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The role of Th17 cells in host defense against intracellular bacterial infection

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The role of Th17 cells in host defense against intracellular bacterial infection.

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Molecular Biology

by

David Isaac Weiss

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ABSTRACT OF THE DISSERTATION

The role of Th17 cells in host defense against

Intracellular bacterial infection.

by

David Isaac Weiss

Doctor of Philosophy in Molecular Biology

University of California, Los Angeles, 2019

Professor Robert L. Modlin, Chair

Human host defense against mycobacteria depends on a functioning immune system. While it is currently established that Th1 cells are major players in host defense against mycobacteria, other cell types such as Th17 cells also correlate strongly with the protective forms of disease. However, the role of Th17 cells in the context of intracellular infection are incompletely understood. Recent work has shown that Th17 cells can secrete an antimicrobial protein IL-26, which can directly lyse extracellular bacteria. Given the established role of antimicrobial peptides against mycobacterial infection, we decided to further investigate IL-26 and determine whether it is antimicrobial against mycobacteria. We find that IL-26 can be secreted by PBMCs and purified T cells in response to IL-1β in the absence of T cell receptor activation. This process is also more rapid than TCR stimulation. Among helper T cells, we show that IL-1RI expression was necessary for this response and identified IL-1RI^{+} Th17 cells as a cell type that can secrete IL-26 in response to IL-1β. Furthermore, we establish that IL-26 secreted in response to IL-1β is functionally antimicrobial.

We also examined whether IL-26 is antimicrobial against intracellular bacteria. We find that IL-26 is differentially expressed between clinical forms of leprosy, with higher expression in tuberculoid leprosy, the resistant form of the disease, as compared to lepromatous leprosy, the progressive form of the disease. Incubation of IL-26 with *M. leprae* and attenuated *M. tuberculosis* strain H37RA led to dose dependent killing, both with bacteria in culture and, importantly, while the bacteria resided within infected macrophages. Additionally, we find that IL-26 treatment of infected macrophages stimulates the autophagy response and enhances phagolysosome fusion in a STING dependent manner. Altogether, this work uncovers a novel mechanism by which Th17 cells contribute to defense against mycobacterial infection.

The dissertation of David Isaac Weiss is approved.

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Balin SJ, Pellegrini M, Klechevsky E, et al. Human antimicrobial cytotoxic T lymphocytes, defined by NK receptors and antimicrobial proteins, kill intracellular bacteria. *Sci Immunol.* 2018;3(26)

Lissina E, Weiss D, Young B, et al. A novel small molecule methyltransferase is important for virulence in Candida albicans. *ACS Chem Biol*. 2013;8(12):2785-93.

Young BD, Weiss DI, Zurita-lopez CI, Webb KJ, Clarke SG, Mcbride AE. Identification of methylated proteins in the yeast small ribosomal subunit: a role for SPOUT methyltransferases in protein arginine methylation. *Biochemistry*. 2012;51(25):5091-104

Presentations

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Weiss DI, Dang AT, Montoya D, Gilliet M, Pellegrini M, Modlin RL. Innate activation of Th17 cells triggers IL-26 release. *Immunobiology of Leprosy Symposium.* UCLA, Los Angeles, CA. Short talk and poster presentation, 2017.

CHAPTER 1

INTRODUCTION

The human immune system is a fundamental physiological process that protects the host from a variety of foreign pathogens. It is classified into two distinct parts, the innate immune system and the adaptive immune system. The innate immune system developed early in evolutionary history and provides the initial response to infection (Janeway Jr. & Medzhitov, 2002). It is rapid and nonspecific, recognizing commonly conserved pathogen associated molecular patterns on the surface of pathogens. Typical innate immune cell types are monocytes and macrophages, neutrophils, natural killer cells, and dendritic cells.

Upon exposure to antigens in the early stages of infection, innate immune cells will subsequently activate the adaptive immune response. While the adaptive immune response is slower to become active, it displays specificity and develops memory against a particular pathogen, enabling the host to respond faster and more effectively upon reinfection (Janeway, Travers, Walport, & Shlomchik, 2001). This is due to the fact that T cells and B cells, the major cell types of the adaptive immune system, can physically rearrange sections of their DNA for optimal antigen specificity, and can rapidly multiply to form a pool of effector cells and memory cells.

The two arms of the immune system do not act independently of each other and are both necessary for host defense. This is easily illustrated in the case of mycobacterial infection, our favorite system to study the determinants of effective host defense. As will be expanded upon below, mycobacterial infection can have various outcomes, with a spectrum of longstanding infection or complete disease clearance, all determined upon the type of immune response the patient is mounting.

Mycobacterial diseases

Mycobacteria account for significant morbidity and mortality worldwide. According to 2014 World Health Organization estimates, one third of the world's population is infected with *M. tuberculosis*, the causative agent of tuberculosis (WHO, 2014). *M. leprae*, which causes the skin and nerve disease leprosy, is also endemic in underdeveloped countries, and can lead to debilitating disability and social stigma. Other mycobacterial infections include "atypical" pathogens such as *M. kansasii* and *Mycobacterium avium* complex, which typically infect immunocompromised individuals, and have become more prevalent in areas of high HIV coinfection.

Mycobacteria are successful human pathogens due to several virulence factors, including their unique cell wall structure, intracellular localization within the host, and effective inhibition of host mechanisms for destruction (Forrellad et al., 2013). Their cell walls are rich with long and complex lipids, such as mycolic acids and lipoarabinomannans, as well as lipoproteins. These outer wall lipids and lipoproteins can bind to mannose receptors and complement receptors on the surface of the macrophage, enhancing entry into the cell (Schlesinger, 1993; Schlesinger, Bellinger-Kawahara, Payne, & Horwitz, 1990). Once inside the host cell, the bacteria are protected from extracellular antibodies, antimicrobial peptides, and complement. In order to persist and replicate inside the phagosome, however, fusion of the phagosome with the lysosome must be prevented. Blockade of phagosome maturation by various mechanisms, including blocking Rab5 exchange for Rab7 and removing PI3P from the phagosome membrane are ways the bacterium inhibits the phagosomes ability to fuse with lysosomes (Vergne et al., 2005; Via et al., 1997).

Once infection is established, the interaction between the bacteria and the host immune response determines whether the infection is cleared or if infection persists in a "latent" manner

(Barry III et al., 2009; Modlin & Bloom, 2013). In tuberculosis infection, latent *M. tuberculosis* resides in lung granulomas, and spontaneous reactivation to symptomatic disease is possible, typically after an event that decreases immune function (Ai, Ruan, Liu, & Zhang, 2016). In the disease leprosy, an analogous state of "latency" is described as tuberculoid leprosy disease, whereas the analog of active disease is lepromatous leprosy disease. The disease spectrum of leprosy, and the host immune response to *M. leprae* are a major focus of this dissertation and is presented in further depth below.

