaved IL-1β (p17) levels were analyzed in the cell lysates and the supernatants, respectively, using Western blotting.

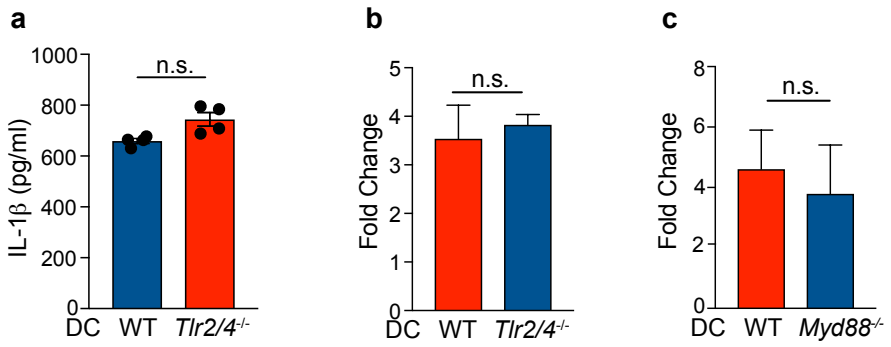

Figure 4-4. IL-1β production during DC-T cell interaction is independent of TLR activation in DCs

(a) WT and *Tlr2/4*$^{-/-}$ DCs were stimulated with WT Th0 cells using αCD3. Secreted IL-1β was quantified 6h post stimulation (b and c) WT, *Tlr2/4*$^{-/-}$ or *Myd88*$^{-/-}$ DCs were stimulated with WT Th0 cells using αCD3. Intracellular pro-IL-1β was analyzed 6h post stimulation. Fold change indicates proportion of pro-IL-1β+ve DCs, compared to PBS controls. Error bars indicate SEM; paired *t*-test.

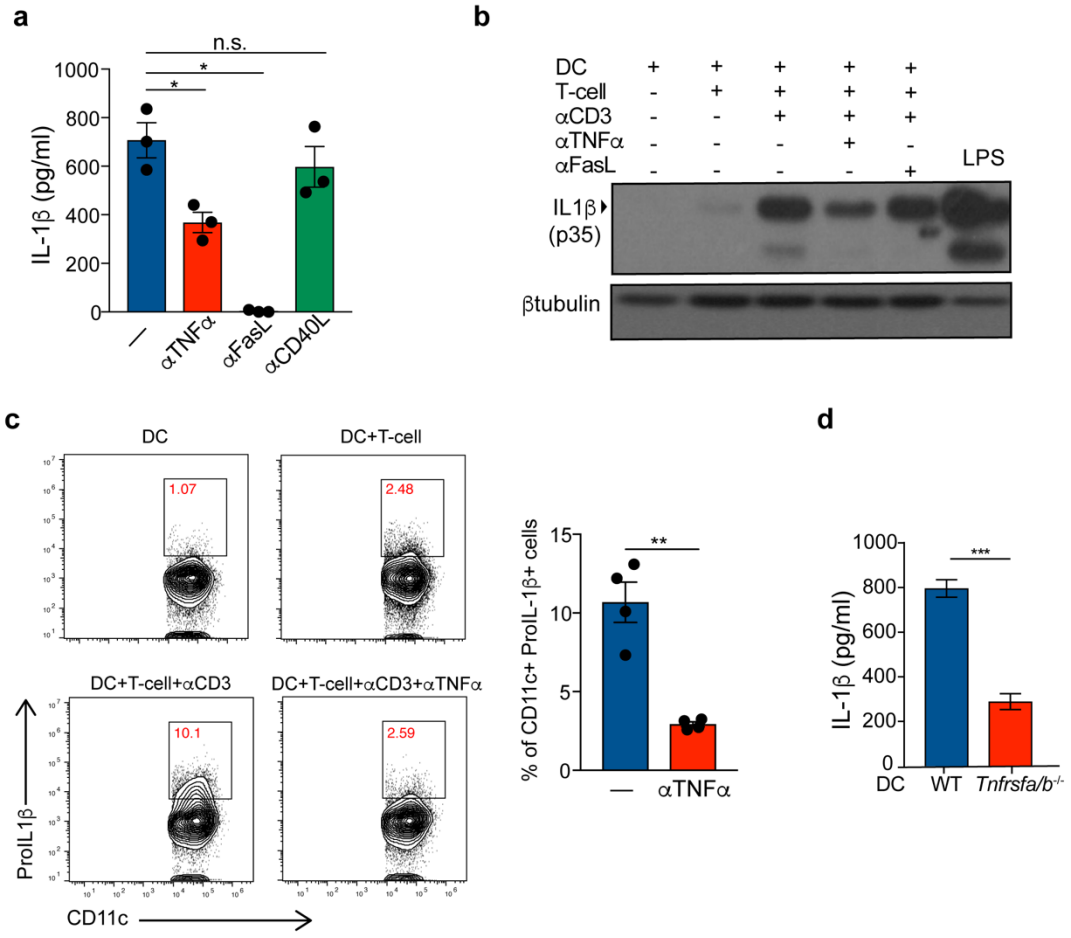

Figure 4-5. DC intrinsic TNFR signaling mediates T cell induced IL-1β production

(a) WT Th0 effector cells were stimulated with αCD3 and WT DCs in the presence or absence of anti- TNFα (20 μg/ml), anti-FasL (10 μg/ml), and anti-CD40L (20 μg/ml), neutralizing antibodies. Secreted IL-1β was quantified after 6h of culture (b) Whole cell lysates from DCs cultured as indicated with effector CD4 T cells (Th0) in the presence or absence of antibodies were subject to Western blot analysis to detect pro-IL-1β. (c) Effector CD4 T cells (Th0) were stimulated with WT DCs using αCD3 in the presence or absence of anti-TNFα (20 μg/ml) for 6h. Intracellular pro-IL-1β was quantified using flow cytometry. (d) WT or *Tnfrsf1a/b*$^{-/-}$ DCs were used to stimulate

WT effector CD4 T cells (Th0) for 6h in the presence of αCD3 and supernatants were collected for IL-1β ELISA. Error bars indicate SEM; (a,c,d) paired *t*-test, (b) Data are representative of two independent experiments.

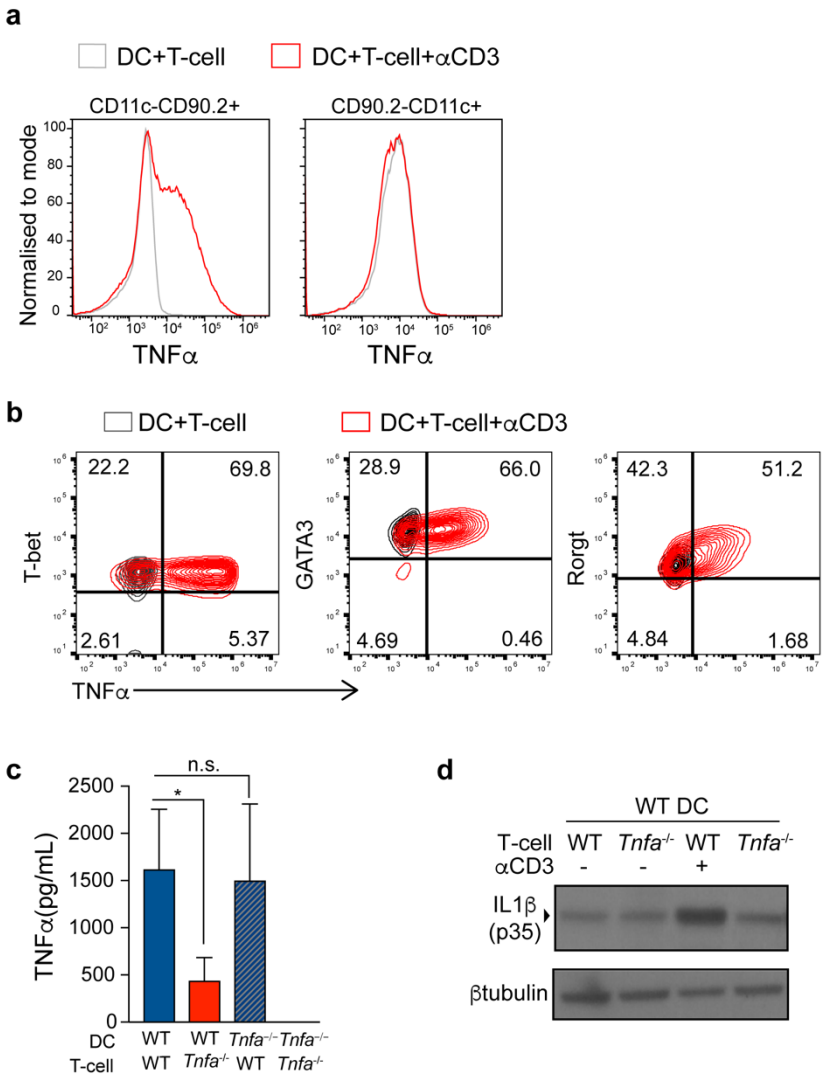

Figure 4-6. CD4 T cells of all lineages can provide TNFα required pro-IL-1β synthesis in DCs

(a) Th0 cells were stimulated with CD3 using WT DCs in the presence of brefeldin A for 3h. Intracellular TNFα in T cells and DCs was quantified using flow cytometry. (b) Th1, Th2 and Th17 polarized CD4 T cells were stimulated with CD3 using WT DCs in the presence of brefeldin A for 3h. Intracellular TNFα in T cells was analyzed using flow cytometry. Cells were considered to be transcription factor positive based on Isotype control antibody staining. (c) WT or *Tnfa*$^{-/-}$ T

cells were stimulated with WT or *Tnfa*$^{-/-}$ DCs. Supernatants from cultures of indicated genotypes (all in the presence of αCD3) were assayed for presence of TNFα. (d) WT DCs were used to stimulate WT or *Tnfa*$^{-/-}$ effector CD4 T cells and Western blot was performed on whole cell lysates (WCL) following 6h after αCD3 stimulation. (a, b and d) Data are representative of three independent experiments. Error bars indicate SEM; paired *t*-test.

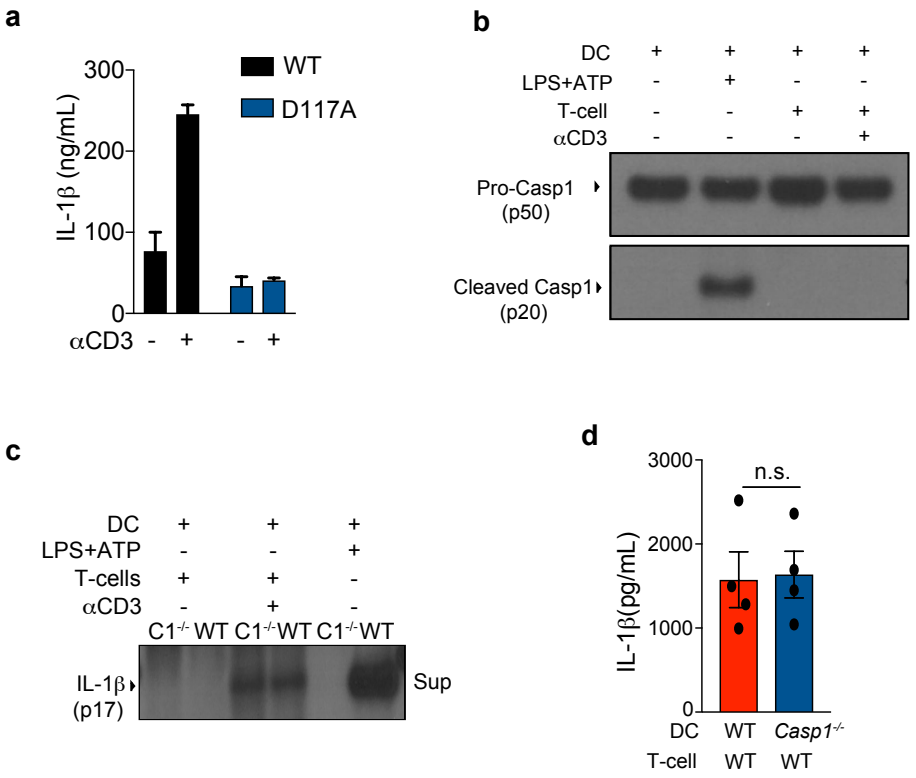

Figure 4-7. T cells elicit bioactive IL-1β production by interacting DCs in an inflammasome independent manner

(a) *Il1b*$^{-/-}$ bone marrow precursors were transduced with MSCV construct expressing WT or D117A *Il1b* cDNA and differentiated into DCs *in vitro*. Transduced DCs were used to activate *in vitro* primed CD4 T cells (Th0) in the presence of αCD3. Secreted IL-1β was measured 18h post stimulation. Error bars indicate SEM. (b) WT DCs were stimulated with LPS+ATP or primed CD4 T cells (Th0). Cleaved caspase-1 was analyzed in the whole cell lysates 18h post stimulation (c and d) WT Th0 cells were stimulated with αCD3 in the presence of either WT or *Casp1*$^{-/-}$ DCs and supernatants were collected after 18h to measure IL-1β using (c) western blot analysis and (d) ELISA. Error bars indicate SEM; (d) paired *t*-test, (a-c) Data are representative of two independent experiments.

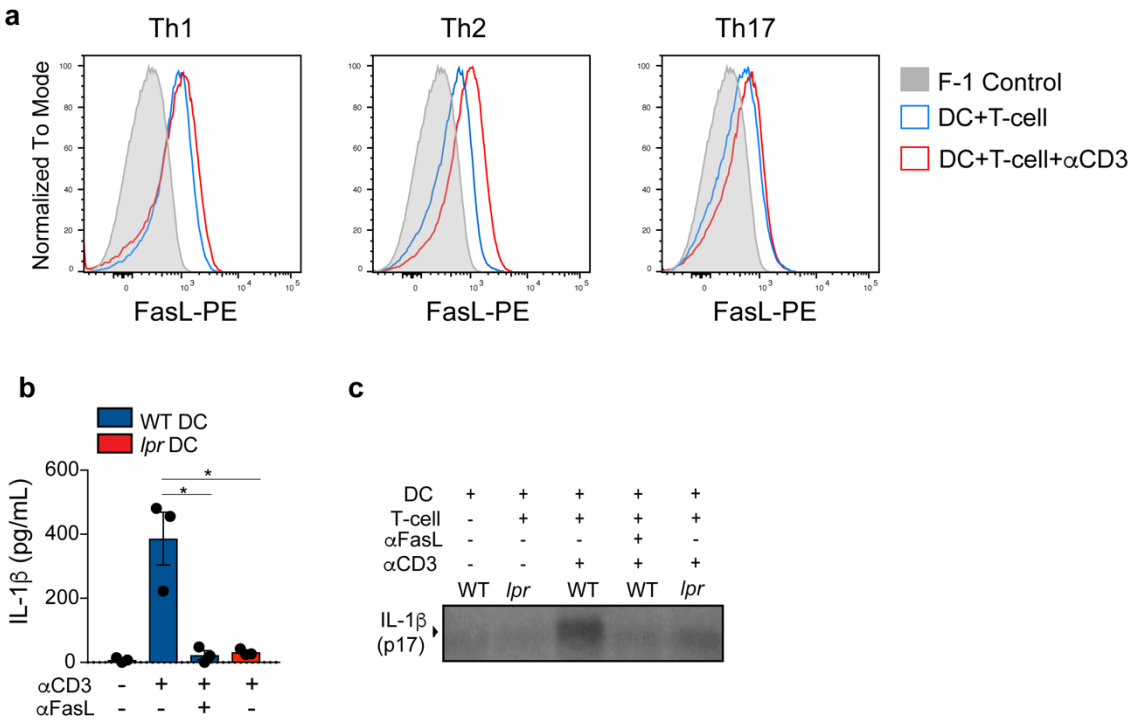

Figure 4-8. T cells engage Fas signaling in DCs leading to active IL-1β production

(a) *In vitro* primed Th1, Th2 and Th17 cells were co-cultured with WT DCs in the presence of αCD3. Surface FasL staining on live CD90.2+ve cells is shown with or without αCD3 stimulation. Data are representative of two independent experiments. (b) WT or *lpr* DCs were used to stimulate effector CD4 T cells (Th0) using αCD3 in the presence or absence of anti-FasL (10 μg/ml) for 18h. Secreted cleaved IL-1β was quantified by (b) ELISA and (c) Western blot. Error bars indicate SEM; (b) paired *t*-test, Data are representative of two independent experiments.