Leprosy

Leprosy, which is caused by the bacillus *Mycobacterium leprae*, is one of the oldest diseases in the human historical record, appearing in the bible. *M. leprae* is an obligate intracellular pathogen that infects and resides within skin macrophages and Schwann cells of the peripheral nerves (Lastória & Abreu, 2014). It is believed to favor cooler temperatures for growth, therefore the extremities, ears, nose, and eyebrows are often sites of infection. Infection of the Schwann cells causes demyelination and inflammation of the nerves, which can lead to significant nerve damage and disability(Chan, Uong, Nassiri, & Gupta, 2019; Modlin et al., 1988). In fact, leprosy is a leading infectious cause of disability worldwide (Chaptini & Marshman, 2015). The disease does not present as a single syndrome, but rather presents as a spectrum of disease that can be described using the Ridley-Jopling classification system (Ridley & Jopling, 1966). At one pole is tuberculoid leprosy (TT), which is characterized by few, well defined papules or plaques, that are typically erythematous or hypopigmented and anesthetic. Biopsies of the lesions show characteristic granulomas with epithelioid macrophages and Langhans giant cells with absent or very few acid-fast bacilli visible (Massone, Belachew, & Schettini, 2015). On the other end of the spectrum is lepromatous leprosy, in which the patients present with numerous skin lesions. Biopsies of lepromatous leprosy lesions reveal loss of

granuloma organization and abundant acid fast bacilli within foamy, lipid droplet filled macrophages. Between the two poles of the spectrum are a variety of borderline forms, including borderline tuberculous (BT), borderline (BB), and borderline lepromatous leprosy (BL), each based on the number of lesions and pathology of those lesions. Nerve damage is a feature throughout the spectrum, and there is additionally a purely neural form of leprosy, with neuropathy without skin lesions. The World Health Organization has more recently simplified the classification of leprosy into paucibacillary disease (includes TT and BT) and multibacillary disease (includes BL and LL) (WHO, 2016). Patients with both paucibacillary or, more frequently, multibacillary disease, can undergo leprosy reactions, sometimes associated treatment (Scollard et al., 2006). One type of reaction, called type I or reversal reaction (RR), is characterized by the appearance of inflamed, erythematous, and painful lesions and increased inflammation of existing lesions, along with systemic symptoms of fever and muscle aches. Type II reactions, also called erythema nodosum leprosum (ENL), are characterized by systemic symptoms and the appearance of painful skin nodules. A third reactional state, called Lucio's phenomenon, is a severe necrotizing vasculitis that can cause significant morbidity and even mortality.

Classification of the patients into the Ridley-Jopling or WHO classifications is useful for guiding the duration of antibiotic treatment for the disease (WHO, 2016). Multi-drug therapy with rifampin, dapsone, and clofazimine is the WHO recommendation for most patients, with a six month regimen of treatment for paucibacillary disease and 12-month regimen for multibacillary patients. These courses of treatment are typically curative, although a minority of patients can have relapses of disease, sometimes decades after the conclusion of therapy. Despite effective antimicrobial therapy for leprosy, the global prevalence of leprosy has persisted over the last decade at roughly 200,000 cases. The majority of new cases are reported in India, Brazil, Indonesia, and other developing/underdeveloped nations. Leprosy is primarily a disease of

poverty, and its persistence despite antimicrobial therapy is largely due to problems of poverty, including lack of access to healthcare and health education (WHO, 2016). Social stigma associated with leprosy is still prevalent, and while quarantine into colonies is becoming much less frequent in modern times, a diagnosis of leprosy can severely impact the social standing of the patient and their family as well. Lack of education and public health screening allows patients with paucibacillary forms of the disease, who may not recognize their lesions as symptoms of leprosy, to avoid seeking treatment, and multibacillary patients may not have access to the treatment they need. There is currently no preventative vaccine for leprosy.

The immunology of leprosy

Aside from the interesting clinical features of the disease leprosy, it is also a wonderful model with which we can study immunology and the components of effective host responses against mycobacteria. This is because the clinical presentation of the patient depends upon the type of immune response the patient is mounting and the skin lesions are readily accessible for study. At the site of disease, paucibacillary patients are mounting an effective resistance against the bacteria characterized by a predominant $CD4^+$ T helper type 1 (Th1) response (Longley et al., 1985; Modlin, 1994; Modlin et al., 1984). This is evidenced by the development delayed type hypersensitivity reactions to subcutaneously injected *M. leprae* sonicate (termed a positive Mitsuda reaction) by paucibacillary patients, indicating a strong T cell mediated immune response (Cooper et al., 1989). Conversely, multibacillary patients mount a predominantly humoral response with circulating antibodies against *M. leprae* antigens and are anergic to subcutaneously injected *M. leprae* antigens (Mutis, Cornelisse, & Ottenhoff, 1993; Sieling et al., 1993; Teles et al., 2013).

Th1 cells have been well characterized as essential for protection against a variety of intracellular pathogens including *Listeria monocytogenes, Salmonella enterica, M. tuberculosis*

and *M. leprae* (Daugelat, Ladel, Schoel, & Kaufmann, 1994; Hess, Ladel, Miko, & Kaufmann, 1996; Modlin & Bloom, 2013). The characteristic cytokines produced by Th1 cells are interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α) and IL-2 (Janeway et al., 2001). Exposure to IFN-γ induces phagolysosomal fusion, antimicrobial peptide production, and a process called autophagy in macrophages (M Fabri et al., 2011; Teles et al., 2013). Autophagy is typically used by cells to recycle macronutrients in organelles during times of stress or starvation, but it is also an effective means by which host cells can degrade phagosome resident bacterial pathogens (Mario Fabri, Realegeno, Jo, & Modlin, 2011; Gutierrez et al., 2004; Liu & Modlin, 2008; Silva et al., 2017). Lesions from patients with tuberculoid leprosy have much greater expression of *IFNG*, *IL2, and TNF* than lesions from patients with lepromatous leprosy (Cooper et al., 1989; Yamamura et al., 1991, 1992). Immunohistochemistry within lesions shows an increase in the number of IFN-γ positive cells in paucibacillary lesions compared to multibacillary lesions (Arnoldi, Gerdes, & Flad, 1990; Volc-Platzer, Stemberger, Luger, Radaszkiewicz, & Wiedermann, 1988). Furthermore, circulating T cells from TT patients produce more IL-2 and IFN-γ protein than T cells from LL patients upon exposure to *M. leprae* antigens *in vitro*. On the contrary, LL lesions have higher expression of *IL10, IL4,* and *IL5,* cytokines characteristic of a Th2 immune response (Yamamura et al., 1991). These cytokines are inhibitory to the development of Th1 responses and likely contribute to the defect in Th1 cytokine production in multibacillary leprosy (D'Andrea et al., 1993; Ito et al., 1999; Zheng & Flavell, 1997). Furthermore, they stimulate B cell antibody production, contributing to the increase in anti-*M. leprae* antibodies in the circulation of multibacillary patients (Fernandez Botran, Sanders, Mosmann, & Vitetta, 1988; Touw, Langendijk, Stoner, & Belehu, 1982; Yokota et al., 1987). Patients undergoing reversal reactions have a dramatic increase in the amount of Th1 cytokines in the lesions, and ability of circulating T cells to produce these cytokines, further indicating the

protective role of Th1 mediate immunity in leprosy (Sreenivasan, Misra, Wilfred, & Nath, 1998; Yamamura et al., 1992).

Despite the clear relationship between Th1 cells and resistance to disseminated *M. leprae* infection, the idea that Th1 cells are the only T cell subset providing protection in this context is overly simplistic. This is easily highlighted by the fact that vaccines against tuberculosis, despite inducing strong IFN-γ responses, fail to confer protective immunity (Tameris et al., 2013).

Th17 cells in leprosy

Th17 cells are a distinct lineage of helper T cell that have been shown to be important for combatting extracellular pathogens. Human naïve $CD4^+$ T cells differentiate into Th17 cells through exposure to IL-6, IL-1β, TGF-β, and IL-23, which induce expression of the transcription factor RORγt (Acosta-Rodriguez, Napolitani, Lanzavecchia, & Sallusto, 2007; Wilson et al., 2007). Activated Th17 cells characteristically secrete the cytokines IL-17A, IL-17F, and IL-22, which induce epithelial cell production of IL-1β, IL-6, CXCL2, and CXCL8 to attract and activate inflammatory cells at the site of infection (Cho et al., 2010; Happel et al., 2005; Jones & Chan, 2002; Kawaguchi et al., 2001; Laan et al., 1999; Milner et al., 2008; Ye et al., 2001). IL-17 and IL-22 from Th17 cells are also able to stimulate the secretion of directly antimicrobial peptides, such as defensins and S100A proteins, from epithelial cells (Jones & Chan, 2002; Kawaguchi et al., 2001; Meller et al., 2015; Milner et al., 2008). Genetic disturbances of Th17 differentiation and function in humans results in susceptibility to chronic infections with a variety of bacterial and fungal pathogens, especially *Staphylococcus aureus* and *Candida albicans* (de Beaucoudrey et al., 2008; Ma et al., 2008; Okada et al., 2015).

There have been several studies investigating the role of Th17 cells in *M. leprae* infection, most of which associate Th17 cells with paucibacillary forms of the disease. The

serum of leprosy patients, regardless of the clinical subtype, have lower concentrations of IL-17A compared to healthy controls, perhaps indicating that dysfunction of Th17 responses contributes to leprosy pathogenesis (Abdallah, Emam, Attia, Hussein, & Mohamed, 2013; Attia et al., 2014). Within clinical subtypes of leprosy, serum concentrations of IL-17A are significantly elevated in paucibacillary disease vs. multibacillary disease. When stimulated with sonicated *M. leprae*, PBMCs from patients with TT and BT leprosy express significantly greater amounts of *IL17A*, *IL17F*, *IL22*, and *IL23A* mRNA transcripts compared to PBMCs from BL and LL patients (Saini, Ramesh, & Nath, 2013). This is mirrored at the protein level, with PBMC from tuberculoid leprosy patients secreting significantly more IL-17A, IL-17F, IL-23, and IL-6 than PBMC from lepromatous leprosy patients in response to *M. leprae* (Saini et al., 2013; Saini, Siddiqui, Ramesh, & Nath, 2016). This increase in Th17 mRNAs and proteins in activated PBMC from PB patients is due in part to a greater frequency of Th17 cells (Sadhu et al., 2016). Within leprosy lesions, there are also greater numbers of Th17 cells and Th17 cytokine mRNA and protein secretion within PB lesions vs. MB lesions (Quaresma et al., 2015; Saini et al., 2013, 2016; Santos et al., 2017)(Santos 2017, Quaresma 2014, Saini 2013, Saini 2016). This correlation of Th17 responses with the clinical forms of leprosy is similar to that of Th1 cells, indicating an association between Th17 cells and effective defense against *M. leprae*.

The reactional states of leprosy, the hallmarks of which are increased inflammation and cellular infiltration of existing and new lesions, are typically associated with a transient shift in the immune response of the patient towards the Th1 response (Yamamura et al., 1992). Interestingly, the characteristic Th17 cytokines IL-17A, IL-17F, IL-22, and IL-26 all act on epithelial cells to produce inflammatory cytokines IL-1β, IL-6, and TNF-α and a variety of chemokines that induce inflammation and promote the recruitment of inflammatory immune cells, suggesting a role for Th17 cells in reactional states of leprosy (Hor et al., 2004; Jones & Chan, 2002; Kawaguchi et al., 2001; Laan et al., 1999). When comparing *M. leprae* antigen

stimulated PBMCs from patients with RR or ENL, levels of *IL17A, IL17F, IL23, IL6,* and *IL21* mRNA transcripts are significantly higher as compared to stimulated PBMCs from patients with BT or LL leprosy, respectively (Saini et al., 2016). Furthermore, IL-17A, IL-21, IL-23A, and IL-6 secretion and IL-17A^{+}CD4^{+} T cell frequency are significantly greater in *M. leprae* stimulated PBMC from patients in reactional leprosy states as compared to non-reactional states. Enhanced secretion of IL-6 and TGF-β by macrophages may account for the increased frequency of circulating Th17 cells during leprosy reactions. Within granulomas, IL-17A and TGF-β were also more abundant in biopsies from RR and ENL patients compared to BT and LL patients (Saini et al., 2016). The association of Th17 cells with paucibacillary forms of the disease and the increase in Th17 activity during reactional states of leprosy highlight the fact that patients who are mounting resistance to *M. leprae* to restrict the infection have a greater frequency of Th17 cells in both circulation and resident at the site of infection.

Antimicrobial peptides and proteins

Aside from the cytokine mediators produced by cells of the adaptive and innate immune system, the production of antimicrobial peptides is also important for defense against mycobacteria. Cationic antimicrobial peptides and proteins like human cathelicidin LL37, beta defensin 2, and granulysin have been specifically shown to kill mycobacteria (Liu et al., 2009, 2006; Stenger et al., 1998). These proteins and peptides tend to be positively charged, for example cathelicidin has a charge of +6 at a pH of 7.4 (Johansson, Gudmundsson, Rottenberg, Berndt, & Agerberth, 1998). The positive charge allows for binding of the peptides to the surface of negatively charged bacteria. Cationic residues, like arginine and lysine, tend to be clustered on one side of the molecule, whereas another side has clustered hydrophobic residues (De Smet & Contreras, 2005). This allows for multimer formation, which enables the peptides to form pore-like

structures in the membranes of the bacteria. This leads to ionic and osmotic imbalance that is lethal for the organism.

Within macrophages, beta defensin 2, encoded by the gene *DEFB4*, and cathelicidin have been demonstrated to have antimicrobial properties against *M. tuberculosis* (Liu et al., 2009, 2006). Both are induced via the vitamin D pathway downstream of TLR2/1 activation (M Fabri et al., 2011). Activation of the enzyme CYP27B1 in the macrophage converts pro-vitamin D into its active form, which binds to the vitamin D receptor and stimulates transcription. Vitamin D alone is sufficient to cause cathelicidin secretion, while synergy with IL-1β is also necessary for *DEFB4* upregulation (Liu et al., 2009). Transcription of beta defensin 2 and cathelicidin were subsequently shown to be necessary for vitamin D and TLR2/1L induced antimicrobial activity against mycobacteria within infected macrophages (Liu et al., 2012)

Another antimicrobial protein with demonstrated activity against *M. tuberculosis* and *M. leprae* is granulysin (Balin et al., 2018; Stenger et al., 1998). Cytotoxic $CD8^+$ T cells that were specific for *M. tuberculosis* antigens were demonstrated to have cytotoxic granules containing both granulysin and the pore forming protein perforin. Upon activation, the $CD8^+$ T cells degranulate and perforin forms pores in the macrophage cell membrane and phagosome membrane, allowing for granulysin to access and kill the bacilli (Stenger et al., 1998). Granulysin expression was found to be upregulated in lesions of tuberculoid leprosy, where again it colocalized with perforin in some cells, however it was found to be more associated with $CD4^+$ CTLs (Ochoa et al., 2001). Further studies defined a new population of $CD8^+$ CTLs that are more abundant in the blood of tuberculoid leprosy patients that express three cytotoxic granule proteins: granzyme B, perforin, and granulysin (Balin et al., 2018). These Tri-cytotoxic T cells were more effective at killing *M. leprae* and *L. monocytogenes* infected macrophages than T cells expressing granzyme B and perforin together, or granzyme B alone, highlighting the importance of granulysin in CTL mediated control of mycobacterial infection.

Interleukin-26

As stated previously, Th17 cells have become implicated in host defense against mycobacteria, and interestingly, one of the characteristic cytokines produced by human Th17 cells, interleukin 26 (IL-26), is also a cationic antimicrobial peptide. The 171 amino acid long protein is a member of the IL-10 family by sequence homology and structure (Knappe, Hor, Wittmann, & Fickenscher, 2000). However, unlike other family members, it is highly positively charged, with a charge of +18 at pH 7 and a PI of 11 (Meller et al., 2015). It also has a hydrophobic face, giving it structural similarities to the other antimicrobial peptides mentioned above (Meller et al., 2015). It has been demonstrated to be directly cytotoxic to a variety of both Gram positive and negative bacteria including *E. coli, S. aureus,* and *P. aeruginosa*, as it is expected to multimerize into a pore forming structure. In most vertebrates, including humans, the *IL26* gene is located on chromosome 22 in between *IFNG* and *IL22* (Collins, Henderson, & Aune, 2012). Unusually, despite conservation of *IL26* from bony fish to humans, the gene is absent in rodents.