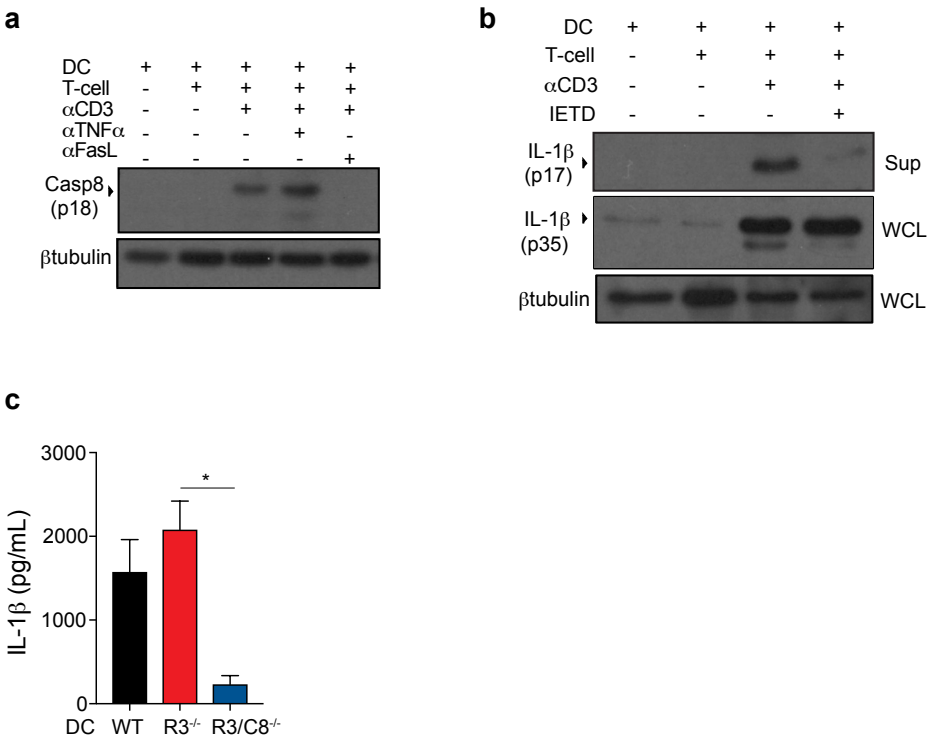

Figure 4-9. DC intrinsic Fas-Caspase-8 axis is responsible for bioactive IL-1β production during DC-T cell interaction

(a) WT DCs were used to stimulate (αCD3) effector CD4 T cells (Th0) in the presence of anti-FasL (10 μg/ml) or anti-TNFα (20 μg/ml) for 12h. Caspase-8 activation was measured by assessing cleavage of caspase-8 in the WCL. (b) WT DCs were used to stimulate (using αCD3) effector CD4 T cells in the presence of IETD (10μM) for 18 h. Cleaved IL-1β (p17) and pro-IL-1β (p35) was measured by Western blot of culture supernatants and WCL, respectively. (c) WT, *Rip3*$^{-/-}$(R3) and *Rip3*$^{-/-}$*Casp8*$^{-/-}$ (R3/C8) DCs were used to stimulate effector CD4 T cells (Th0) in the presence of αCD3 for 18h and secreted IL-1β was quantified using ELISA. Error bars indicate SEM; paired *t*-test, (a and b) Data are representative of two independent experiments.

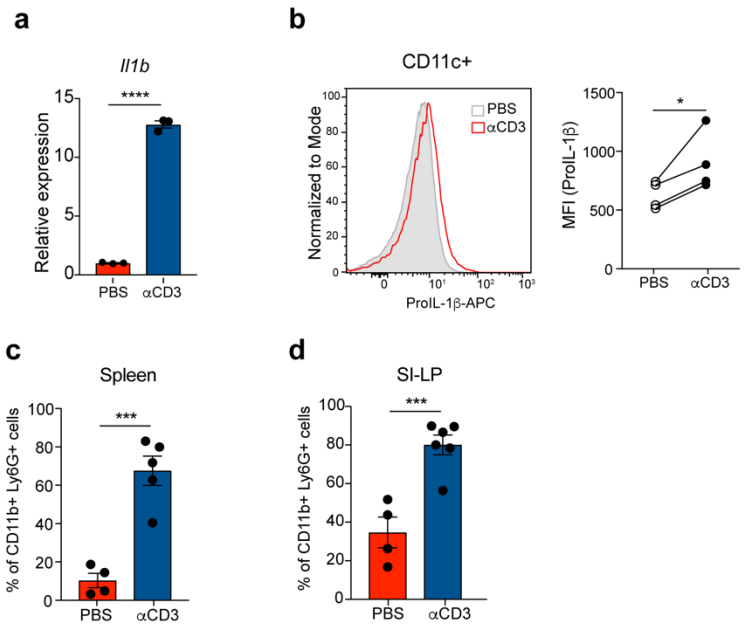

Figure 4-10. Systemic T cell activation in vivo leads to IL-1β induction and neutrophil recruitment

(a) WT mice were injected with αCD3 (50μg) intravenously. Spleen cells were harvested 3-4h post injection and immediately lysed for RNA isolation. Data are normalized to *Hprt1*. (b) Spleen cells from (a) were stained for DC markers and intracellular pro-IL-1β. Flow plot is pre-gated on live CD11c+ve cells. Data are representative of 2 independent experiments. (c and d) WT mice were injected with αCD3 (20μg) i.p. Neutrophil infiltration was quantified in the (c) spleen and (d) SI-LP. Error bars indicate SEM; Unpaired *t*- test.

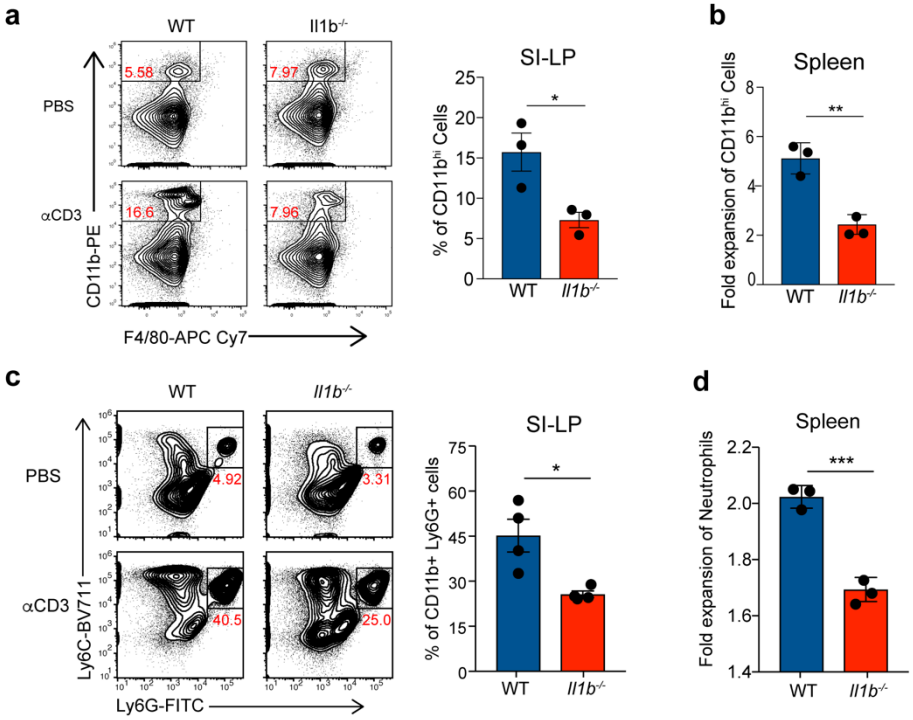

Figure 4-11. Systemic T cell activation in vivo leads to IL-1β dependent inflammatory leukocyte recruitment

WT and *Il1b*$^{-/-}$ mice were injected with αCD3 (20μg) i.p. and (top) monocyte and (bottom) neutrophil infiltration was analyzed in (a, c) SI-LP and (b, d) spleen, 18h later, using flow cytometry. Error bars indicate SEM; Unpaired *t*- test.

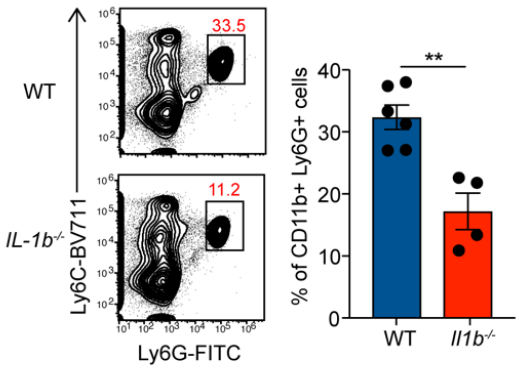

Figure 4-12. Antigen specific T cell reactivation in vivo leads to IL-1β dependent inflammatory leukocyte recruitment

WT OT-II T cells were differentiated into Th17 cells *in vitro*. $5x10^6$ OT-II Th17 cells were transferred i.v. into WT or *Il1b*$^{-/-}$ mice. 24h later 50μg of $OVA_{323-339}$ peptide was injected i.v. into recipients. 12h after peptide injection, spleens were harvested and analyzed for neutrophil infiltration. Error bars indicate SEM; Unpaired *t*- test.

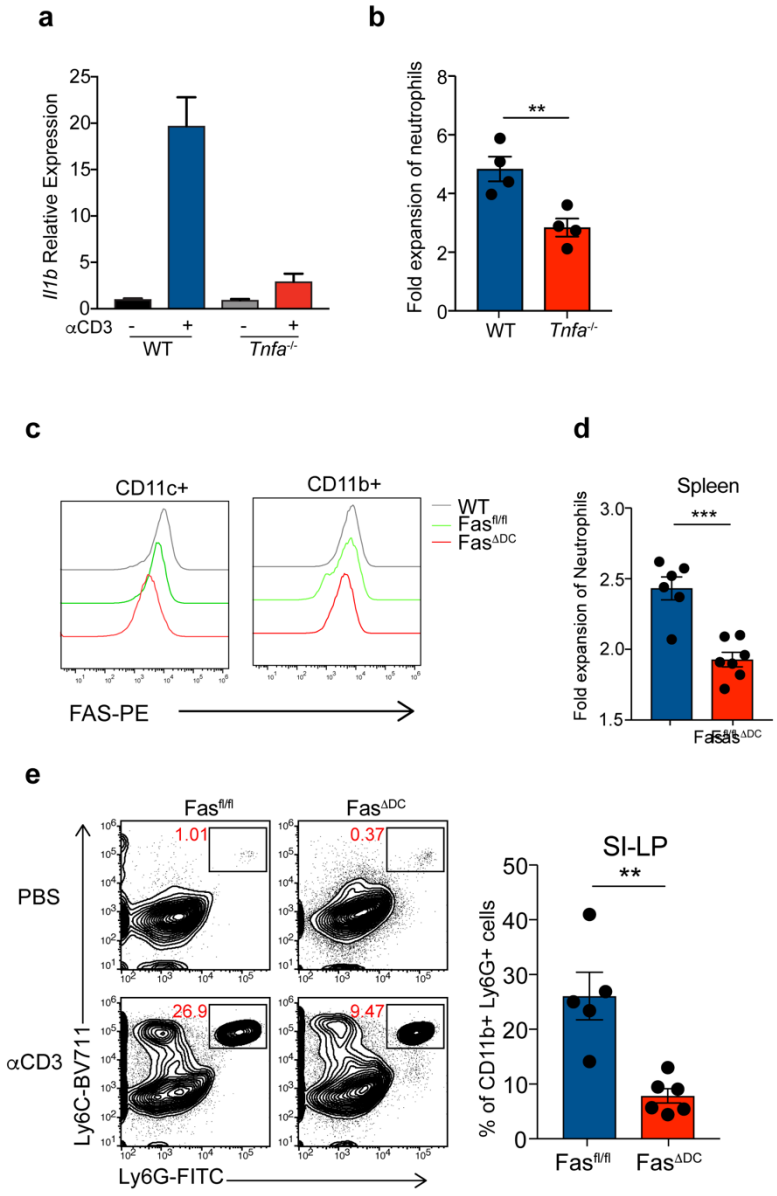

Figure 4-13. T cells activate TNFR-Fas signaling pathway to elicit IL-1β production and subsequent leukocyte requirement

(a) WT or *Tnfa*$^{-/-}$ mice were injected with αCD3 (50μg) intravenously. Spleen cells were harvested 3-4h post injection and immediately lysed for RNA isolation. Data are normalized to *Hprt1*. (b) WT or *Tnfa*$^{-/-}$ mice were treated with αCD3 i.p. Spleens were harvested 3h later and analyzed for

neutrophil infiltration. (c) Fas expression on DCs from given genotypes shows CD11c+ cell specific Fas deletion. (d, e) Fasfl/fl or Fasfl/fl x CD11c-cre ($Fas^{\Delta DC}$) mice were treated with αCD3 i.p.; 18h later (d) Spleen and (e) SI-LP analyzed for neutrophil infiltration. Error bars indicate SEM; Unpaired *t*- test.

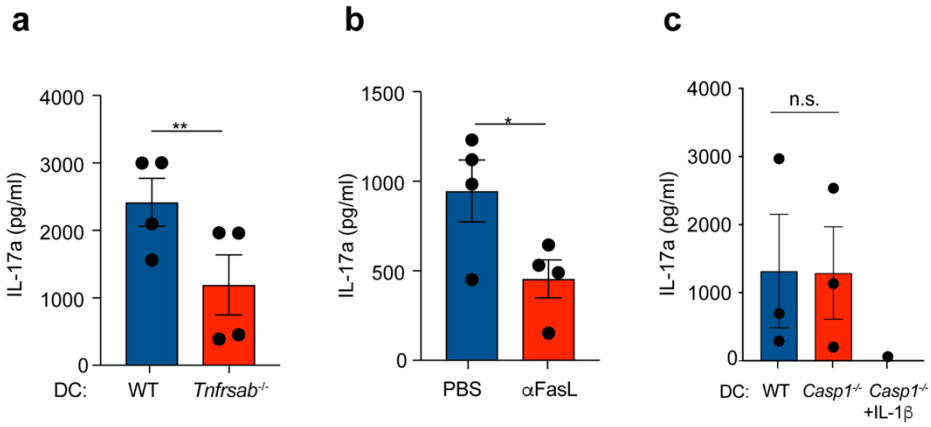

Figure 4-14. Memory CD4 T cell reactivation is dependent on DC intrinsic TNFR and Fas signaling

(a) $CD44^{hi}$ $CD62L^{lo}$ effector CD4 T cells were isolated from WT mice and re-stimulated with (a) WT or *Tnfrab*$^{-/-}$ CD11c+ve splenic DCs (b) WT DCs were used to stimulate $CD44^{hi}$ $CD62L^{lo}$ effector CD4 T cells in the presence or absence of anti-FasL neutralizing antibody and αCD3 (30ng/ml) or (c) WT or *Casp1*$^{-/-}$ CD11c+ve splenic DCs were used to stimulate $CD44^{hi}$ $CD62L^{lo}$ effector CD4 T cells. Quantities of IL-17a were measured in the culture supernatants 48h post stimulation. Error bars indicate SEM; paired *t*-test

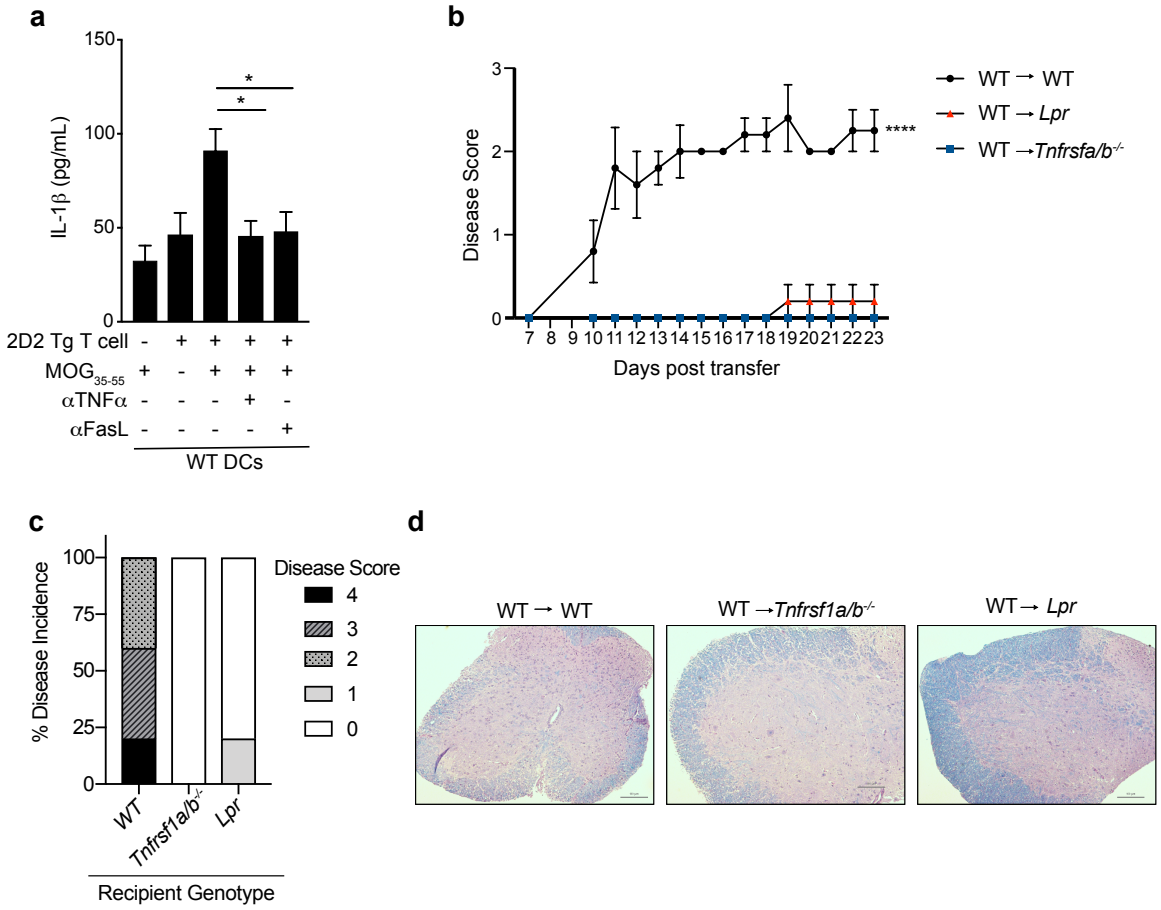

Figure 4-15. TNFR and Fas deficient mice are show significantly reduced T cell driven IL-1β production and associated autoimmune inflammation

(a) Th17 polarized 2D2 TCR Tg cells were re-stimulated with WT BMDCs and MOG_{35-55} (30μM) in the presence of absence of αTNFα (20 μg/ml) and αFasL (10 μg/ml). Secreted IL-1β was measured 18h post-stimulation. (b) Mean EAE clinical disease scores of WT, *Tnfrsfa/b*$^{-/-}$ or *Lpr* recipients after adoptive transfer of $7x10^6$ WT CD4 T cells primed *in vivo* with MOG_{35-55} n=5 (c) Percentage of mice with given disease scores in each recipient group (d) Luxol fast blue staining of spinal cords from given genotypes, 28 days after T cell transfer, was done to assess

demyelination. Scale bar is 100μm. Error bars indicate SEM; (a, b) unpaired *t*-test, (c) two-way repeated-measures ANOVA test.