Aside from its direct antimicrobial function, IL-26 is also proinflammatory. Binding of IL-26 to its receptor, composed of IL-10R2 and IL-20R1, leads to the secretion of IL-1β, IL-6, and IL-8 (Hor et al., 2004; Sheikh et al., 2004). IL-20R1 expression is limited to epithelial cells, whereas IL-10R2 expression is ubiquitously expressed, which was initially thought to limit IL-26 signaling to epithelial sites (Hor et al., 2004). However, IL-26 has been shown to activate the IL-10R2 receptor subunit on monocytes in the absence of IL-20R1, and lead to IL-1β and IL-6 secretion from the cells (Corvaisier et al., 2012; Poli et al., 2017). Additionally, IL-26 can bind to nucleic acids and enhance their sensing by pattern recognition receptors (Meller et al., 2015; Poli et al., 2017). Binding of either bacterial or human DNA to IL-26 led to greater secretion of type I IFN from plasmacytoid dendritic cells via TLR9 activation (Meller et al., 2015). Another group showed that IL-26-DNA complexes can activate the cytosolic DNA sensor stimulator of

interferon genes (STING) within monocytes (Poli et al., 2017). Other Th17 cytokines like IL-17A and IL-22 are also proinflammatory, making IL-26 typical of Th17 effector function (Laan et al., 1999; Miyamoto et al., 2003).

IL-26 was initially discovered as a protein produced by *herpesvirus saimiri* transformed T cells (Knappe et al., 2000). It has since been shown to be produced by T cell receptor activated Th17 cells (Lee et al., 2010; Meller et al., 2015; Wilson et al., 2007). Additionally, it has been implicated in the pathology of many Th17 mediated autoimmune diseases, including rheumatoid arthritis, inflammatory bowel disease, and psoriasis (Corvaisier et al., 2012; Dambacher et al., 2009; Fujii et al., 2017; Itoh et al., 2019). More recently, some groups have described IL-26 secretion from non-T cell sources. Inflamed smooth muscle cells in the setting of vasculitis secrete IL-26, as do activated lung macrophages in the setting of chronic bronchitis (Che et al., 2014; Poli et al., 2017). The lack of an effective antibody for intracellular flow cytometry staining has precluded a comprehensive study of the various cell types that may secrete IL-26 in blood and dissociated tissues.

Aims and significance.

The components that result in protective immunity against intracellular bacterial infection are incompletely understood. When some people encounter *M. leprae* they develop tuberculoid leprosy and seem to mount an effective cell mediated immune response, while other people develop disseminated disease. Still others clear infection without ever becoming symptomatic. Why does this happen?

Years of research has shown that $CD4^+$ Th1 cells that secrete IFN-γ and TNF-α, cytotoxic $CD8^+$ T cells secreting antimicrobial peptides like granulysin, and the vitamin-D antimicrobial protein axis and autophagy activation in macrophages are playing an active role in fighting *M. tuberculosis* and *M. leprae* infection. However, there is mounting evidence that Th17

cells correlate with protective immune responses against mycobacteria. Furthermore, Th17 cells have been shown to be producers of IL-26, a cytokine that has been demonstrated to be directly antimicrobial to extracellular bacteria and proinflammatory both by receptor binding and by enhancing the recognition of nucleic acid by innate immune sensors. Given this, we sought to 1) further understand the biology of IL-26 secretion from Th17 cells and 2) to investigate whether IL-26 is antimicrobial against intracellular bacteria and the mechanism behind that antimicrobial activity. We hoped to better understand the means by which Th17 cells provide protection against mycobacteria. In the bigger picture, we believe that this knowledge can be used in developing and evaluating therapeutics like vaccines or directed antimicrobial treatments.

References

Abdallah, M., Emam, H., Attia, E., Hussein, J., & Mohamed, N. (2013). Estimation of serum level of interleukin-17 and interleukin-4 in leprosy, towards more understanding of leprosy immunopathogenesis. *Indian Journal of Dermatology, Venereology and Leprology*, *79*(6), 772–776. https://doi.org/10.4103/0378-6323.120723

Acosta-Rodriguez, E. V, Napolitani, G., Lanzavecchia, A., & Sallusto, F. (2007). Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. *Nature Immunology*, *8*(9), 942–949. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17676045

Ai, J.-W., Ruan, Q.-L., Liu, Q.-H., & Zhang, W.-H. (2016). Updates on the risk factors for latent tuberculosis reactivation and their managements. *Emerging Microbes & Infections*, *5*(2), e10. https://doi.org/10.1038/emi.2016.10

Arnoldi, J., Gerdes, J., & Flad, H.-D. (1990). Immunohistologic assessment of cytokine production of infiltrating cells in various forms of leprosy. *American Journal of Pathology*, *137*, 749–753.

Attia, E. A., Abdallah, M., El-Khateeb, E., Saad, A. A., Lotfi, R. A., Abdallah, M., & El-Shennawy, D. (2014). Serum Th17 cytokines in leprosy: correlation with circulating CD4(+) CD25 (high)FoxP3 (+) T-regs cells, as well as down regulatory cytokines. *Archives for Dermatological Research. Archiv Für Dermatologische Forschung*, *306*(9), 793–801. https://doi.org/10.1007/s00403-014-1486-2

Balin, S. J., Pellegrini, M., Klechevsky, E., Won, S. T., Weiss, D. I., Choi, A. W., … Modlin, R. L. (2018). Human antimicrobial cytotoxic T lymphocytes, defined by NK receptors and antimicrobial proteins, kill intracellular bacteria. *Sci Immunol*, *3*(26). https://doi.org/10.1126/sciimmunol.aat7668

Barry III, C. E., Boshoff, H. I., Dartois, V., Dick, T., Ehrt, S., Flynn, J., … Young, D. (2009). The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *Nature Reviews: Microbiology*, *7*(12), 845–855. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19855401

Chan, J. P., Uong, J., Nassiri, N., & Gupta, R. (2019). Lessons From Leprosy: Peripheral

Neuropathies and Deformities in Chronic Demyelinating Diseases. *The Journal of Hand Surgery*, *44*(5), 411–415. https://doi.org/10.1016/j.jhsa.2018.07.007
Chaptini, C., & Marshman, G. (2015). Leprosy: a review on elimination, reducing the disease burden, and future research. *Leprosy Review*, *86*(4), 307–315. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/26964426
Che, K. F., Tengvall, S., Levanen, B., Silverpil, E., Smith, M. E., Awad, M., … Linden, A. (2014). Interleukin-26 in antibacterial host defense of human lungs. Effects on neutrophil mobilization. *American Journal of Respiratory and Critical Care Medicine*, *190*(9), 1022–1031. https://doi.org/10.1164/rccm.201404-0689OC
Cho, J. S., Pietras, E. M., Garcia, N. C., Ramos, R. I., Farzam, D. M., Monroe, H. R., … Miller, L. S. (2010). IL-17 is essential for host defense against cutaneous Staphylococcus aureus infection in mice. *Journal of Clinical Investigation*, *120*(5), 1762–1773. https://doi.org/10.1172/jci40891
Collins, P. L., Henderson, M. a, & Aune, T. M. (2012). Lineage-specific adjacent IFNG and IL26 genes share a common distal enhancer element. *Genes and Immunity*, *13*(6), 481–488. https://doi.org/10.1038/gene.2012.22
Cooper, C. L., Mueller, C., Sinchaisri, T. A., Pirmez, C., Chan, J., Kaplan, G., … Modlin, R. L. (1989). Analysis of naturally occurring delayed-type hypersensitivity reactions in leprosy by in situ hybridization. *Journal of Experimental Medicine*, *169*(5), 1565–1581. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2523952
Corvaisier, M., Delneste, Y., Jeanvoine, H., Preisser, L., Blanchard, S., Garo, E., … Jeannin, P. (2012). IL-26 is overexpressed in rheumatoid arthritis and induces proinflammatory cytokine production and Th17 cell generation. *PLoS Biology*, *10*(9), e1001395. https://doi.org/10.1371/journal.pbio.1001395
D'Andrea, A., Aste-Amezaga, M., Valiante, N. M., Ma, X., Kubin, M., & Trinchieri, G. (1993). Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. *Journal of Experimental Medicine*, *178*, 1041–1048.
Dambacher, J., Beigel, F., Zitzmann, K., De Toni, E. N., Goke, B., Diepolder, H. M., … Brand, S. (2009). The role of the novel Th17 cytokine IL-26 in intestinal inflammation. *Gut*, *58*(9), 1207–1217. https://doi.org/10.1136/gut.2007.130112
Daugelat, S., Ladel, C. H., Schoel, B., & Kaufmann, S. H. (1994). Antigen-specific T-cell responses during primary and secondary Listeria monocytogenes infection. *Infection and Immunity*, *62*(5), 1881–1888. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8168953
de Beaucoudrey, L., Puel, A., Filipe-Santos, O., Cobat, A., Ghandil, P., Chrabieh, M., … Casanova, J. L. (2008). Mutations in STAT3 and IL12RB1 impair the development of human IL-17-producing T cells. *Journal of Experimental Medicine*, *205*(7), 1543–1550. https://doi.org/10.1084/jem.20080321
De Smet, K., & Contreras, R. (2005). Human Antimicrobial Peptides: Defensins, Cathelicidins and Histatins. *Biotechnology Letters*, *27*(18), 1337–1347. https://doi.org/10.1007/s10529-005-0936-5
Fabri, M, Stenger, S., Shin, D. M., Yuk, J. M., Liu, P. T., Realegeno, S., … Modlin, R. L. (2011). Vitamin D Is Required for IFN-gamma-Mediated Antimicrobial Activity of Human Macrophages. *Science Translational Medicine*, *3*(104), 104ra102. https://doi.org/ARTN 104ra102DOI 10.1126/scitranslmed.3003045
Fabri, Mario, Realegeno, S. E., Jo, E.-K., & Modlin, R. L. (2011). Role of autophagy in the host response to microbial infection and potential for therapy. *Current Opinion in Immunology*, *23*(1), 65–70. https://doi.org/10.1016/j.coi.2010.10.010
Fernandez Botran, R., Sanders, V. M., Mosmann, T. R., & Vitetta, E. S. (1988). Lymphokine-mediated regulation of the proliferative response of clones of T helper 1 and T helper 2

cells. *Journal of Experimental Medicine*, *168*, 543–558.
Forrellad, M. A., Klepp, L. I., Gioffré, A., Sabio y García, J., Morbidoni, H. R., de la Paz Santangelo, M., … Bigi, F. (2013). Virulence factors of the Mycobacterium tuberculosis complex. *Virulence*, *4*(1), 3–66. https://doi.org/10.4161/viru.22329
Fujii, M., Nishida, A., Imaeda, H., Ohno, M., Nishino, K., Sakai, S., … Andoh, A. (2017). Expression of Interleukin-26 is upregulated in inflammatory bowel disease. *World Journal of Gastroenterology*, *23*(30), 5519–5529. https://doi.org/10.3748/wjg.v23.i30.5519
Gutierrez, M. G., Master, S. S., Singh, S. B., Taylor, G. A., Colombo, M. I., & Deretic, V. (2004). Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. *Cell*, *119*(6), 753–766. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15607973
Happel, K. I., Dubin, P. J., Zheng, M., Ghilardi, N., Lockhart, C., Quinton, L. J., … Kolls, J. K. (2005). Divergent roles of IL-23 and IL-12 in host defense against Klebsiella pneumoniae. *Journal of Experimental Medicine*, *202*(6), 761–769. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16157683
Hess, J., Ladel, C., Miko, D., & Kaufmann, S. H. (1996). Salmonella typhimurium aroA- infection in gene-targeted immunodeficient mice: major role of CD4+ TCR-alpha beta cells and IFN-gamma in bacterial clearance independent of intracellular location. *Journal of Immunology (Baltimore, Md. : 1950)*, *156*(9), 3321–3326. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8617956
Hor, S., Pirzer, H., Dumoutier, L., Bauer, F., Wittmann, S., Sticht, H., … Fickenscher, H. (2004). The T-cell lymphokine interleukin-26 targets epithelial cells through the interleukin-20 receptor 1 and interleukin-10 receptor 2 chains. *Journal of Biological Chemistry*, *279*(32), 33343–33351. https://doi.org/10.1074/jbc.M405000200
Ito, S., Ansari, P., Sakatsume, M., Dickensheets, H., Vazquez, N., Donnelly, R. P., … Finbloom, D. S. (1999). Interleukin-10 inhibits expression of both interferon alpha- and interferon gamma- induced genes by suppressing tyrosine phosphorylation of STAT1. *Blood*, *93*(5), 1456–1463. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10029571
Itoh, T., Hatano, R., Komiya, E., Otsuka, H., Narita, Y., Aune, T. M., … Ohnuma, K. (2019). Biological Effects of IL-26 on T Cell–Mediated Skin Inflammation, Including Psoriasis. *Journal of Investigative Dermatology*, *139*(4), 878–889. https://doi.org/10.1016/j.jid.2018.09.037
Janeway, C. A., Travers, P., Walport, M., & Shlomchik, M. J. (2001). Adaptive immunity to infection. In *Immunobiology 5 : the immune system in health and disease*.
Janeway Jr., C. A., & Medzhitov, R. (2002). Innate immune recognition. *Annual Review of Immunology*, *20*, 197–216. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11861602
Johansson, J., Gudmundsson, G. H., Rottenberg, M. E., Berndt, K. D., & Agerberth, B. (1998). Conformation-dependent antibacterial activity of the naturally occurring human peptide LL-37. *Journal of Biological Chemistry*, *273*(6), 3718–3724. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9452503
Jones, C. E., & Chan, K. (2002). Interleukin-17 stimulates the expression of interleukin-8, growth-related oncogene-alpha, and granulocyte-colony-stimulating factor by human airway epithelial cells. *American Journal of Respiratory Cell and Molecular Biology*, *26*(6), 748–753.
Kawaguchi, M., Kokubu, F., Kuga, H., Matsukura, S., Hoshino, H., Ieki, K., … Huang, S. K. (2001). Modulation of bronchial epithelial cells by IL-17. *Journal of Allergy and Clinical Immunology*, *108*(5), 804–809. https://doi.org/10.1067/mai.2001.119027
Knappe, A., Hor, S., Wittmann, S., & Fickenscher, H. (2000). Induction of a novel cellular homolog of interleukin-10, AK155, by transformation of T lymphocytes with herpesvirus saimiri. *Journal of Virology*, *74*(8), 3881–3887. Retrieved from