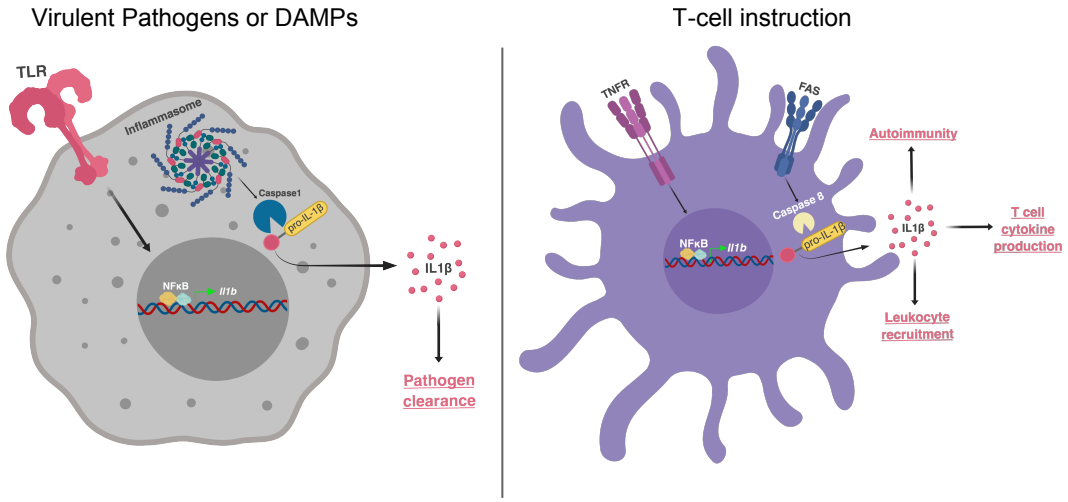

Figure 4-16. Illustration of "T cell instructed" IL-1β production by DCs and its comparison to inflammasome induced IL-1β production by macrophages

(Left) During inflammasome activation in macrophages, TLR and NLR-Caspase-1 activation leads to synthesis and cleavage of pro-IL-1β, respectively. Robust production of IL-1β as a result of inflammasome activation is critical for pathogen clearance. (Right) In contrast, "T cell instructed" IL-1β production by DCs utilizes TNFR signaling for pro-IL1β synthesis while the cleavage signal is provided by Fas-Caspase-8 axis. The IL-1β produced upon such T cell instruction drives cytokine production by effector CD4 T cells systemic leukocyte recruitment and autoimmunity. Figure was created with BioRender®.

CHAPTER FIVE

T Cells Activate Dendritic Cells Upon Cognate Interaction Bypassing the Need for Microbial Recognition for Innate Immune Activation

Introduction

DCs are professional antigen presenting cells that express a slew of surface, endosomal as well as cytosolic PRRs that recognize the conserved microbial ligands associated with pathogens (Takeuchi and Akira, 2010). During infection, activation of PRRs by microbial ligands leads to innate immune activation. Consequently, DCs undergo the process of maturation that includes, but is not limited to, upregulation of antigen presentation machinery, costimulatory molecules and innate cytokine production. Only mature DCs are able to successfully activate and prime a T cell response. DC activation relies on sensing of non-self-ligands by PRRs thus preventing triggering of T cell responses towards self-antigens. However, it remains unclear how DCs get activated occur during autoimmune scenarios. Autoimmune pathology is always associated with presence innate cytokines as well as chemokines in the damaged tissues. Moreover, the most successful treatment strategies for several autoimmune diseases are anti-cytokine biologics such as anti-TNFα and Anakinra underscoring the importance of understanding the mechanism of innate immune activation during autoimmunity.

Our data suggests that effector CD4 T cells can use TNF superfamily ligands to instruct IL-1β production by DCs independently of PRR activation. Induction of IL-1β is dependent on NF-κB activation which occurs downstream of TNFR signaling during DC-T cell interaction. It is conceivable NF-κB activation in DCs will result in transcriptional changes DCs beyond pro-IL-

1β induction. In addition to DCs, T cells might also interact with monocytes and macrophages to further contribute to inflammation. We hypothesized that the dysregulated innate immune inflammation seen in T cell mediated autoimmunity is caused by the "T cell instruction" of antigen presenting cells and that interaction with T cells can overcome the need for microbial sensing for innate immune activation.

Results

TNFRSF enables PRR independent DC activation by T cells

We first tested if DC-T cell interaction results in innate cytokine production in addition to IL-1β. We found that engagement with T cells also led to IL-6 and IL-12 production. Both IL-6 and IL-12 are mediators of inflammation as they can not only activate endothelial cells, myeloid cells but also skew T cell response away from Tregs. Interestingly, unlike IL-1β, loss of TNFR in DCs did not affect T cell induced IL-6 and IL-12 suggesting that additional signaling pathways are operating in DCs following their interaction with T cells (Figure 5-1a). CD40 is another TNFR superfamily member that leads to non-canonical NF-κB signaling. Although CD40 neutralization did not compromise IL-1β, we decided to test the role of CD40 signaling in IL-6 and IL-12 induction. Indeed, CD40 deficient DCs displayed comprised IL-6 as well as IL-12 (Figure 5-1b). Together this data indicates a division of labor within the TNFR super family for PRR independent innate immune activation. Necessity of Fas signaling for pro-IL-1β cleavage but not for its transcriptional induction also supports this idea. Unique function for different TNFRSF members also offers a good handle to surgically modulate DC function during sterile DC-T cell interaction.

DCs undergo unique transcriptional programming upon cognate interaction with T cells

In order to reveal the global effects of T cell instruction on DCs, we analyzed the transcriptional profile of DCs following their interaction with T cells (Figure 5-2). Additionally, we also stimulated DCs with LPS to directly compare PRR versus T cell instructed DC activation. Principle component analysis showed that the transcriptional landscape of DCs following LPS stimulation as well as T cell interaction was significantly distinct from their unstimulated controls. More importantly, LPS activated and T cell instructed DCs clustered separately implying unique features in the two treatment groups (Figure 5-3). Hierarchical clustering analysis of differentially expressed genes gave rise to a cluster that contained genes that are upregulated upon LPS treatment as well as T cell interaction (Figure 5-4). Gene ontology analysis of T cell induced genes showed enrichment in innate immune response genes (Figure 5-4). In agreement with our previous data this set of genes included *il6, il12 and il1b* suggesting that T cell elicit innate cytokine transcription in interacting DCs (Figure 5-5). Furthermore, this cluster contained also genes related to DC maturation such as *Cd80, Cd86* and *Cd40* showing the DCs can undergo maturation independently of PRR activation (Figure 5-5). However, the relative upregulation was significantly higher in LPS treated DCs compared to T cell interaction (Figure 5-5). The functional consequence of such quantitative difference remains to be investigated. The two treatment groups also share a large number of downregulated genes as shown in cluster two. Together, this data suggests that high avidity interaction with cognate T cells can, to a large extent, mimic PRR activation in DCs resulting in DC maturation and innate immune activation.

Surprisingly, there was a set of genes that were differentially expressed only in DCs only upon interaction with T cells but not by LPS stimulation suggesting that T cell instruction is not simply

milder version of PRR stimulation but is biologically distinct (Figure 5-6). Genes uniquely upregulated DCs upon T cell interaction included *Insig, Hmgcs, Hmgcr1* and *Tmem173* (Figure 5-6). Gene ontology analysis of this cluster showed enrichment of genes in the cholesterol synthesis pathway (Figure 5-6). Since these genes were not induced by PRR signaling, it will be interesting to study the biological significance of such T cell dependent transcriptional programming of DCs. *Tmem173* which encodes for the protein STING is one of the most intriguing hits in this transcriptomic analysis. STING is adaptor protein in the DNA sensing pathway (Barber, 2014). It is critical antiviral immune response to DNA viruses but can also be activated by endogenous host DNA causing interferonopathy. STING localizes at the endoplasmic reticulum membrane where the proteins in the cholesterol biosynthetic pathway such as INSIG and SCAP also reside. Moreover, it was recently shown that limiting lipid biosynthesis leads to STING activation as well as induction of ISGs further suggesting a link between STING and cholesterol biosynthesis (York et al., 2015). Interestingly, while STING was induced by T cell interaction, an associated interferon program was absent (Figure 5-7). On the other hand, LPS stimulation did not result in STING upregulation but elicited strong interferon signature, probably via TRIF signaling pathway (Figure 5-7). Together this data points to an interferon independent function of STING in DCs during their cognate interaction with T cells that needs systematic investigation.

Discussion

We know that innate control of adaptive immunity relies on microbial recognition by DCs through PRRs. Innate immune activation by PRRs is critical for innate cytokine production and thus for the generation of effector CD4 T cells. We have now discovered that effector T cells can in turn activate DCs to produce innate cytokines independently of PRR signaling. This is a consequential

extension to the concept of innate control of adaptive immunity. While we discovered the phenomena of T cell instruction of innate immunity in the context of autoreactive T cell activation, the implications of this work are likely to extend beyond autoimmunity. High avidity T cell interactions with DCs can also occur when the T cell suppression mechanism are absent. For example, over active T cells are observed in immune checkpoint blockade therapy. Although unhinged T cell function is beneficial for anti-tumor immunity, activated T cells can plausibly engage TNFRSF dependent inflammatory program in myeloid cells. Patients treated with anti-PD1 and anti-CTLA4 blocking antibodies often experience autoimmune like symptoms and excessive innate inflammation. However, the mechanism of innate activation following immunotherapy is not clear. It might be interesting to see if the TNFR or related signaling pathways are responsible for immunotherapy associated innate immune activation. Similar mechanism could also be operative in CAR-T driven immunopathology which remains an immense challenge in CAR-T cell therapy. Overall, our discovery of T cell instruction of innate immunity lays the conceptual groundwork for investigation in areas of research beyond autoimmunity and present a several therapeutic targets, like TNFα, CD40 and Fas, to treat sterile inflammation.

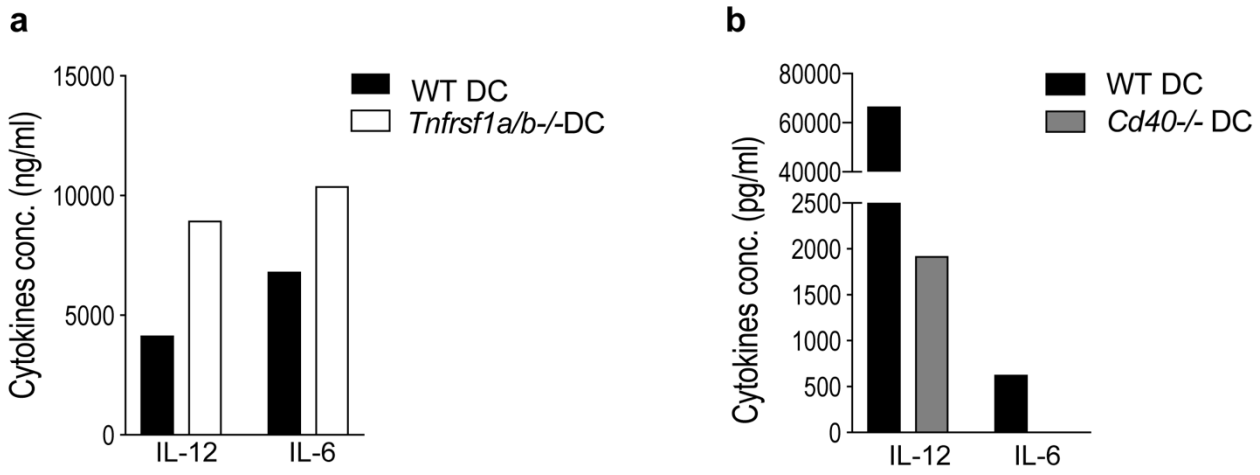

Figure 5-1. Unique roles of DC intrinsic TNFR and CD40 signaling in T cell induced innate cytokine secretion

Effector CD4 T cells (Th0) were stimulated with WT, (a) TNFRKO DC or (b) CD40 KO DC in the presence of αCD3 (200ng/ml). Secreted cytokines IL-6 and IL-12 were analyzed 6 hrs. post stimulation. Data representative of two independent experiments.

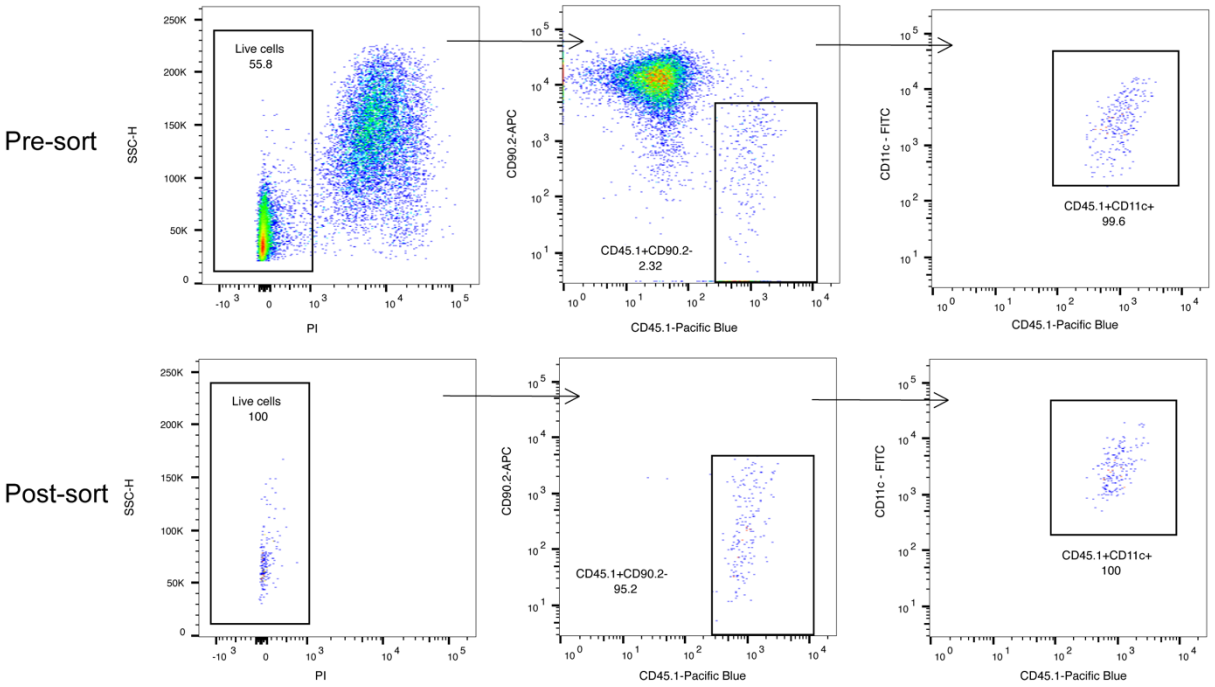

Figure 5-2. Gating strategy for sorting CD11c+ DCs following interaction with effector CD4 T cells for 3hrs

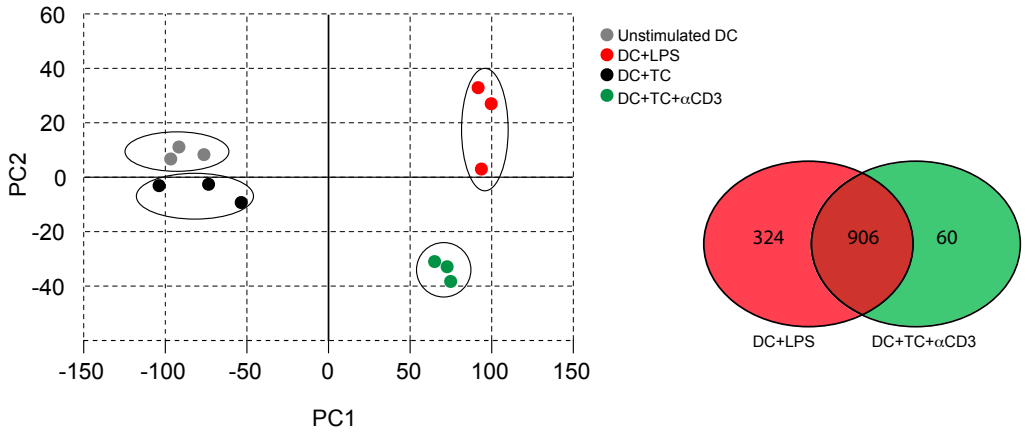

Figure 5-3. Comparison of differentially expressed genes in DCs following LPS stimulation versus T cell interaction

Principle component analysis of differentially expressed genes in DCs after given treatments. Venn diagram showing total number of commonly and uniquely upregulated genes in DCs after LPS treatment versus T cell interaction.