http://www.ncbi.nlm.nih.gov/pubmed/10729163
Laan, M., Cui, Z. H., Hoshino, H., Lotvall, J., Sjostrand, M., Gruenert, D. C., … Linden, A. (1999). Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. *Journal of Immunology*, *162*(4), 2347–2352. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9973514
Lastória, J. C., & Abreu, M. A. M. M. de. (2014). Leprosy: review of the epidemiological, clinical, and etiopathogenic aspects - part 1. *Anais Brasileiros de Dermatologia*, *89*(2), 205–218. https://doi.org/10.1590/ABD1806-4841.20142450
Lee, W. W., Kang, S. W., Choi, J., Lee, S. H., Shah, K., Eynon, E. E., … Kang, I. (2010). Regulating human Th17 cells via differential expression of IL-1 receptor. *Blood*, *115*(3), 530–540. https://doi.org/10.1182/blood-2009-08-236521
Liu, P. T., & Modlin, R. L. (2008). Human macrophage host defense against Mycobacterium tuberculosis. *Current Opinion in Immunology*, *20*(4), 371–376. https://doi.org/10.1016/j.coi.2008.05.014
Liu, P. T., Schenk, M., Walker, V. P., Dempsey, P. W., Kanchanapoomi, M., Wheelwright, M., … Modlin, R. L. (2009). Convergence of IL-1beta and VDR activation pathways in human TLR2/1-induced antimicrobial responses. *PloS One*, *4*(6), e5810. https://doi.org/10.1371/journal.pone.0005810
Liu, P. T., Stenger, S., Li, H., Wenzel, L., Tan, B. H., Krutzik, S. R., … Modlin, R. L. (2006). Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science*, *311*(5768), 1770–1773. https://doi.org/10.1126/science.1123933
Liu, P. T., Wheelwright, M., Teles, R., Komisopoulou, E., Edfeldt, K., Ferguson, B., … Modlin, R. L. (2012). MicroRNA-21 targets the vitamin D-dependent antimicrobial pathway in leprosy. *Nature Medicine*, *18*(2), 267–273. https://doi.org/10.1038/nm.2584
Longley, J., Haregewoin, A., Yemaneberhan, T., van Diepen, T. W., Nsibami, J., Knowles, D., … Godal, T. (1985). In vivo responses to Mycobacterium leprae: antigen presentation, interleukin-2 production, and immune cell phenotypes in naturally occuring leprosy lesions. *International Journal of Leprosy*, *53*, 385–394.
Ma, C. S., Chew, G. Y., Simpson, N., Priyadarshi, A., Wong, M., Grimbacher, B., … Cook, M. C. (2008). Deficiency of Th17 cells in hyper IgE syndrome due to mutations in STAT3. *Journal of Experimental Medicine*, *205*(7), 1551–1557. https://doi.org/10.1084/jem.20080218
Massone, C., Belachew, W. A., & Schettini, A. (2015). Histopathology of the lepromatous skin biopsy. *Clinics in Dermatology*, *33*(1), 38–45. https://doi.org/10.1016/j.clindermatol.2014.10.003
Meller, S., Di Domizio, J., Voo, K. S., Friedrich, H. C., Chamilos, G., Ganguly, D., … Gilliet, M. (2015). T(H)17 cells promote microbial killing and innate immune sensing of DNA via interleukin 26. *Nature Immunology*, *16*(9), 970–979. https://doi.org/10.1038/ni.3211
Milner, J. D., Brenchley, J. M., Laurence, A., Freeman, A. F., Hill, B. J., Elias, K. M., … Douek, D. C. (2008). Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. *Nature*, *452*(7188), 773–776. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18337720
Miyamoto, M., Prause, O., Sjostrand, M., Laan, M., Lotvall, J., & Linden, A. (2003). Endogenous IL-17 as a mediator of neutrophil recruitment caused by endotoxin exposure in mouse airways. *Journal of Immunology*, *170*(9), 4665–4672. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12707345
Modlin, R. L. (1994). Th1-Th2 paradigm: insights from leprosy. *Journal of Investigative Dermatology*, *102*(6), 828–832. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8006444
Modlin, R. L., & Bloom, B. R. (2013). TB or not TB: that is no longer the question. *Science Translational Medicine*, *5*(213), 213sr6. https://doi.org/10.1126/scitranslmed.3007402

Modlin, R. L., Hofman, F. M., Horwitz, D. A., Husmann, L. A., Gillis, S., Taylor, C. R., & Rea, T. H. (1984). In situ identification of cells in human leprosy granulomas with monoclonal antibodies to interleukin 2 and its receptor. *Journal of Immunology*, *132*(6), 3085–3090. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6327818

Modlin, R. L., Melancon-Kaplan, J., Young, S. M., Pirmez, C., Kino, H., Convit, J., … Bloom, B. R. (1988). Learning from lesions: patterns of tissue inflammation in leprosy. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(4), 1213–1217. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3257577

Mutis, T., Cornelisse, Y. E., & Ottenhoff, T. H. (1993). Mycobacteria induce CD4+ T cells that are cytotoxic and display Th1-like cytokine secretion profile: heterogeneity in cytotoxic activity and cytokine secretion levels. *European Journal of Immunology*, *23*, 2189–2195.

Ochoa, M. T., Stenger, S., Sieling, P. A., Thoma-Uszynski, S., Sabet, S., Cho, S. G., … Modlin, R. L. (2001). T-cell release of granulysin contributes to host defense in leprosy. *Nature Medicine*, *7*(2), 174–179. https://doi.org/10.1038/84620

Okada, S., Markle, J. G., Deenick, E. K., Mele, F., Averbuch, D., Lagos, M., … Casanova, J.-L. (2015). IMMUNODEFICIENCIES. Impairment of immunity to Candida and Mycobacterium in humans with bi-allelic RORC mutations. *Science (New York, N.Y.)*, *349*(6248), 606–613. https://doi.org/10.1126/science.aaa4282

Poli, C., Augusto, J. F., Dauve, J., Adam, C., Preisser, L., Larochette, V., … Jeannin, P. (2017). IL-26 Confers Proinflammatory Properties to Extracellular DNA. *Journal of Immunology*, *198*(9), 3650–3661. https://doi.org/10.4049/jimmunol.1600594

Quaresma, J. A., Aarao, T. L., Sousa, J. R., Botelho, B. S., Barros, L. F., Araujo, R. S., … Fuzii, H. T. (2015). T-helper 17 cytokines expression in leprosy skin lesions. *British Journal of Dermatology*, *173*(2), 565–567. https://doi.org/10.1111/bjd.13608

Ridley, D. S., & Jopling, W. H. (1966). Classification of leprosy according to immunity. A five-group system. *Int J Lepr*, *34*, 255–273.

Sadhu, S., Khaitan, B. K., Joshi, B., Sengupta, U., Nautiyal, A. K., & Mitra, D. K. (2016). Reciprocity between Regulatory T Cells and Th17 Cells: Relevance to Polarized Immunity in Leprosy. *PLOS Neglected Tropical Diseases*, *10*(1), e0004338. https://doi.org/10.1371/journal.pntd.0004338

Saini, C., Ramesh, V., & Nath, I. (2013). CD4+ Th17 cells discriminate clinical types and constitute a third subset of non Th1, Non Th2 T cells in human leprosy. *PLoS Neglected Tropical Diseases*, *7*(7), e2338. https://doi.org/10.1371/journal.pntd.0002338

Saini, C., Siddiqui, A., Ramesh, V., & Nath, I. (2016). Leprosy Reactions Show Increased Th17 Cell Activity and Reduced FOXP3+ Tregs with Concomitant Decrease in TGF-beta and Increase in IL-6. *PLoS Neglected Tropical Diseases*, *10*(4), e0004592. https://doi.org/10.1371/journal.pntd.0004592

Santos, M. B., de Oliveira, D. T., Cazzaniga, R. A., Varjão, C. S., dos Santos, P. L., Santos, M. L. B., … de Jesus, A. R. (2017). Distinct Roles of Th17 and Th1 Cells in Inflammatory Responses Associated with the Presentation of Paucibacillary Leprosy and Leprosy Reactions. *Scandinavian Journal of Immunology*, *86*(1), 40–49. https://doi.org/10.1111/sji.12558

Schlesinger, L. S. (1993). Macrophage phagocytosis of virulent but not attenuated strains of Mycobacterium tuberculosis is mediated by mannose receptors in addition to complement receptors. *Journal of Immunology*, *150*(7), 2920–2930. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/0008454864

Schlesinger, L. S., Bellinger-Kawahara, C. G., Payne, N. R., & Horwitz, M. A. (1990). Phagocytosis of M. tuberculosis is mediated by human monocyte complement receptors and complement component C3. *Journal of Immunology*, *144*, 2771–2780.

Scollard, D. M., Adams, L. B., Gillis, T. P., Krahenbuhl, J. L., Truman, R. W., & Williams, D. L.

(2006). The continuing challenges of leprosy. *Clinical Microbiology Reviews*, *19*(2), 338–381. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16614253

Sheikh, F., Baurin, V. V, Lewis-Antes, A., Shah, N. K., Smirnov, S. V, Anantha, S., … Kotenko, S. V. (2004). Cutting edge: IL-26 signals through a novel receptor complex composed of IL-20 receptor 1 and IL-10 receptor 2. *Journal of Immunology*, *172*(4), 2006–2010. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14764663

Sieling, P. A., Abrams, J. S., Yamamura, M., Salgame, P., Bloom, B. R., Rea, T. H., & Modlin, R. L. (1993). Immunosuppressive roles for IL-10 and IL-4 in human infection. In vitro modulation of T cell responses in leprosy. *Journal of Immunology*, *150*(12), 5501–5510.

Silva, B. J., Barbosa, M. G., Andrade, P. R., Ferreira, H., Nery, J. A., Corte-Real, S., … Pinheiro, R. O. (2017). Autophagy Is an Innate Mechanism Associated with Leprosy Polarization. *PLoS Pathogens*, *13*(1), e1006103. https://doi.org/10.1371/journal.ppat.1006103

Sreenivasan, P., Misra, R. S., Wilfred, D., & Nath, I. (1998). Lepromatous leprosy patients show T helper 1-like cytokine profile with differential expression of interleukin-10 during type 1 and 2 reactions. *Immunology*, *95*(4), 529–536. https://doi.org/10.1046/j.1365-2567.1998.00634.x

Stenger, S., Hanson, D. A., Teitelbaum, R., Dewan, P., Niazi, K. R., Froelich, C. J., … Modlin, R. L. (1998). An antimicrobial activity of cytolytic T cells mediated by granulysin. *Science*, *282*(5386), 121–125. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9756476

Tameris, M. D., Hatherill, M., Landry, B. S., Scriba, T. J., Snowden, M. A., Lockhart, S., … McShane, H. (2013). Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. *Lancet*, *381*(9871), 1021–1028. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23391465

Teles, R. M., Graeber, T. G., Krutzik, S. R., Montoya, D., Schenk, M., Lee, D. J., … Modlin, R. L. (2013). Type I interferon suppresses type II interferon-triggered human anti-mycobacterial responses. *Science*, *339*(6126), 1448–1453. https://doi.org/10.1126/science.1233665

Touw, J., Langendijk, E. M., Stoner, G. L., & Belehu, A. (1982). Humoral immunity in leprosy: immunoglobulin G and M antibody responses to Mycobacterium leprae in relation to various disease patterns. *Infection and Immunity*, *36*(3), 885–892. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7047400

Vergne, I., Chua, J., Lee, H.-H., Lucas, M., Belisle, J., & Deretic, V. (2005). Mechanism of phagolysosome biogenesis block by viable Mycobacterium tuberculosis. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(11), 4033–4038. https://doi.org/10.1073/pnas.0409716102

Via, L. E., Deretic, D., Ulmer, R. J., Hibler, N. S., Huber, L. A., & Deretic, V. (1997). Arrest of mycobacterial phagosome maturation is caused by a block in vesicle fusion between stages controlled by rab5 and rab7. *The Journal of Biological Chemistry*, *272*(20), 13326–13331. https://doi.org/10.1074/jbc.272.20.13326

Volc-Platzer, B., Stemberger, H., Luger, T., Radaszkiewicz, T., & Wiedermann, G. (1988). Defective intralesional interferon-gamma activity in lepromatous leprosy. *Clinical and Experimental Immunology*, *71*, 235–240.

WHO. (2014). Global tuberculosis report.

WHO. (2016). *Global leprosy strategy 2016-2020: Accelerating towards a leprosy-free world*.

Wilson, N. J., Boniface, K., Chan, J. R., McKenzie, B. S., Blumenschein, W. M., Mattson, J. D., … de Waal, M. R. (2007). Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nature Immunology*, *8*(9), 950–957. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17676044

Yamamura, M., Uyemura, K., Deans, R. J., Weinberg, K., Rea, T. H., Bloom, B. R., & Modlin, R.

L. (1991). Defining protective responses to pathogens: cytokine profiles in leprosy lesions. *Science*, *254*(5029), 277–279. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1925582

Yamamura, M., Wang, X. H., Ohmen, J. D., Uyemura, K., Rea, T. H., Bloom, B. R., & Modlin, R. L. (1992). Cytokine patterns of immunologically mediated tissue damage. *Journal of Immunology*, *149*(4), 1470–1475. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1500726

Ye, P., Rodriguez, F. H., Kanaly, S., Stocking, K. L., Schurr, J., Schwarzenberger, P., … Kolls, J. K. (2001). Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. *Journal of Experimental Medicine*, *194*(4), 519–527. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11514607

Yokota, T., Coffman, R. L., Hagiwara, H., Rennick, D. M., Takebe, Y., Yokota, K., … Arai, K. (1987). Isolation and characterization of lymphokine cDNA clones encoding mouse and human IgA-enhancing factor and eosinophil colony-stimulating factor activities: relationship to interleukin 5. *Proceedings of the National Academy of Sciences of the United States of America*, *84*, 7388–7392.

Zheng, W., & Flavell, R. A. (1997). The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell*, *89*(4), 587–596.

CHAPTER 2

IL-1β induces the rapid secretion of the antimicrobial protein IL-26 from Th17 cells

Abstract: Th17 cells play a critical role in the adaptive immune response against extracellular bacteria and the possible mechanisms by which they can protect against infection are of particular interest. Here we describe a novel IL-1β dependent pathway for secretion of the antimicrobial peptide IL-26 from human Th17 cells that is independent of and more rapid than classical T cell receptor activation. We find that IL-26 is secreted by 3 hours after treating peripheral blood mononuclear cells with *Mycobacterium leprae*, as compared to 48 hours for IFN-γ and IL-17A. IL-1β was required for microbial ligand induction of IL-26 and was sufficient to stimulate IL-26 release from Th17 cells. Only the IL-1 receptor 1 (IL-1RI) positive Th17 cells responded to IL-1β, inducing an NF-kB regulated transcriptome. Finally, supernatants from IL-1β treated memory T cells killed *E. coli* in an IL-26 dependent manner. These results identify a mechanism by which human IL-1RI$^+$ "antimicrobial Th17 cells" can be rapidly activated by IL-1β as part of the innate immune response to produce IL-26 in order to kill extracellular bacteria.

Introduction

T cells are crucial for effective host defense against a wide range of infectious pathogens, triggering an antimicrobial activity against the foreign invader. T cells release antimicrobial proteins, which directly kill the invading pathogen such as granulysin (Stenger et al., 1998), or cytokines that act indirectly, by causing the induction of antimicrobial effector molecules in other cell types. It is generally accepted that Th17 cells play a critical role in host defense against extracellular bacteria (Cho et al., 2010; Happel et al., 2005; Milner et al., 2008; Ye et al., 2001). The IL-17 family of cytokines, including IL-17A and IL-17F, bind to receptors on a variety of cell types including epithelial cells, fibroblasts, macrophages, and neutrophils.

This leads to the secretion of chemokines like CXCL2, CXCL5, and IL-8 and inflammatory cytokines IL-6, G-CSF and GM-CSF, attracting and activating myeloid cells at the site of infection (Jones & Chan, 2002; Kawaguchi et al., 2001; Laan et al., 1999). IL-17 and IL-22 from Th17 cells are also able to stimulate antimicrobial defensin secretion from epithelial cells (Jones & Chan, 2002; Kawaguchi et al., 2001; Meller et al., 2015; Milner et al., 2008). We have previously reported that Th17 cells also secrete IL-26, a cytokine that has been shown to be directly antimicrobial against a variety of Gram positive and Gram negative bacteria (Meller et al., 2015).