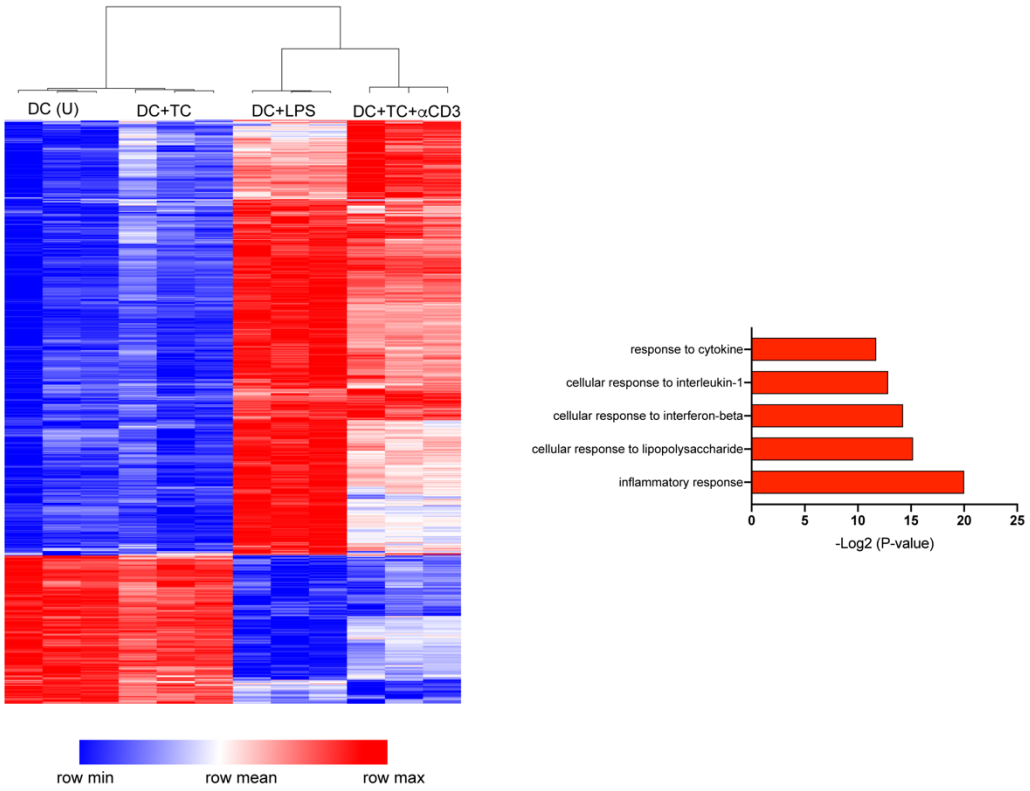

Figure 5-4. Hierarchical clustering of differentially expressed genes in DCs following LPS stimulation versus T cell interaction

Heatmap showing hierarchical clustering of differentially expressed genes common to both treatments. Gene ontology analysis of gene set containing all genes upregulated in DCs upon T cell interaction. Yajing Gao (Pasare lab) performed the clustering Hierarchical analysis.

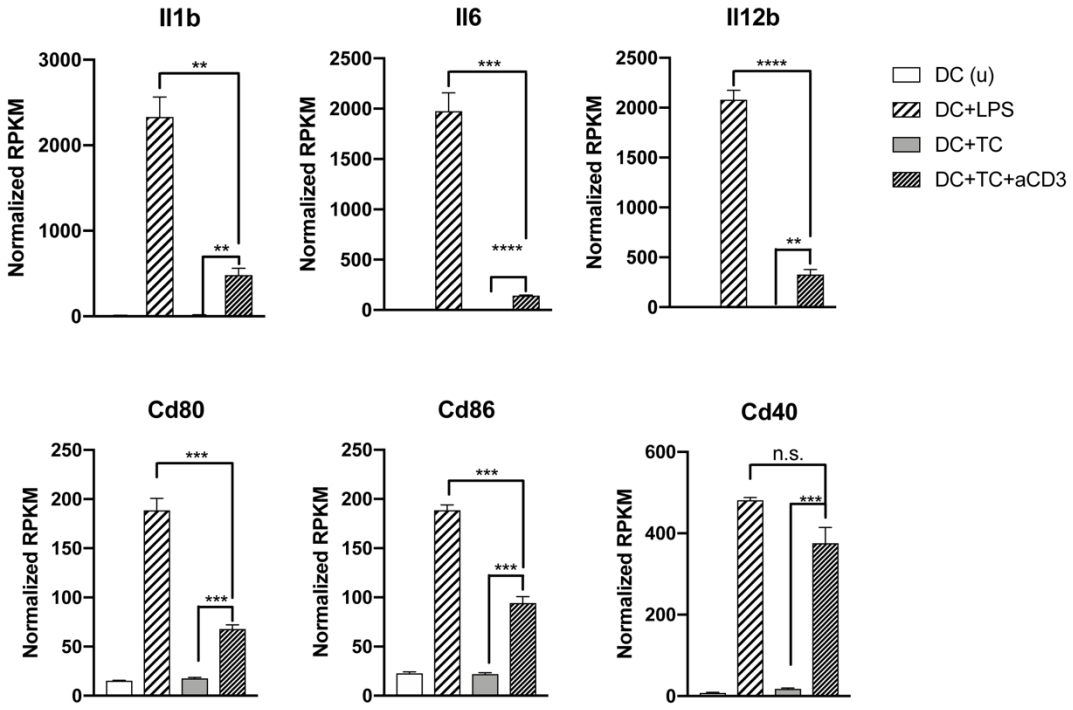

Figure 5-5. Normalized RPKM values of gene transcripts involved in DC maturation obtained from RNA sequencing analysis

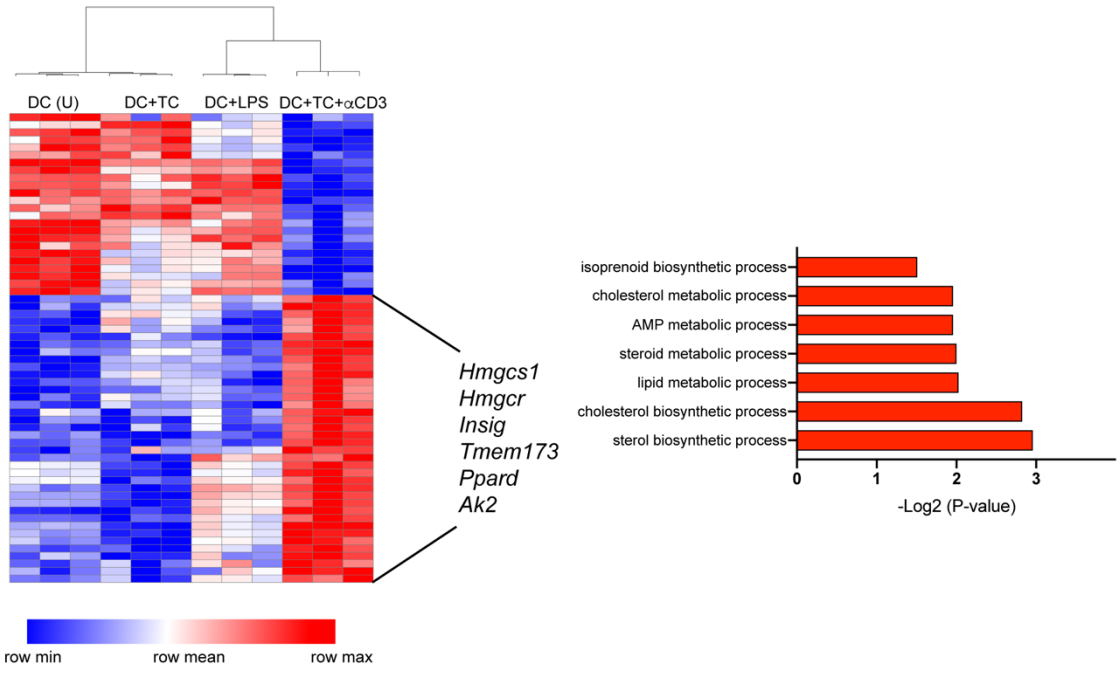

Figure 5-6. Hierarchical clustering of differentially expressed genes in DCs following T cell interaction but not LPS stimulation

Heatmap showing hierarchical clustering of differentially expressed genes common to both treatments. Gene ontology analysis of gene set containing gene uniquely upregulated in DCs upon T cell interaction. Yajing Gao (Pasare lab) performed the clustering Hierarchical analysis.

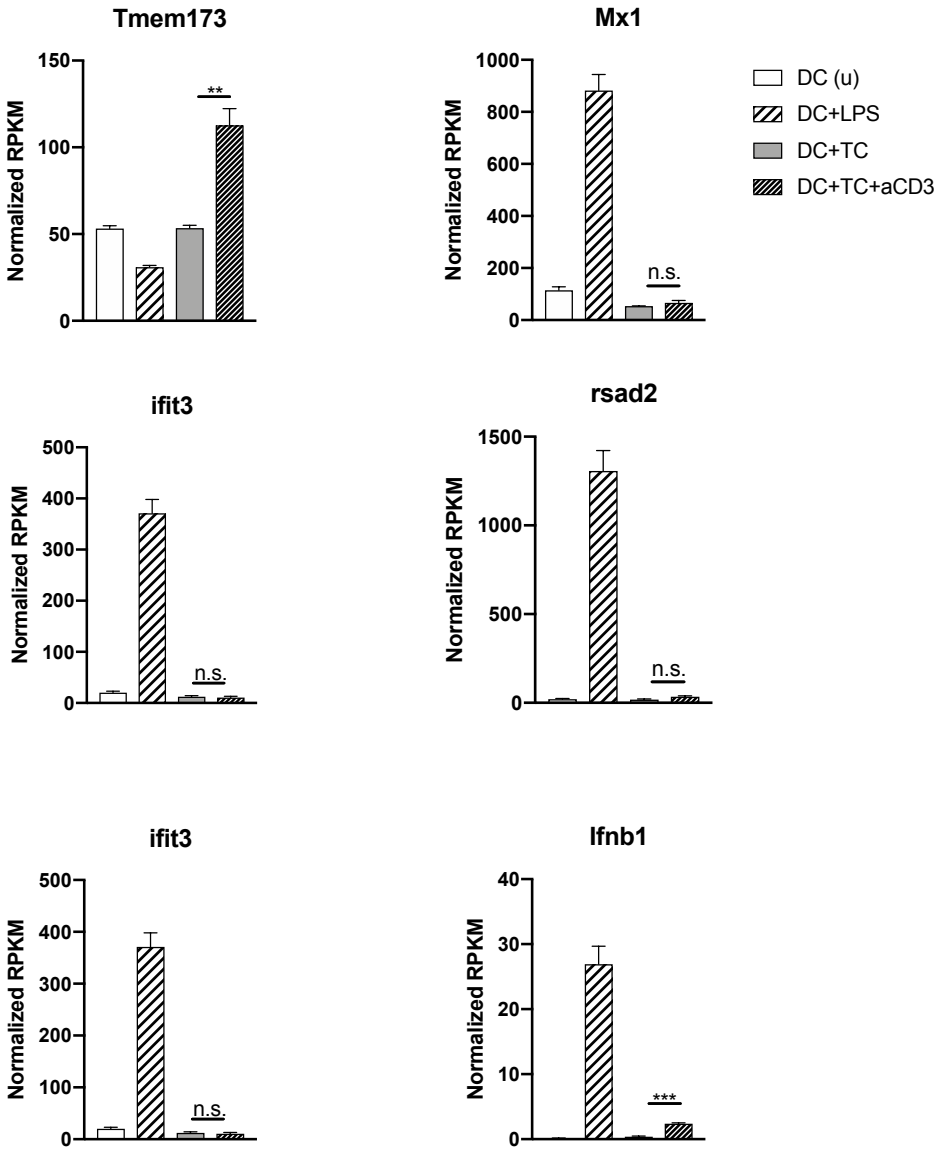

Figure 5-7. Normalized RPKM values of gene transcripts in the STING pathway obtained from RNA sequencing analysis

DISCUSSION

Overview

A productive immune response is a result of a highly sophisticated cross-talk between the innate and the adaptive immune system. Macrophages and DCs sense microbial presence through the expression of PRRs and instruct an appropriate T cell response. Although the fundamental principles of innate control of adaptive immunity are well established in the context of naïve CD4 T cell differentiation by DCs, the molecular nature of DCs and memory CD4 T cell interaction is poorly studied. In this dissertation research, I asked two fundamental question. First, what innate cues are required for memory CD4 T cell reactivation? and second, how does memory CD4 T cell reactivation can occur in the absence of microbial sensing by DCs, particularly in the case of autoimmune diseases? These questions are not only relevant to our understanding of T cell biology but also have immense clinical implications as autoimmune flares are often associated with dysregulated memory T cell responses.

The data I have presented in chapter three provides compelling evidence that analogous to naïve CD4 T cell differentiation, memory CD4 T cell reactivation also rely on three signal-paradigm for optimal function. I found that, in addition to TCR and co-stimulation, memory CD4 T cell require innate cytokine cues reactivation. In particular, IL-1R and related signaling was found to be necessary for licensing CD4 T cell effector function. More importantly, I showed that IL-1R signaling is critical for cytokine production by all CD4 T cell lineages, Th1, Th2 and Th17 (Figure 6-1) (Jain et al., 2018). Since memory CD4 T cells were thought to be independent of innate cytokine cues, this discovery provides a conceptual leap in our understanding of innate regulation of memory CD4 T cells. It also has implications on human health as it sheds light on the previously

unclear mechanism of action of widely used IL-1R blockade in CD4 T cell mediated autoimmune disease.

IL-1β is an inflammatory innate cytokine which is produced when myeloid cells sense a virulent pathogen. The findings above led us to ask how IL-1β is produced during T cell driven autoimmunity in the absence of pathogen invasion. Therefore, I decided to delineate the mechanism of IL-1β secretion specifically during sterile inflammation caused by autoreactive T cell activation. The results presented in chapter four show that effector CD4 T cells can instruct DCs to produce innate cytokine, IL-1β, independently of microbial sensing by DCs. Self-reactive T cell engage on TNFR and Fas-Caspase-8 signaling in interacting DCs to induce pro-IL-1β and its bioactive cleavage, respectively (Figure 6-1). Consequently, TNFR and Fas deficient mice were also found to be resistant to T cell mediated autoimmune inflammation. More importantly, T cell induced IL-1β production was completely independent of canonical as well as non-canonical inflammasomes.

I further extended these findings, in chapter five, showing that DCs undergo global transcriptional changes mimicking PRR activation when they interact with high avidity effector CD4 T cells. This is a consequential extension to our understanding of innate and adaptive immune cross-talk and thus, the implications of this work are likely to extend beyond autoimmunity. It is conceivable that TNFR dependent mechanism could also be responsible for innate immune activation as seen in tumor immunotherapy as well as graft versus host disease. The research presented here could thus prove to be crucial in novel drug discovery for therapeutic intervention during dysregulated immune activation.

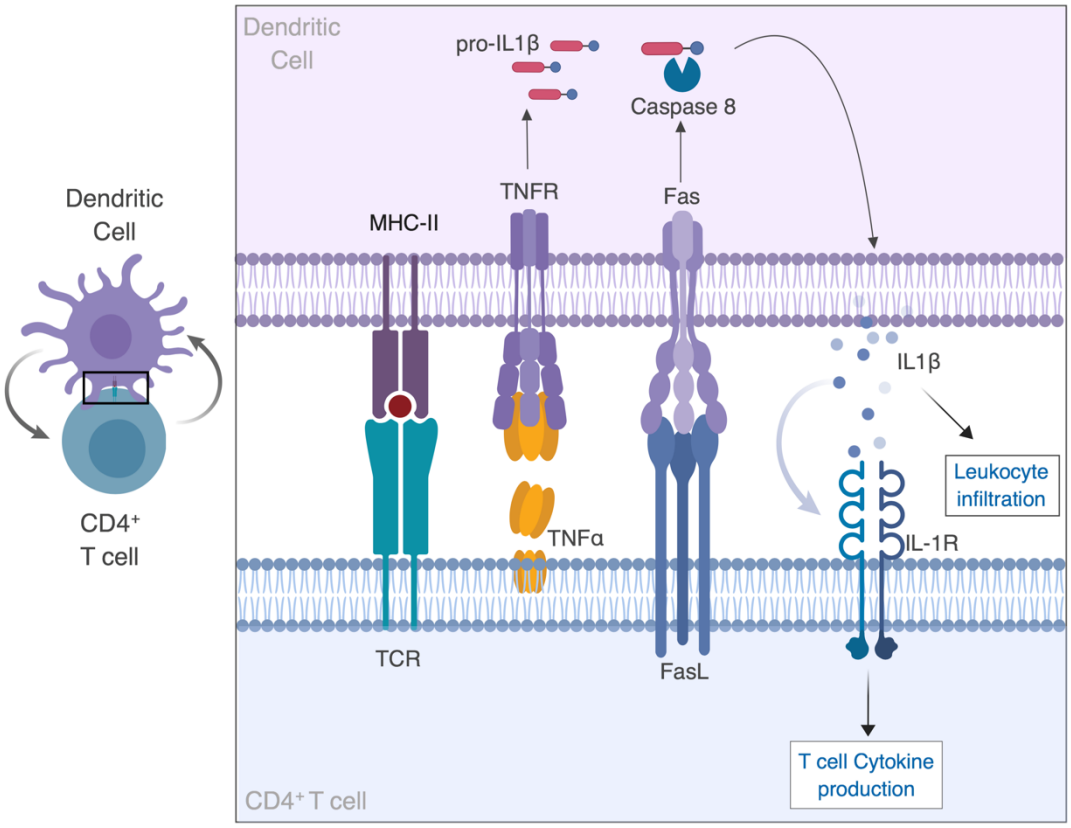

Figure 6-1. Inflammasome independent IL-1β drives CD4 T cell effector function and leukocyte infiltration

Innate control of memory CD4 T cell effector function

Naïve CD4 T cell differentiation is known to be tightly regulated by the innate immune system. In my dissertation research, I aimed to understand the role of innate cytokines during memory CD4 T cell reactivation. To tease out the cytokine dependence within different T cell populations, I analyzed their cytokine receptor profile. We found that memory CD4 T cells downregulated receptors for priming cytokines but constitutively expressed IL-1 family receptors. In agreement with this data, we discovered that while previously differentiated CD4 T cells are minimally dependent on priming cytokines, they require IL-1 family of cytokines for optimal effector function. This data points to a qualitative disparity between the mechanism of innate regulation of naïve and memory CD4 T cells. There appears to be a shift from dependence on JAK-STAT signaling during priming phase to MyD88 signaling during the reactivation phase. Furthermore, while MyD88 serves as a central node that regulates reactivation of all CD4 T cell lineages, the necessity for a specific IL-1 family member seem to be dependent on the nature of immune challenge. I found that *C. rodentium* specific CD4 T cells were dependent on IL-1R signaling while *L. monocytogenes* specific CD4 T cells required IL-18R signaling for optimal cytokine secretion. This distinction in cytokine requirement could also be a result of route of infection as mice were infected with *C. rodentium* orally while *L. monocytogenes* was administered intraperitoneally. Microenvironment and pathogen dependent cytokine requirements could be dictated by either the availability of specific innate cytokine or its receptor expression on CD4 T cells or both. A thorough cytokine receptor expression analysis could provide insights into innate cytokine mediated regulation of T cell function.

Extending findings from CD4 T cell to innate lymphoid cells

The data in chapter three shows that IL-1R signaling stabilizes *Il17a, Il17f, Il22, Il13* and *Il5* transcripts. These cytokines are also produced by innate lymphoid cells upon stimulation with specific IL-1 family of cytokines. IL-18, IL-33 and IL-1β are known to promote cytokine production by ILC1, ILC2 and ILC3 populations, respectively (Cortez et al., 2015). However, the mechanism of IL-1 family dependent cytokine production is not properly investigated. Since T cell cytokines are inherently highly susceptible to RNA decay and are post transcriptionally stabilized by IL-1R signaling, it is possible that IL-1 family also licenses ILC function via a similar post-transcriptional mechanism.

Inherent challenges in studying memory CD4 T cell function

A memory immune response provides rapid protection to the host against reinfection. Generation of memory cells has demonstrated in B cell, CD8 T cell as well as CD4 T cell compartments. Memory is manifested by the generation of long-lived antigen specific cells, higher precursor frequency due to primary expansion phase and rapid production of cytokines. Expression profile of surface markers is widely used to identify memory T cells. Memory CD4 T cells are distinguished from their naïve counterparts based on their high expression of CD44 and low levels of CD62L.

The necessity of antigen specific memory CD8 T cell function is often demonstrated by heightened host immunity upon antigen re-exposure. Since CD8 T cells are directly involved in killing the infected cells or tumors, one can simply analyze the pathogen or tumor burden during re-challenge to test the effectivity of memory CD8 T cells following biological perturbations. Due to the

simplicity in studying direct CD8 T cell function, significant amount of work has been done to establish cellular and molecular players required for CD8 memory T cells generation and reactivation. However, the same is not true for CD4 T cell memory. While we know that a fraction of CD4 T cells survive the contraction phase to become long lived, the relevance of CD4 T cell memory has remained elusive. A successful primary CD4 T cell response enables development of memory CD8 T cells, memory B cells as well as antibody producing plasma cells. Once generated, memory CD8 T cells and B cells do not require memory CD4 T cell help for antigen-specific function. Therefore, whether memory CD4 T cell contribute at all to enhance overall memory responses remains elusive.

One potential scenario where memory CD4 T cells appear to provide protection is in the case of influenza infection. The influenza virus incorporates mutations to escape antibody and CD8 mediated immunity however, its CD4 T cell epitopes remain intact (Teijaro et al., 2010). While there is some correlative evidence, the necessity of memory CD4 T cells function in influenza immunity has not been adequately tested. Recently, a strain of streptococcus of pneumonia was also shown to induce long lived CD4 memory that could be reactivated to produce IL-17a upon reinfection. Although there is limited *in vivo* evidence for memory CD4 T cell function, higher number of CD4 T cells are correlated with improves anti-pathogen and anti-tumor immunity. While I used *in vitro* reactivation methods to measure memory CD4 T cells responses, the development of MHCII tetramers and sensitive cytokine detection techniques can be helpful to study of CD4 T cell memory response *in vivo*.

Unique functions of IL-1α and IL-1β

IL-1R1 serves as cognate receptor for both IL-1α and IL-1β. Even though both of these proteins bind to the same receptor and have similar structures, distinctions do exist. Differences between IL-1α and IL-1β are largely due to their cellular sources and mechanism of production. IL-1α is constitutively expressed in the mucosal barriers, astrocytes and endothelial cells. Upon necrosis, IL-1α is released and acts as alarmin. Unlike IL-1β, Il-1α precursor shows biological activity which accounts for sterile inflammation during tissue injury. IL-1β, on the other hand, is induced in myeloid cells upon PRR stimulation. IL-1β precursor requires maturational cleavage which can other in both inflammasome dependent or independent manner as described in chapter 4. Several reports shed light on the differences between the biological roles of IL-1α and IL-1β. IL-1β deficient mice develop fewer tumors as compared to WT and IL-1α deficient mice (Voronov et al., 2013). Moreover, it was recently shown that IL-1α but not IL-1β is associated with atherosclerosis (Freigang et al., 2013).

Data shown in chapter three suggests that the IL-1β but not IL-1α is required for licensing CD4 T cell effector function during their reactivation with DCs. However, it is important to note that the *in vitro* experimental setup in that study did not include stromal cells that are likely provide IL-1α to an ongoing immune response *in vivo*. The data showing that exogenous addition of IL-1α can also license cytokine production by CD4 T cells also suggests that IL-1α can play a role in CD4 T cell reactivation. Therefore, it is critical to study the unique roles of IL-1α and IL-1β in CD4 T cell effector function over a wide range of pathogens and route of infections. Since IL-1α and IL-1β share their cognate receptor their signaling consequences is also thought to be largely

overlapping. However, given their distinct biological function it might be interesting to look at global transcriptional effects of IL-1α and IL-1β.

Significance inflammasome independent mechanisms of IL-1β production

Interleukin-1β is critical for anti-microbial host defense and also serves as a key mediator of autoimmune inflammation. IL-1β production requires induction of transcription by innate immune receptor signaling and processing of pro-IL-1β by proteases. Inflammasome activation is the most well studied mechanism of active IL-1β production. So far functional inflammasomes has been reported in macrophages, monocytes as well as epithelial cells. In these cell types, inflammasome is activated in response to virulent pathogens or damage associated molecular patterns. There are two major cellular consequences of inflammasome activation. First the multimeric inflammasome complex recruits the protease caspase-1 that undergoes autoproteolytic cleavage for activation. Caspase-1 activation is a hallmark of inflammasome activation and leads to bioactive IL-1β and IL-18 production. While many substrates for caspase-1 have been predicted, IL-1β and IL-18 remain its most prominent targets (Keller et al., 2008). Second, once the cell commits to the inflammasome pathway, it undergoes inflammatory cells death that is associated with spillage of its cytosolic contents. This type of cell death is called pyroptosis and is mediated by Gasdermin D that initiates cell membrane rupture. Altogether, inflammasome is highly inflammatory process which is accompanied by the elimination of the host cell. While such a process is advantageous to remove the replicative niche of virulent pathogens and trigger downstream immune response, the associated inflammation can cause immunopathology.

While IL-1β contributes to inflammation it also plays several homeostatic functions. IL-1R signaling is critical for intestinal homeostasis, tissue repair as well as optimal neuronal response. Since these processes are immunologically silent, it is conceivable that additional mechanisms of IL-1β production have emerged over the course of evolution. In addition to caspase-1, cathepsin C and Caspase-8 have also been shown to cleave pro-IL-1β. In my dissertation research I uncovered a previously unknown mechanism of IL-1β production by DCs that is triggered upon DC-T cell interaction. TNFR and Fas are expressed on most cell types, it is possible that TNFR-Fas dependent mechanism described in chapter 4 could also be employed for homeostatic IL-1β production.

Limited models to study *bona fide* T cell mediated sterile autoimmunity

Animal models are the core of autoimmune disease research as they provide insights into underlying etiologies and involvement of specific signaling pathways. The autoimmune animal models can be broadly divided into three type: Spontaneous model, genetically engineered models, antigen-induced models. Some mouse strains have genetic susceptibility to autoimmunity such as NOD mice develop spontaneous T1D (Atkinson and Leiter, 1999) and NZB/W F1 mouse model exhibit SLE-like pathology (Dubois et al., 1966). Genetic manipulations can include knock-out or knock-in mouse models and transgenic lines. For example, Mrl/LPR mice develop autoimmune like lymphoproliferation due to lack of Fas mediated cell death (Watanabe-Fukunaga et al., 1992). Foxp3-DTR knock-in mice can have inducible deletion of Tregs leading to autoimmune inflammation (Kim et al., 2007a). However, such models are not available for several autoimmune conditions. In these scenarios, pathogenic T cells are generated via extrinsic immune challenges with a known self-antigen to induce disease. Since injection with the antigen is not sufficient to

activate DCs, antigen is traditionally emulsified with CFA to mature DCs. CFA is a potent DC stimulant and therefore enables activation of self-reactive T cells that will normally ignore self-peptides. This strategy is used to induce EAE by immunizing with myelin peptides (Stromnes and Goverman, 2006a). Collagen-induced arthritis is triggered following immunization with collagen II with immunostimulants like CFA (Brand et al., 2007). While these models allow for reductionist approach, they do not faithfully represent the innate and adaptive immune activation that occurs in the absence of extrinsic immune challenge during autoimmunity. Therefore, the results from these studies could be confounded by presence of PAMPs during immune activation. One way to circumvent these caveats, at least in T cell mediated autoimmunity, is to adoptively transfer pathogenic T cells into otherwise unmanipulated recipients. This strategy is often used to induce EAE as well as type 1 diabetes where TCR Tg T cells have been shown to be sufficient in initiating paralysis or insulitis, respectively (Haskins, 2005; Stromnes and Goverman, 2006b). Overall, it is critical to take into account the extrinsic stimulus used in animal models of autoimmunity in order for appropriately infer the results.

TNFR superfamily in adaptive instruction of innate immunity

The expression of TNFRSF members is widespread yet their requirement for protective immunity seems to be contextual, suggesting that they could be key players in modulating innate and adaptive immune interactions (Ward-Kavanagh et al., 2016). The adaptive instruction of autoimmunity illustrated in chapter four and chapter five has led to several intriguing questions of both conceptual and clinical significance. Date presented here show that TNFR, Fas and CD40 signaling contribute to T cells induced DC activation via distinct mechanisms. While TNFR signaling leads to pro-IL-1β synthesis, Fas signaling is required for maturational cleavage of pro-IL-1β. Moreover, even

though abrogation of CD40 does not significantly affect IL-1β production, it leads to complete loss of T cell induced IL-6 and IL-12 by DCs. These data suggest that TNFR super family members have evolved to accomplish distinct role in adaptive instruction of innate immunity. Studying the global transcriptional profile of DCs following their cognate interaction with T cells in the absence of individual TNFR super family members could unravel the division of labor that seems to exist within the TNFRSF.

It will also be interesting to analyze the contribution of TNFRSF in response to tumor immunotherapy. There is often widespread inflammation and extensive stromal and hematopoietic cell cross-talk in tumor beds following immunotherapy (Grivennikov et al., 2010). The fact that TNFRSF signaling leads to NF-kB activation and is largely employed in intercellular communication offers a compelling case for studying this receptor family in the context of anti-tumor immunity. Moreover, TNF superfamily are also expressed by many other immune cells as well as stromal cells (Croft et al., 2012). Therefore, non-immune cells might utilize TNFRSF to contribute to ongoing immune response or restoration of homeostasis.

Overall Conclusions

The goal for studying innate and adaptive immune cross-talk is to reveal how this interaction gives rises to a functional immune response. Insights into the innate cues necessary for T cell immunity have proven to be instrumental to our understanding of anti-microbial responses as well as autoimmune inflammation. A great body of research led to in-depth understanding of the innate cytokines made by DCs and their impact on naïve CD4 T cell differentiation. However, the role of innate cytokine for memory CD4 T cell function has not been rigorously tested. Additionally,

there has been clinical success in anti-cytokine therapies for autoimmune disease, yet the exact mechanisms responsible for innate cytokine production in T cell mediated autoinflammation remain obscure. The work presented here provides compelling evidence that IL-1β is required for memory CD4 T cell function. Mechanistically, IL-1R signaling was found to be critical for post-transcriptional stabilization of T cell cytokines, a previously unknown mechanism of regulation of T cell responses. Furthermore, I found that while memory CD4 T cell rely on IL-1β for their function, their reactivation was independent of PRR signaling in interacting DCs. This is paradoxical since IL-1β is an innate cytokine made by myeloid cells in response to inflammasome activation. The findings in this dissertation research establish the existence of a TLR and inflammasome-independent pathway of IL-1β production. Effector T cell were found to engage TNFR-Fas signaling in DCs to trigger a novel pathway of mature IL-1β secretion. Together, the current study has uncovered fundamental principles of innate and adaptive immune cross-talk. This work is also immediately applicable to human health as it presents the mechanism of action of IL-1β blockade therapies as well as novel targets for tempering autoimmune inflammation. Future studies will focus on exploring the vast implications of "T cell-instruction" on myeloid cells in autoinflammation observed in immune checkpoint blockade therapy, CAR-T therapy, as well as graft versus host disease.

BIBLIOGRAPHY

Afonina, I.S., Muller, C., Martin, S.J., and Beyaert, R. (2015). Proteolytic Processing of Interleukin-1 Family Cytokines: Variations on a Common Theme. Immunity *42*, 991-1004.

Ainscough, J.S., Frank Gerberick, G., Zahedi-Nejad, M., Lopez-Castejon, G., Brough, D., Kimber, I., and Dearman, R.J. (2014). Dendritic cell IL-1alpha and IL-1beta are polyubiquitinated and degraded by the proteasome. J Biol Chem *289*, 35582-35592.

Anderson, M.S., Venanzi, E.S., Klein, L., Chen, Z., Berzins, S.P., Turley, S.J., von Boehmer, H., Bronson, R., Dierich, A., Benoist, C.*, et al*. (2002). Projection of an immunological self shadow within the thymus by the aire protein. Science *298*, 1395-1401.

Anderson, P. (2008). Post-transcriptional control of cytokine production. Nat Immunol *9*, 353-359.

Ariotti, S., Hogenbirk, M.A., Dijkgraaf, F.E., Visser, L.L., Hoekstra, M.E., Song, J.Y., Jacobs, H., Haanen, J.B., and Schumacher, T.N. (2014). T cell memory. Skin-resident memory CD8(+) T cells trigger a state of tissue-wide pathogen alert. Science *346*, 101-105.

Atarashi, K., Nishimura, J., Shima, T., Umesaki, Y., Yamamoto, M., Onoue, M., Yagita, H., Ishii, N., Evans, R., Honda, K.*, et al*. (2008). ATP drives lamina propria T(H)17 cell differentiation. Nature *455*, 808-U810.

Atkinson, M.A., and Leiter, E.H. (1999). The NOD mouse model of type 1 diabetes: as good as it gets? Nat Med *5*, 601-604.

Bachmann, M.F., Gallimore, A., Linkert, S., Cerundolo, V., Lanzavecchia, A., Kopf, M., and Viola, A. (1999). Developmental regulation of Lck targeting to the CD8 coreceptor controls signaling in naive and memory T cells. Journal of Experimental Medicine *189*, 1521-1529.

Bachmann, M.F., and Kopf, M. (2001). On the role of the innate immunity in autoimmune disease. J Exp Med *193*, F47-50.

Baldassare, J.J., Bi, Y., and Bellone, C.J. (1999). The role of p38 mitogen-activated protein kinase in IL-1 beta transcription. J Immunol *162*, 5367-5373.

Barber, G.N. (2014). STING-dependent cytosolic DNA sensing pathways. Trends Immunol *35*, 88-93.

Basu, R., O'Quinn, D.B., Silberger, D.J., Schoeb, T.R., Fouser, L., Ouyang, W., Hatton, R.D., and Weaver, C.T. (2012). Th22 cells are an important source of IL-22 for host protection against enteropathogenic bacteria. Immunity *37*, 1061-1075.

Basu, R., Whitley, S.K., Bhaumik, S., Zindl, C.L., Schoeb, T.R., Benveniste, E.N., Pear, W.S., Hatton, R.D., and Weaver, C.T. (2015). IL-1 signaling modulates activation of STAT transcription factors to antagonize retinoic acid signaling and control the TH17 cell-iTreg cell balance. Nat Immunol *16*, 286-295.

Ben-Sasson, S.Z., Hu-Li, J., Quiel, J., Cauchetaux, S., Ratner, M., Shapira, I., Dinarello, C.A., and Paul, W.E. (2009). IL-1 acts directly on CD4 T cells to enhance their antigen-driven expansion and differentiation. P Natl Acad Sci USA *106*, 7119-7124.

Berg, R.E., Crossley, E., Murray, S., and Forman, J. (2003). Memory CD8(+) T cells provide innate immune protection against Listeria monocytogenes in the absence of cognate antigen. Journal of Experimental Medicine *198*, 1583-1593.

Bettelli, E., Pagany, M., Weiner, H.L., Linington, C., Sobel, R.A., and Kuchroo, V.K. (2003). Myelin oligodendrocyte glycoprotein-specific T cell receptor transgenic mice develop spontaneous autoimmune optic neuritis. J Exp Med *197*, 1073-1081.

Beuscher, H.U., Gunther, C., and Rollinghoff, M. (1990). IL-1 beta is secreted by activated murine macrophages as biologically inactive precursor. J Immunol *144*, 2179-2183.

Bevan, M.J. (2004). Helping the CD8(+) T-cell response. Nat Rev Immunol *4*, 595-602.

Biondo, C., Mancuso, G., Midiri, A., Signorino, G., Domina, M., Lanza Cariccio, V., Mohammadi, N., Venza, M., Venza, I., Teti, G., *et al*. (2014). The interleukin-1beta/CXCL1/2/neutrophil axis mediates host protection against group B streptococcal infection. Infect Immun *82*, 4508-4517.

Birnbaum, M.E., Mendoza, J.L., Sethi, D.K., Dong, S., Glanville, J., Dobbins, J., Ozkan, E., Davis, M.M., Wucherpfennig, K.W., and Garcia, K.C. (2014). Deconstructing the peptide-MHC specificity of T cell recognition. Cell *157*, 1073-1087.

Blom, L., and Poulsen, L.K. (2012). IL-1 family members IL-18 and IL-33 upregulate the inflammatory potential of differentiated human Th1 and Th2 cultures. J Immunol *189*, 4331-4337.

Bluestone, J.A., Bour-Jordan, H., Cheng, M., and Anderson, M. (2015). T cells in the control of organ-specific autoimmunity. J Clin Invest *125*, 2250-2260.

Blum, J.S., Wearsch, P.A., and Cresswell, P. (2013). Pathways of antigen processing. Annu Rev Immunol *31*, 443-473.

Boesteanu, A.C., and Katsikis, P.D. (2009). Memory T cells need CD28 costimulation to remember. Semin Immunol *21*, 69-77.

Borowski, A.B., Boesteanu, A.C., Mueller, Y.M., Carafides, C., Topham, D.J., Altman, J.D., Jennings, S.R., and Katsikis, P.D. (2007). Memory CD8+ T cells require CD28 costimulation. J Immunol *179*, 6494-6503.

Bossaller, L., Chiang, P.I., Schmidt-Lauber, C., Ganesan, S., Kaiser, W.J., Rathinam, V.A., Mocarski, E.S., Subramanian, D., Green, D.R., Silverman, N., *et al*. (2012). Cutting edge: FAS (CD95) mediates noncanonical IL-1beta and IL-18 maturation via caspase-8 in an RIP3-independent manner. J Immunol *189*, 5508-5512.

Brand, D.D., Latham, K.A., and Rosloniec, E.F. (2007). Collagen-induced arthritis. Nat Protoc *2*, 1269-1275.

Brennan, F.M., Chantry, D., Jackson, A., Maini, R., and Feldmann, M. (1989). Inhibitory effect of TNF alpha antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. Lancet *2*, 244-247.

Bretscher, P., and Cohn, M. (1970). A theory of self-nonself discrimination. Science *169*, 1042-1049.

Byrne, J.A., Butler, J.L., and Cooper, M.D. (1988). Differential activation requirements for virgin and memory T cells. J Immunol *141*, 3249-3257.

Casolaro, V., Fang, X., Tancowny, B., Fan, J., Wu, F., Srikantan, S., Asaki, S.Y., De Fanis, U., Huang, S.K., Gorospe, M., *et al*. (2008). Posttranscriptional regulation of IL-13 in T cells: role of the RNA-binding protein HuR. J Allergy Clin Immunol *121*, 853-859 e854.

Cayrol, C., and Girard, J.P. (2014). IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy. Curr Opin Immunol *31*, 31-37.

Chang, J., Burkett, P.R., Borges, C.M., Kuchroo, V.K., Turka, L.A., and Chang, C.H. (2013). MyD88 is essential to sustain mTOR activation necessary to promote T helper 17 cell proliferation by linking IL-1 and IL-23 signaling. P Natl Acad Sci USA *110*, 2270-2275.

Chatila, T., Silverman, L., Miller, R., and Geha, R. (1989). Mechanisms of T-Cell Activation by the Calcium Ionophore Ionomycin. Journal of Immunology *143*, 1283-1289.

Chen, C.Y., and Shyu, A.B. (1995). AU-rich elements: characterization and importance in mRNA degradation. Trends Biochem Sci *20*, 465-470.

Chen, J., Cascio, J., Magee, J.D., Techasintana, P., Gubin, M.M., Dahm, G.M., Calaluce, R., Yu, S., and Atasoy, U. (2013). Posttranscriptional gene regulation of IL-17 by the RNA-binding protein HuR is required for initiation of experimental autoimmune encephalomyelitis. J Immunol *191*, 5441-5450.

Chung, Y., Chang, S.H., Martinez, G.J., Yang, X.X.O., Nurieva, R., Kang, H.S., Ma, L., Watowich, S.S., Jetten, A.M., Tian, Q., *et al*. (2009). Critical Regulation of Early Th17 Cell Differentiation by Interleukin-1 Signaling. Immunity *30*, 576-587.

Cogswell, J.P., Godlevski, M.M., Wisely, G.B., Clay, W.C., Leesnitzer, L.M., Ways, J.P., and Gray, J.G. (1994). NF-kappa B regulates IL-1 beta transcription through a consensus NF-kappa B binding site and a nonconsensus CRE-like site. J Immunol *153*, 712-723.

Coombes, J.L., Siddiqui, K.R., Arancibia-Carcamo, C.V., Hall, J., Sun, C.M., Belkaid, Y., and Powrie, F. (2007). A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. J Exp Med *204*, 1757-1764.

Cortez, V.S., Robinette, M.L., and Colonna, M. (2015). Innate lymphoid cells: new insights into function and development. Curr Opin Immunol *32*, 71-77.

Croft, M., Duan, W., Choi, H., Eun, S.Y., Madireddi, S., and Mehta, A. (2012). TNF superfamily in inflammatory disease: translating basic insights. Trends Immunol *33*, 144-152.

Crotty, S. (2015). A brief history of T cell help to B cells. Nat Rev Immunol *15*, 185-189.

Curtsinger, J.M., and Mescher, M.F. (2010). Inflammatory cytokines as a third signal for T cell activation. Curr Opin Immunol *22*, 333-340.

Curtsinger, J.M., Schmidt, C.S., Mondino, A., Lins, D.C., Kedl, R.M., Jenkins, M.K., and Mescher, M.F. (1999). Inflammatory cytokines provide a third signal for activation of naive CD4+ and CD8+ T cells. J Immunol *162*, 3256-3262.

De Jager, P.L., Jia, X., Wang, J., de Bakker, P.I., Ottoboni, L., Aggarwal, N.T., Piccio, L., Raychaudhuri, S., Tran, D., Aubin, C., *et al*. (2009). Meta-analysis of genome scans and replication

identify CD6, IRF8 and TNFRSF1A as new multiple sclerosis susceptibility loci. Nat Genet *41*, 776-782.

DeSilva, D.R., Jones, E.A., Feeser, W.S., Manos, E.J., and Scherle, P.A. (1997). The p38 mitogen-activated protein kinase pathway in activated and anergic Th1 cells. Cell Immunol *180*, 116-123.

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

Dinarello, C.A. (2011). Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. Blood *117*, 3720-3732.

Dinarello, C.A., Simon, A., and van der Meer, J.W. (2012). Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. Nat Rev Drug Discov *11*, 633-652.

Dooms, H., and Abbas, A.K. (2002). Life and death in effector T cells. Nat Immunol *3*, 797-798.

Dozmorov, M.G., Guthridge, J.M., Hurst, R.E., and Dozmorov, I.M. (2010). A comprehensive and universal method for assessing the performance of differential gene expression analyses. PLoS One *5*.

Dubois, E.L., Horowitz, R.E., Demopoulos, H.B., and Teplitz, R. (1966). NZB/NZW mice as a model of systemic lupus erythematosus. JAMA *195*, 285-289.

El-Behi, M., Ciric, B., Dai, H., Yan, Y., Cullimore, M., Safavi, F., Zhang, G.X., Dittel, B.N., and Rostami, A. (2011). The encephalitogenicity of T(H)17 cells is dependent on IL-1- and IL-23-induced production of the cytokine GM-CSF. Nat Immunol *12*, 568-575.

Enouz, S., Carrie, L., Merkler, D., Bevan, M.J., and Zehn, D. (2012). Autoreactive T cells bypass negative selection and respond to self-antigen stimulation during infection. Journal of Experimental Medicine *209*, 1769-1779.

Esplugues, E., Huber, S., Gagliani, N., Hauser, A.E., Town, T., Wan, Y.Y., O'Connor, W., Jr., Rongvaux, A., Van Rooijen, N., Haberman, A.M., *et al*. (2011). Control of TH17 cells occurs in the small intestine. Nature *475*, 514-518.

Ferreira, M.A., Vonk, J.M., Baurecht, H., Marenholz, I., Tian, C., Hoffman, J.D., Helmer, Q., Tillander, A., Ullemar, V., van Dongen, J., *et al*. (2017). Shared genetic origin of asthma, hay fever and eczema elucidates allergic disease biology. Nat Genet *49*, 1752-1757.

Fletcher, J.M., Lalor, S.J., Sweeney, C.M., Tubridy, N., and Mills, K.H. (2010). T cells in multiple sclerosis and experimental autoimmune encephalomyelitis. Clin Exp Immunol *162*, 1-11.

Franchi, L., Eigenbrod, T., Munoz-Planillo, R., and Nunez, G. (2009). The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. Nat Immunol *10*, 241-247.

Freigang, S., Ampenberger, F., Weiss, A., Kanneganti, T.D., Iwakura, Y., Hersberger, M., and Kopf, M. (2013). Fatty acid-induced mitochondrial uncoupling elicits inflammasome-independent IL-1alpha and sterile vascular inflammation in atherosclerosis. Nat Immunol *14*, 1045-1053.

Fujinami, R.S., and Oldstone, M.B. (1985). Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. Science *230*, 1043-1045.

Furlan, R., Martino, G., Galbiati, F., Poliani, P.L., Smiroldo, S., Bergami, A., Desina, G., Comi, G., Flavell, R., Su, M.S., *et al*. (1999). Caspase-1 regulates the inflammatory process leading to autoimmune demyelination. J Immunol *163*, 2403-2409.

Garlanda, C., Dinarello, C.A., and Mantovani, A. (2013). The interleukin-1 family: back to the future. Immunity *39*, 1003-1018.

Gaublomme, J.T., Yosef, N., Lee, Y., Gertner, R.S., Yang, L.V., Wu, C., Pandolfi, P.P., Mak, T., Satija, R., Shalek, A.K., *et al*. (2015). Single-Cell Genomics Unveils Critical Regulators of Th17 Cell Pathogenicity. Cell *163*, 1400-1412.

Gebhardt, T., Wakim, L.M., Eidsmo, L., Reading, P.C., Heath, W.R., and Carbone, F.R. (2009). Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. Nat Immunol *10*, 524-530.

Grivennikov, S.I., Greten, F.R., and Karin, M. (2010). Immunity, inflammation, and cancer. Cell *140*, 883-899.

Guarda, G., Dostert, C., Staehli, F., Cabalzar, K., Castillo, R., Tardivel, A., Schneider, P., and Tschopp, J. (2009). T cells dampen innate immune responses through inhibition of NLRP1 and NLRP3 inflammasomes. Nature *460*, 269-273.

Guma, M., Ronacher, L., Liu-Bryan, R., Takai, S., Karin, M., and Corr, M. (2009). Caspase 1-independent activation of interleukin-1beta in neutrophil-predominant inflammation. Arthritis Rheum *60*, 3642-3650.

Gurung, P., Anand, P.K., Malireddi, R.K.S., Walle, L.V., Van Opdenbosch, N., Dillon, C.P., Weinlich, R., Green, D.R., Lamkanfi, M., and Kanneganti, T.D. (2014). FADD and Caspase-8 Mediate Priming and Activation of the Canonical and Noncanonical Nlrp3 Inflammasomes. Journal of Immunology *192*, 1835-1846.

Hardle, L., Bachmann, M., Bollmann, F., Pautz, A., Schmid, T., Eberhardt, W., Kleinert, H., Pfeilschifter, J., and Muhl, H. (2015). Tristetraprolin regulation of interleukin-22 production. Sci Rep *5*, 15112.

Harkiolaki, M., Holmes, S.L., Svendsen, P., Gregersen, J.W., Jensen, L.T., McMahon, R., Friese, M.A., van Boxel, G., Etzensperger, R., Tzartos, J.S., *et al*. (2009). T Cell-Mediated Autoimmune

Disease Due to Low-Affinity Crossreactivity to Common Microbial Peptides. Immunity *30*, 348-357.

Harrington, L.E., Janowski, K.M., Oliver, J.R., Zajac, A.J., and Weaver, C.T. (2008). Memory CD4 T cells emerge from effector T-cell progenitors. Nature *452*, 356-360.

Haskins, K. (2005). Pathogenic T-cell clones in autoimmune diabetes: more lessons from the NOD mouse. Adv Immunol *87*, 123-162.

Havenar-Daughton, C., Kolumam, G.A., and Murali-Krishna, K. (2006). Cutting Edge: The direct action of type I IFN on CD4 T cells is critical for sustaining clonal expansion in response to a viral but not a bacterial infection. J Immunol *176*, 3315-3319.

Helmby, H., and Grencis, R.K. (2004). Interleukin-1 plays a major role in the development of Th2-mediated immunity. Eur J Immunol *34*, 3674-3681.

Hermann-Kleiter, N., and Baier, G. (2010). NFAT pulls the strings during CD4(+) T helper cell effector functions. Blood *115*, 2989-2997.

Hinks, A., Cobb, J., Marion, M.C., Prahalad, S., Sudman, M., Bowes, J., Martin, P., Comeau, M.E., Sajuthi, S., Andrews, R., *et al*. (2013). Dense genotyping of immune-related disease regions identifies 14 new susceptibility loci for juvenile idiopathic arthritis. Nat Genet *45*, 664-669.

Hirota, K., Turner, J.E., Villa, M., Duarte, J.H., Demengeot, J., Steinmetz, O.M., and Stockinger, B. (2013). Plasticity of Th17 cells in Peyer's patches is responsible for the induction of T cell-dependent IgA responses. Nat Immunol *14*, 372-379.

Hoffman, H.M. (2009). Therapy of autoinflammatory syndromes. J Allergy Clin Immun *124*, 1129-1138.

Hoffman, H.M., Mueller, J.L., Broide, D.H., Wanderer, A.A., and Kolodner, R.D. (2001). Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome. Nat Genet *29*, 301-305.

Hsieh, C.S., Macatonia, S.E., Tripp, C.S., Wolf, S.F., Ogarra, A., and Murphy, K.M. (1993). Development of Th1 Cd4+ T-Cells through Il-12 Produced by Listeria-Induced Macrophages. Science *260*, 547-549.

Hu, W., and Pasare, C. (2013). Location, location, location: tissue-specific regulation of immune responses. J Leukocyte Biol *94*, 409-421.

Hu, W., Troutman, T.D., Edukulla, R., and Pasare, C. (2011). Priming microenvironments dictate cytokine requirements for T helper 17 cell lineage commitment. Immunity *35*, 1010-1022.

Hugot, J.P., Chamaillard, M., Zouali, H., Lesage, S., Cezard, J.P., Belaiche, J., Almer, S., Tysk, C., O'Morain, C.A., Gassull, M.*, et al*. (2001). Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature *411*, 599-603.

Hutcheson, J., Scatizzi, J.C., Siddiqui, A.M., Haines, G.K., 3rd, Wu, T., Li, Q.Z., Davis, L.S., Mohan, C., and Perlman, H. (2008). Combined deficiency of proapoptotic regulators Bim and Fas results in the early onset of systemic autoimmunity. Immunity *28*, 206-217.

International Multiple Sclerosis Genetics, C., Beecham, A.H., Patsopoulos, N.A., Xifara, D.K., Davis, M.F., Kemppinen, A., Cotsapas, C., Shah, T.S., Spencer, C., Booth, D.*, et al*. (2013). Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. Nat Genet *45*, 1353-1360.

Ippagunta, S.K., Brand, D.D., Luo, J., Boyd, K.L., Calabrese, C., Stienstra, R., Van de Veerdonk, F.L., Netea, M.G., Joosten, L.A., Lamkanfi, M.*, et al*. (2010). Inflammasome-independent role of

apoptosis-associated speck-like protein containing a CARD (ASC) in T cell priming is critical for collagen-induced arthritis. J Biol Chem *285*, 12454-12462.

Itoh, N., Imagawa, A., Hanafusa, T., Waguri, M., Yamamoto, K., Iwahashi, H., Moriwaki, M., Nakajima, H., Miyagawa, J., Namba, M., *et al*. (1997). Requirement of Fas for the development of autoimmune diabetes in nonobese diabetic mice. J Exp Med *186*, 613-618.

Ivanov, II, Zhou, L., and Littman, D.R. (2007). Transcriptional regulation of Th17 cell differentiation. Semin Immunol *19*, 409-417.

Ivanov, I.I., Atarashi, K., Manel, N., Brodie, E.L., Shima, T., Karaoz, U., Wei, D.G., Goldfarb, K.C., Santee, C.A., Lynch, S.V., *et al*. (2009). Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria. Cell *139*, 485-498.

Ivanov, I.I., Frutos, R.D., Manel, N., Yoshinaga, K., Rifkin, D.B., Sartor, R.B., Finlay, B.B., and Littman, D.R. (2008). Specific Microbiota Direct the Differentiation of IL-17-Producing T-Helper Cells in the Mucosa of the Small Intestine. Cell Host Microbe *4*, 337-349.

Iwasaki, A., and Medzhitov, R. (2004). Toll-like receptor control of the adaptive immune responses. Nat Immunol *5*, 987-995.

Iwasaki, A., and Medzhitov, R. (2015). Control of adaptive immunity by the innate immune system. Nat Immunol *16*, 343-353.

Jain, A., and Pasare, C. (2017). Innate Control of Adaptive Immunity: Beyond the Three-Signal Paradigm. J Immunol *198*, 3791-3800.

Jain, A., Song, R., Wakeland, E.K., and Pasare, C. (2018). T cell-intrinsic IL-1R signaling licenses effector cytokine production by memory CD4 T cells. Nat Commun *9*, 3185.

Janeway, C.A. (1989a). Approaching the Asymptote - Evolution and Revolution in Immunology. Cold Spring Harb Sym *54*, 1-13.

Janeway, C.A., Jr. (1989b). The priming of helper T cells. Semin Immunol *1*, 13-20.

Janeway, C.A., Jr. (1992). The immune system evolved to discriminate infectious nonself from noninfectious self. Immunol Today *13*, 11-16.

Jenkins, M.K., and Moon, J.J. (2012). The Role of Naive T Cell Precursor Frequency and Recruitment in Dictating Immune Response Magnitude. Journal of Immunology *188*, 4135-4140.

Jesus, A.A., and Goldbach-Mansky, R. (2014). IL-1 blockade in autoinflammatory syndromes. Annu Rev Med *65*, 223-244.

Jiang, X., Clark, R.A., Liu, L., Wagers, A.J., Fuhlbrigge, R.C., and Kupper, T.S. (2012). Skin infection generates non-migratory memory CD8+ T(RM) cells providing global skin immunity. Nature *483*, 227-231.

Jin, Y., Andersen, G., Yorgov, D., Ferrara, T.M., Ben, S., Brownson, K.M., Holland, P.J., Birlea, S.A., Siebert, J., Hartmann, A.*, et al*. (2016). Genome-wide association studies of autoimmune vitiligo identify 23 new risk loci and highlight key pathways and regulatory variants. Nat Genet *48*, 1418-1424.

Joosten, L.A., Netea, M.G., Fantuzzi, G., Koenders, M.I., Helsen, M.M., Sparrer, H., Pham, C.T., van der Meer, J.W., Dinarello, C.A., and van den Berg, W.B. (2009). Inflammatory arthritis in caspase 1 gene-deficient mice: contribution of proteinase 3 to caspase 1-independent production of bioactive interleukin-1beta. Arthritis Rheum *60*, 3651-3662.

Kaiser, W.J., Upton, J.W., Long, A.B., Livingston-Rosanoff, D., Daley-Bauer, L.P., Hakem, R., Caspary, T., and Mocarski, E.S. (2011). RIP3 mediates the embryonic lethality of caspase-8-deficient mice. Nature *471*, 368-372.

Kawai, T., and Akira, S. (2007). Signaling to NF-kappa B by Toll-like receptors. Trends Mol Med *13*, 460-469.

Kawai, T., and Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol *11*, 373-384.

Keller, M., Ruegg, A., Werner, S., and Beer, H.D. (2008). Active caspase-1 is a regulator of unconventional protein secretion. Cell *132*, 818-831.

Khabar, K.S.A. (2007). Rapid transit in the immune cells: the role of mRNA turnover regulation. J Leukocyte Biol *81*, 1335-1344.

Kim, J.M., Rasmussen, J.P., and Rudensky, A.Y. (2007a). Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. Nat Immunol *8*, 191-197.

Kim, K.D., Zhao, J., Auh, S., Yang, X., Du, P., Tang, H., and Fu, Y.X. (2007b). Adaptive immune cells temper initial innate responses. Nat Med *13*, 1248-1252.

Kopf, M., Le Gros, G., Bachmann, M., Lamers, M.C., Bluethmann, H., and Kohler, G. (1993). Disruption of the murine IL-4 gene blocks Th2 cytokine responses. Nature *362*, 245-248.

Kruglov, A.A., Lampropoulou, V., Fillatreau, S., and Nedospasov, S.A. (2011). Pathogenic and protective functions of TNF in neuroinflammation are defined by its expression in T lymphocytes and myeloid cells. J Immunol *187*, 5660-5670.

Kuhn, R., Lohler, J., Rennick, D., Rajewsky, K., and Muller, W. (1993). Interleukin-10-deficient mice develop chronic enterocolitis. Cell *75*, 263-274.

Kuno, K., and Matsushima, K. (1994). The IL-1 receptor signaling pathway. J Leukoc Biol *56*, 542-547.

Kuswanto, W., Burzyn, D., Panduro, M., Wang, K.K., Jang, Y.C., Wagers, A.J., Benoist, C., and Mathis, D. (2016). Poor Repair of Skeletal Muscle in Aging Mice Reflects a Defect in Local, Interleukin-33-Dependent Accumulation of Regulatory T Cells. Immunity *44*, 355-367.

Lalor, S.J., Dungan, L.S., Sutton, C.E., Basdeo, S.A., Fletcher, J.M., and Mills, K.H. (2011). Caspase-1-processed cytokines IL-1beta and IL-18 promote IL-17 production by gammadelta and CD4 T cells that mediate autoimmunity. J Immunol *186*, 5738-5748.

Le Gros, G., Ben-Sasson, S.Z., Seder, R., Finkelman, F.D., and Paul, W.E. (1990). Generation of interleukin 4 (IL-4)-producing cells in vivo and in vitro: IL-2 and IL-4 are required for in vitro generation of IL-4-producing cells. J Exp Med *172*, 921-929.

Lee, H.H., Yoon, N.A., Vo, M.T., Kim, C.W., Woo, J.M., Cha, H.J., Cho, Y.W., Lee, B.J., Cho, W.J., and Park, J.W. (2012a). Tristetraprolin down-regulates IL-17 through mRNA destabilization. FEBS Lett *586*, 41-46.

Lee, Y., Awasthi, A., Yosef, N., Quintana, F.J., Xiao, S., Peters, A., Wu, C., Kleinewietfeld, M., Kunder, S., Hafler, D.A., *et al*. (2012b). Induction and molecular signature of pathogenic TH17 cells. Nat Immunol *13*, 991-999.

Lemmers, B., Salmena, L., Bidere, N., Su, H., Matysiak-Zablocki, E., Murakami, K., Ohashi, P.S., Jurisicova, A., Lenardo, M., Hakem, R., *et al*. (2007). Essential role for caspase-8 in Toll-like receptors and NFkappaB signaling. J Biol Chem *282*, 7416-7423.

Lenschow, D.J., Walunas, T.L., and Bluestone, J.A. (1996). CD28/B7 system of T cell costimulation. Annu Rev Immunol *14*, 233-258.

Levandowski, C.B., Mailloux, C.M., Ferrara, T.M., Gowan, K., Ben, S., Jin, Y., McFann, K.K., Holland, P.J., Fain, P.R., Dinarello, C.A., *et al*. (2013). NLRP1 haplotypes associated with vitiligo and autoimmunity increase interleukin-1beta processing via the NLRP1 inflammasome. Proc Natl Acad Sci U S A *110*, 2952-2956.

Li, C.R., Mueller, E.E., and Bradley, L.M. (2014). Islet antigen-specific Th17 cells can induce TNF-alpha-dependent autoimmune diabetes. J Immunol *192*, 1425-1432.

Lichtman, A.H., Chin, J., Schmidt, J.A., and Abbas, A.K. (1988). Role of Interleukin-1 in the Activation of Lymphocytes-T. P Natl Acad Sci USA *85*, 9699-9703.

Lin, C.C., and Edelson, B.T. (2017). New Insights into the Role of IL-1beta in Experimental Autoimmune Encephalomyelitis and Multiple Sclerosis. J Immunol *198*, 4553-4560.

Liston, A., Lesage, S., Wilson, J., Peltonen, L., and Goodnow, C.C. (2003). Aire regulates negative selection of organ-specific T cells. Nat Immunol *4*, 350-354.

Liu, X., Nemeth, D.P., McKim, D.B., Zhu, L., DiSabato, D.J., Berdysz, O., Gorantla, G., Oliver, B., Witcher, K.G., Wang, Y., *et al*. (2019). Cell-Type-Specific Interleukin 1 Receptor 1 Signaling in the Brain Regulates Distinct Neuroimmune Activities. Immunity *50*, 317-333 e316.

London, C.A., Lodge, M.P., and Abbas, A.K. (2000). Functional responses and costimulator dependence of memory CD4(+) T cells. Journal of Immunology *164*, 265-272.

Lopalco, G., Cantarini, L., Vitale, A., Iannone, F., Anelli, M.G., Andreozzi, L., Lapadula, G., Galeazzi, M., and Rigante, D. (2015). Interleukin-1 as a common denominator from autoinflammatory to autoimmune disorders: premises, perils, and perspectives. Mediators Inflamm *2015*, 194864.

Lukens, J.R., Gross, J.M., and Kanneganti, T.D. (2012). IL-1 family cytokines trigger sterile inflammatory disease. Front Immunol *3*.

MacLeod, M.K., Kappler, J.W., and Marrack, P. (2010). Memory CD4 T cells: generation, reactivation and re-assignment. Immunology *130*, 10-15.

Maeda, S., Hsu, L.C., Liu, H., Bankston, L.A., Iimura, M., Kagnoff, M.F., Eckmann, L., and Karin, M. (2005). Nod2 mutation in Crohn's disease potentiates NF-kappaB activity and IL-1beta processing. Science *307*, 734-738.

Mailer, R.K., Joly, A.L., Liu, S., Elias, S., Tegner, J., and Andersson, J. (2015). IL-1beta promotes Th17 differentiation by inducing alternative splicing of FOXP3. Sci Rep *5*, 14674.

Mannie, M.D., Dinarello, C.A., and Paterson, P.Y. (1987). Interleukin 1 and myelin basic protein synergistically augment adoptive transfer activity of lymphocytes mediating experimental autoimmune encephalomyelitis in Lewis rats. J Immunol *138*, 4229-4235.

Martin, B.N., Wang, C., Zhang, C.J., Kang, Z., Gulen, M.F., Zepp, J.A., Zhao, J., Bian, G., Do, J.S., Min, B., *et al*. (2016). T cell-intrinsic ASC critically promotes T(H)17-mediated experimental autoimmune encephalomyelitis. Nat Immunol *17*, 583-592.

Martinon, F., Burns, K., and Tschopp, J. (2002). The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell *10*, 417-426.

Martinon, F., Petrilli, V., Mayor, A., Tardivel, A., and Tschopp, J. (2006). Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature *440*, 237-241.

Masopust, D., Vezys, V., Marzo, A.L., and Lefrancois, L. (2001). Preferential localization of effector memory cells in nonlymphoid tissue. Science *291*, 2413-2417.

McGeachy, M.J., Chen, Y., Tato, C.M., Laurence, A., Joyce-Shaikh, B., Blumenschein, W.M., McClanahan, T.K., O'Shea, J.J., and Cua, D.J. (2009). The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. Nat Immunol *10*, 314-324.

Merica, R., Khoruts, A., Pape, K.A., Reinhardt, R.L., and Jenkins, M.K. (2000). Antigen-experienced CD4 T cells display a reduced capacity for clonal expansion in vivo that is imposed by factors present in the immune host. Journal of Immunology *164*, 4551-4557.

Miller, L.S., O'Connell, R.M., Gutierrez, M.A., Pietras, E.M., Shahangian, A., Gross, C.E., Thirumala, A., Cheung, A.L., Cheng, G., and Modlin, R.L. (2006). MyD88 mediates neutrophil recruitment initiated by IL-1R but not TLR2 activation in immunity against Staphylococcus aureus. Immunity *24*, 79-91.

Moriwaki, K., Bertin, J., Gough, P.J., and Chan, F.K. (2015). A RIPK3-caspase 8 complex mediates atypical pro-IL-1beta processing. J Immunol *194*, 1938-1944.

Mueller, S.N., Gebhardt, T., Carbone, F.R., and Heath, W.R. (2013). Memory T cell subsets, migration patterns, and tissue residence. Annu Rev Immunol *31*, 137-161.

Mufazalov, I.A., Regen, T., Schelmbauer, C., Kuschmann, J., Muratova, A.M., Nikolaev, A., Muller, W., Pinteaux, E., and Waisman, A. (2016). Generation of a Novel T Cell Specific Interleukin-1 Receptor Type 1 Conditional Knock Out Mouse Reveals Intrinsic Defects in Survival, Expansion and Cytokine Production of CD4 T Cells. Plos One *11*.

Naik, S., Bouladoux, N., Wilhelm, C., Molloy, M.J., Salcedo, R., Kastenmuller, W., Deming, C., Quinones, M., Koo, L., Conlan, S.*, et al*. (2012). Compartmentalized control of skin immunity by resident commensals. Science *337*, 1115-1119.

Naik, S., Larsen, S.B., Gomez, N.C., Alaverdyan, K., Sendoel, A., Yuan, S., Polak, L., Kulukian, A., Chai, S., and Fuchs, E. (2017). Inflammatory memory sensitizes skin epithelial stem cells to tissue damage. Nature *550*, 475-480.

Ndejembi, M.P., Teijaro, J.R., Patke, D.S., Bingaman, A.W., Chandok, M.R., Azimzadeh, A., Nadler, S.G., and Farber, D.L. (2006). Control of memory CD4 T cell recall by the CD28/B7 costimulatory pathway. J Immunol *177*, 7698-7706.

Nelson, R.W., Beisang, D., Tubo, N.J., Dileepan, T., Wiesner, D.L., Nielsen, K., Wuthrich, M., Klein, B.S., Kotov, D.I., Spanier, J.A.*, et al*. (2015). T cell receptor cross-reactivity between

similar foreign and self peptides influences naive cell population size and autoimmunity. Immunity *42*, 95-107.

Netea, M.G., van de Veerdonk, F.L., van der Meer, J.W.M., Dinarello, C.A., and Joosten, L.A.B. (2015). Inflammasome-Independent Regulation of IL-1-Family Cytokines. Annual Review of Immunology Vol 33 *33*, 49-77.

Ogura, Y., Bonen, D.K., Inohara, N., Nicolae, D.L., Chen, F.F., Ramos, R., Britton, H., Moran, T., Karaliuskas, R., Duerr, R.H., *et al.* (2001). A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature *411*, 603-606.

Okamura, H., Tsutsi, H., Komatsu, T., Yutsudo, M., Hakura, A., Tanimoto, T., Torigoe, K., Okura, T., Nukada, Y., Hattori, K., *et al.* (1995). Cloning of a new cytokine that induces IFN-gamma production by T cells. Nature *378*, 88-91.

Okamura, H., Tsutsui, H., Kashiwamura, S., Yoshimoto, T., and Nakanishi, K. (1998). Interleukin-18: a novel cytokine that augments both innate and acquired immunity. Adv Immunol *70*, 281-312.

Pagan, A.J., Peters, N.C., Debrabant, A., Ribeiro-Gomes, F., Pepper, M., Karp, C.L., Jenkins, M.K., and Sacks, D.L. (2013). Tracking antigen-specific CD4+T cells throughout the course of chronic Leishmania major infection in resistant mice. Eur J Immunol *43*, 427-438.

Pasare, C., and Medzhitov, R. (2004). Toll-dependent control mechanisms of CD4 T cell activation. Immunity *21*, 733-741.

Pasare, C., and Medzhitov, R. (2005). Toll-dependent control mechanisms of CD4 T cell activation (vol 21, pg 733, 2004). Immunity *22*, 397-398.

Paul, W.E., and Ohara, J. (1987). B-cell stimulatory factor-1/interleukin 4. Annu Rev Immunol *5*, 429-459.

Paust, S., and Cantor, H. (2005). Regulatory T cells and autoimmune disease. Immunol Rev *204*, 195-207.

Peine, M., Marek, R.M., and Lohning, M. (2016). IL-33 in T Cell Differentiation, Function, and Immune Homeostasis. Trends Immunol *37*, 321-333.

Pepper, M., Linehan, J.L., Pagan, A.J., Zell, T., Dileepan, T., Cleary, P.P., and Jenkins, M.K. (2010). Different routes of bacterial infection induce long-lived TH1 memory cells and short-lived TH17 cells. Nat Immunol *11*, 83-89.

Philip, N.H., DeLaney, A., Peterson, L.W., Santos-Marrero, M., Grier, J.T., Sun, Y., Wynosky-Dolfi, M.A., Zwack, E.E., Hu, B., Olsen, T.M., *et al*. (2016). Activity of Uncleaved Caspase-8 Controls Anti-bacterial Immune Defense and TLR-Induced Cytokine Production Independent of Cell Death. PLoS Pathog *12*, e1005910.

Poussier, P., Ning, T., Banerjee, D., and Julius, M. (2002). A unique subset of self-specific intraintestinal T cells maintains gut integrity. Journal of Experimental Medicine *195*, 1491-1497.

Pugliese, A. (2017). Autoreactive T cells in type 1 diabetes. J Clin Invest *127*, 2881-2891.

Raphael, I., Nalawade, S., Eagar, T.N., and Forsthuber, T.G. (2015). T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine *74*, 5-17.

Rathinam, V.A., Vanaja, S.K., and Fitzgerald, K.A. (2012). Regulation of inflammasome signaling. Nat Immunol *13*, 333-342.

Reiner, S.L. (2007). Development in motion: helper T cells at work. Cell *129*, 33-36.

Rider, P., Carmi, Y., Guttman, O., Braiman, A., Cohen, I., Voronov, E., White, M.R., Dinarello, C.A., and Apte, R.N. (2011). IL-1alpha and IL-1beta recruit different myeloid cells and promote different stages of sterile inflammation. J Immunol *187*, 4835-4843.

Rogers, P.R., Dubey, C., and Swain, S.L. (2000). Qualitative changes accompany memory T cell generation: faster, more effective responses at lower doses of antigen. J Immunol *164*, 2338-2346.

Rosenblum, M.D., Remedios, K.A., and Abbas, A.K. (2015). Mechanisms of human autoimmunity. J Clin Invest *125*, 2228-2233.

Sallusto, F., Geginat, J., and Lanzavecchia, A. (2004). Central memory and effector memory T cell subsets: function, generation, and maintenance. Annu Rev Immunol *22*, 745-763.

Sallusto, F., Lenig, D., Forster, R., Lipp, M., and Lanzavecchia, A. (1999). Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature *401*, 708-712.

Santarlasci, V., Cosmi, L., Maggi, L., Liotta, F., and Annunziato, F. (2013). IL-1 and T Helper Immune Responses. Front Immunol *4*, 182.

Satoskar, A.R., Okano, M., Connaughton, S., Raisanen-Sokolwski, A., David, J.R., and Labow, M. (1998). Enhanced Th2-like responses in IL-1 type 1 receptor-deficient mice. Eur J Immunol *28*, 2066-2074.

Schenkel, J.M., Fraser, K.A., and Masopust, D. (2014). Cutting edge: resident memory CD8 T cells occupy frontline niches in secondary lymphoid organs. J Immunol *192*, 2961-2964.

Schenten, D., Nish, S.A., Yu, S., Yan, X.T., Lee, H.K., Brodsky, I., Pasman, L., Yordy, B., Wunderlich, F.T., Bruning, J.C., *et al*. (2014). Signaling through the Adaptor Molecule MyD88 in CD4(+) T Cells Is Required to Overcome Suppression by Regulatory T Cells. Immunity *40*, 78-90.

Schiering, C., Krausgruber, T., Chomka, A., Frohlich, A., Adelmann, K., Wohlfert, E.A., Pott, J., Griseri, T., Bollrath, J., Hegazy, A.N., *et al*. (2014). The alarmin IL-33 promotes regulatory T-cell function in the intestine. Nature *513*, 564-+.

Schmitz, J., Owyang, A., Oldham, E., Song, Y.L., Murphy, E., McClanahan, T.K., Zurawski, G., Moshrefi, M., Qin, J.Z., Li, X.X., *et al*. (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*, 479-490.

Schnare, M., Barton, G.M., Holt, A.C., Takeda, K., Akira, S., and Medzhitov, R. (2001). Toll-like receptors control activation of adaptive immune responses. Nat Immunol *2*, 947-950.

Schoenborn, J.R., Dorschner, M.O., Sekimata, M., Santer, D.M., Shnyreva, M., Fitzpatrick, D.R., Stamatoyannopoulos, J.A., and Wilson, C.B. (2007). Comprehensive epigenetic profiling identifies multiple distal regulatory elements directing transcription of the gene encoding interferon-gamma. Nat Immunol *8*, 732-742.

Schoenborn, J.R., and Wilson, C.B. (2007). Regulation of interferon-gamma during innate and adaptive immune responses. Adv Immunol *96*, 41-101.

Schott, W.H., Haskell, B.D., Tse, H.M., Milton, M.J., Piganelli, J.D., Choisy-Rossi, C.M., Reifsnyder, P.C., Chervonsky, A.V., and Leiter, E.H. (2004). Caspase-1 is not required for type 1 diabetes in the NOD mouse. Diabetes *53*, 99-104.

Shaw, M.H., Kamada, N., Kim, Y.G., and Nunez, G. (2012). Microbiota-induced IL-1beta, but not IL-6, is critical for the development of steady-state TH17 cells in the intestine. J Exp Med *209*, 251-258.

Shaw, P.J., McDermott, M.F., and Kanneganti, T.D. (2011). Inflammasomes and autoimmunity. Trends Mol Med *17*, 57-64.

Shenderov, K., Barber, D.L., Mayer-Barber, K.D., Gurcha, S.S., Jankovic, D., Feng, C.G., Oland, S., Hieny, S., Caspar, P., Yamasaki, S., *et al*. (2013). Cord factor and peptidoglycan recapitulate

the Th17-promoting adjuvant activity of mycobacteria through mincle/CARD9 signaling and the inflammasome. J Immunol *190*, 5722-5730.

Shin, H., and Iwasaki, A. (2013). Tissue-resident memory T cells. Immunol Rev *255*, 165-181.

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

Stehlik, C. (2009). Multiple interleukin-1beta-converting enzymes contribute to inflammatory arthritis. Arthritis Rheum *60*, 3524-3530.

Stromnes, I.M., and Goverman, J.M. (2006a). Active induction of experimental allergic encephalomyelitis. Nat Protoc *1*, 1810-1819.

Stromnes, I.M., and Goverman, J.M. (2006b). Passive induction of experimental allergic encephalomyelitis. Nat Protoc *1*, 1952-1960.

Strutt, T.M., McKinstry, K.K., Dibble, J.P., Winchell, C., Kuang, Y., Curtis, J.D., Huston, G., Dutton, R.W., and Swain, S.L. (2010). Memory CD4+ T cells induce innate responses independently of pathogen. Nat Med *16*, 558-564, 551p following 564.

Strutt, T.M., McKinstry, K.K., and Swain, S.L. (2011). Control of innate immunity by memory CD4 T cells. Adv Exp Med Biol *780*, 57-68.

Swain, S.L., Weinberg, A.D., English, M., and Huston, G. (1990). IL-4 directs the development of Th2-like helper effectors. J Immunol *145*, 3796-3806.

Takeuchi, O., and Akira, S. (2010). Pattern recognition receptors and inflammation. Cell *140*, 805-820.

Teijaro, J.R., Turner, D., Pham, Q., Wherry, E.J., Lefrancois, L., and Farber, D.L. (2011). Cutting edge: Tissue-retentive lung memory CD4 T cells mediate optimal protection to respiratory virus infection. J Immunol *187*, 5510-5514.

Teijaro, J.R., Verhoeven, D., Page, C.A., Turner, D., and Farber, D.L. (2010). Memory CD4 T cells direct protective responses to influenza virus in the lungs through helper-independent mechanisms. J Virol *84*, 9217-9226.

Tsoi, L.C., Stuart, P.E., Tian, C., Gudjonsson, J.E., Das, S., Zawistowski, M., Ellinghaus, E., Barker, J.N., Chandran, V., Dand, N., *et al*. (2017). Large scale meta-analysis characterizes genetic architecture for common psoriasis associated variants. Nat Commun *8*, 15382.

VanderBorght, A., Geusens, P., Raus, J., and Stinissen, P. (2001). The autoimmune pathogenesis of rheumatoid arthritis: role of autoreactive T cells and new immunotherapies. Semin Arthritis Rheum *31*, 160-175.

Varanasi, V., Avanesyan, L., Schumann, D.M., and Chervonsky, A.V. (2012). Cytotoxic mechanisms employed by mouse T cells to destroy pancreatic beta-cells. Diabetes *61*, 2862-2870.

Vasanthakumar, A., Moro, K., Xin, A., Liao, Y., Gloury, R., Kawamoto, S., Fagarasan, S., Mielke, L.A., Afshar-Sterle, S., Masters, S.L., *et al*. (2015). The transcriptional regulators IRF4, BATF and IL-33 orchestrate development and maintenance of adipose tissue-resident regulatory T cells. Nat Immunol *16*, 276-285.

Villarino, A.V., Katzman, S.D., Gallo, E., Miller, O., Jiang, S., McManus, M.T., and Abbas, A.K. (2011). Posttranscriptional silencing of effector cytokine mRNA underlies the anergic phenotype of self-reactive T cells. Immunity *34*, 50-60.

Voronov, E., Dotan, S., Krelin, Y., Song, X., Elkabets, M., Carmi, Y., Rider, P., Idan, C., Romzova, M., Kaplanov, I., *et al*. (2013). Unique Versus Redundant Functions of IL-1alpha and IL-1beta in the Tumor Microenvironment. Front Immunol *4*, 177.

Waldner, H., Sobel, R.A., Howard, E., and Kuchroo, V.K. (1997). Fas- and FasL-deficient mice are resistant to induction of autoimmune encephalomyelitis. J Immunol *159*, 3100-3103.

Wan, C.K., Li, P., Spolski, R., Oh, J., Andraski, A.B., Du, N., Yu, Z.X., Dillon, C.P., Green, D.R., and Leonard, W.J. (2015). IL-21-mediated non-canonical pathway for IL-1beta production in conventional dendritic cells. Nat Commun *6*, 7988.

Wang, L., Du, F., and Wang, X. (2008). TNF-alpha induces two distinct caspase-8 activation pathways. Cell *133*, 693-703.

Ward-Kavanagh, L.K., Lin, W.W., Sedy, J.R., and Ware, C.F. (2016). The TNF Receptor Superfamily in Co-stimulating and Co-inhibitory Responses. Immunity *44*, 1005-1019.

Watanabe-Fukunaga, R., Brannan, C.I., Copeland, N.G., Jenkins, N.A., and Nagata, S. (1992). Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature *356*, 314-317.

Watts, T.H. (2005). Tnf/tnfr family members in costimulation of T cell responses. Annu Rev Immunol *23*, 23-68.

Wu, J., and Chen, Z.J. (2014). Innate immune sensing and signaling of cytosolic nucleic acids. Annu Rev Immunol *32*, 461-488.

Yang, C.A., and Chiang, B.L. (2015). Inflammasomes and human autoimmunity: A comprehensive review. J Autoimmun *61*, 1-8.

Yang, J.F., Murphy, T.L., Ouyang, W.J., and Murphy, K.M. (1999). Induction of interferon-gamma production in Th1 CD4(+) T cells: evidence for two distinct pathways for promoter activation. Eur J Immunol *29*, 548-555.

Yang, Y., Ochando, J.C., Bromberg, J.S., and Ding, Y. (2007). Identification of a distant T-bet enhancer responsive to IL-12/Stat4 and IFNgamma/Stat1 signals. Blood *110*, 2494-2500.

York, A.G., Williams, K.J., Argus, J.P., Zhou, Q.D., Brar, G., Vergnes, L., Gray, E.E., Zhen, A., Wu, N.C., Yamada, D.H., *et al*. (2015). Limiting Cholesterol Biosynthetic Flux Spontaneously Engages Type I IFN Signaling. Cell *163*, 1716-1729.

Zehn, D., and Bevan, M.J. (2006). T cells with low avidity for a tissue-restricted antigen routinely evade central and peripheral tolerance and cause autoimmunity. Immunity *25*, 261-270.

Zheng, H., Fletcher, D., Kozak, W., Jiang, M., Hofmann, K.J., Conn, C.A., Soszynski, D., Grabiec, C., Trumbauer, M.E., Shaw, A., *et al*. (1995). Resistance to fever induction and impaired acute-phase response in interleukin-1 beta-deficient mice. Immunity *3*, 9-19.

Zhu, J., Yamane, H., and Paul, W.E. (2010). Differentiation of effector CD4 T cell populations (*). Annu Rev Immunol *28*, 445-489.

Zielinski, C.E., Mele, F., Aschenbrenner, D., Jarrossay, D., Ronchi, F., Gattorno, M., Monticelli, S., Lanzavecchia, A., and Sallusto, F. (2012). Pathogen-induced human TH17 cells produce IFN-gamma or IL-10 and are regulated by IL-1beta. Nature *484*, 514-518.