IL-26, a 19-kDa α -helical protein that belongs to the IL-10 cytokine family, is conserved in most vertebrate species, but is absent in mice (Donnelly et al., 2010). It is secreted primarily by activated T cells, and among $CD4^+$ helper T cells it is more specifically produced by Th17 cells (Meller et al., 2015; Wilson et al., 2007; Wolk, Kunz, Asadullah, & Sabat, 2002). IL-26 displays certain hallmarks of naturally occurring antimicrobial peptides, including amphipathic structure, clustering of cationic charges, and multimer formation, and was found to disrupt bacterial membranes via pore formation (Zasloff, 2002). Aside from its antimicrobial properties, IL-26 is also pro-inflammatory, and signaling through its IL-10R2/IL-20R1 heterodimeric receptor on epithelial cells results in secretion of proinflammatory cytokines such as IL-6, IL-1β, and IL-8 (Hor et al., 2004; Sheikh et al., 2004). Monocytes and macrophages may also respond to IL-26 to produce inflammatory cytokines despite only expressing the IL-10R2 subunit of the receptor (Corvaisier et al., 2012). Furthermore, IL-26 has been shown to bind to both DNA and RNA and enhance their detection by cellular sensors, resulting in increased type I interferon production (Meller et al., 2015; Poli et al., 2017).

Compared to the rapidity of the innate response, the induction of adaptive T cell effector function is relatively slow, preventing T cells from contributing to host defense during the critical early phase of the immune response (Janeway Jr. & Medzhitov, 2002). There is evidence that

some lymphoid cell subpopulations can be rapidly activated by cytokines to contribute to the early immune response, such as innate lymphoid cells (ILCs), which are devoid of T cell receptors (TCR), and γδ T cells (Cua & Tato, 2010; Hasegawa et al., 2013; Sutton et al., 2009). In addition, some of the T cell polarizing cytokines are known to augment activation of differentiated cells. For example, IL-12, which polarize naïve cells to become Th1 cells (Hsieh et al., 1993; Manetti et al., 1993; Seder & Paul, 1994), synergizes with IL-18 to induce IFN-γ production by Th1 cells in the absence of TCR activation(Yoshimoto et al., 1998) and IL-1β, which is a polarizing cytokine for Th17 cells (Acosta-Rodriguez, Napolitani, Lanzavecchia, & Sallusto, 2007; Manel, Unutmaz, & Littman, 2008), enhances TCR induced release of IL-17 from Th17 cells (W. W. Lee et al., 2010). Here we investigated whether Th17 cells can be activated by TCR-independent pathways to secrete the antimicrobial protein IL-26 to defend the host against microbial infection.

Results

IL-26 secretion is rapid after PBMC stimulation with bacterial sonicate

In order to determine the kinetics of IL-26 production in response to invading bacteria, we studied the time course of cytokine release from PBMCs in response to *Mycobacterium leprae*, given that this bacterium, in addition to inducing Th1 cells, activates Th17 cells (Arlehamn et al., 2014; Becattini et al., 2015). We measured the kinetics of IL-26 induction compared with characteristic memory T cell cytokines IL-17A and IFN-γ by stimulating PBMCs from tuberculoid leprosy (T-lep) patients with sonicated *M. leprae*, as these individuals mount strong T cell responses to the pathogen (Modlin et al., 1988). IL-17A and IFN-γ were not detectable until 2 days after *M. leprae* treatment, as is typical for a memory response (Fig. 1A). Surprisingly, IL-26 was detected in the supernatants as early as 3 hours with concentrations

steadily increasing over 4 days. These results indicate that *M. leprae* sonicate rapidly induces IL-26 at an earlier time point than a classic memory recall response.

Innate stimuli trigger IL-26 secretion from PBMC in an IL-1β dependent manner

We next investigated the innate mechanism of IL-26 induction by stimulating PBMCs overnight with a variety of microbial ligands that activate pattern recognition receptors (PRRs), given that mycobacteria express a range of innate ligands. The early secretion of IL-26 by PBMCs in response to *M. leprae* sonicate suggested a mechanism involving an alternative pathway not requiring T cell memory, such that we studied IL-26 induction in healthy donors. We found several PRR ligands that significantly induced IL-26 in high (TLR5L, TLR4L, TLR2/1L, TLR2L), medium (TLR2/6L, NOD2L) and low (TLR8L, TLR9L) amounts (Fig. 1B).

We asked whether PRR induction of IL-26 was a direct effect of PRR engagement or was dependent upon the release of a proinflammatory cytokine. To this end we investigated whether known PRR induced cytokines, as well as T cell modulatory cytokines, were sufficient by themselves to induce IL-26 (Fig. 1C). Strikingly, of nine cytokines tested, only IL-1β was able to induce significant IL-26 secretion from the T cells after 48 hours. Furthermore, analysis of supernatants collected from PRR ligand stimulated PBMC revealed that PRR ligands that stimulated greater amounts of IL-1β also tended to stimulate greater amounts of IL-26 (Fig. 1D). We were able to fit a sigmoidal dose-response curve to this data with an $R^2 = 0.69$, which demonstrated a clear association between IL-1β and IL-26 secretion. Finally, using a suboptimal dose of Pam3CSK4, a TLR2/1 ligand, we find that the addition of anti-IL-1β blocking antibody led to a significant decrease in IL-26 secretion after PBMC stimulation (Fig. 1E). These results demonstrate that the mechanism for the early induction of IL-26 by PRR ligands is mediated by the production of IL-1β.

Memory CD4$^+$ T cells secrete IL-26 in response to IL-1β in the absence of T cell receptor activation

Given that PBMCs are a heterogeneous group of immune cells and that IL-26 secretion is primarily associated with Th17 cells, we asked whether T cells could be induced by IL-1β to secrete IL-26 in the absence of TCR stimulation. We found that when treated with IL-1β, both purified CD3$^+$ T cells and CD4$^+$ T cells produced IL-26, while there was no IL-26 secretion with IL-6 or IL-23 treatment (Fig. 2A). Further experiments demonstrated that purified CD45RO$^+$ memory CD4$^+$ T cells accounted for all of the IL-26 production seen following IL-1β stimulation (Fig. 2B). Within the CD4+ memory T cell compartment, IL-1R1$^+$ T cells were required for the IL-1β-induced IL-26 secretion as depletion of these cells abrogated the response (fig. S1).

Investigation of the response of memory CD4$^+$ T cells to varying doses of IL-1β revealed that after 48 hours, concentrations of IL-1β as low as 0.032 ng/ml resulted in significant levels of IL-26 secretion from the cells compared to untreated cells (p-adj ≤ 0.001) (Fig. 2C, upper left panel). Higher doses of IL-1β resulted in increasing amounts of IL-26 secretion, with the effect plateauing at concentrations of 0.8 ng/ml and above. We also performed a dose response analysis with Immunocult human T cell activator (StemCell Inc.), a solution of tetrameric antibody complexes against CD3 and CD28 (subsequently referred to as anti-CD3/CD28), to compare IL-26 secretion in response to IL-1β or T cell receptor crosslinking (Fig. 2C, upper left panel). The addition of increasing doses of anti-CD3/CD28 led to increasing amounts of IL-26 secretion such that at the two highest concentrations IL-26 secretion was enhanced compared to IL-1β, but this is not statistically significant.

To characterize the kinetics of the IL-26 response, we longitudinally sampled T cell cultures stimulated in parallel with either IL-1β or anti-CD3/CD28 at concentrations that had yielded similar levels of IL-26 after 48 hours (20 ng/ml and 0.5 µl/ml, respectively). When cultured with IL-1β, significant IL-26 secretion became detectable at 6 hours (p = 0.0003 as

compared to untreated cells), with IL-26 concentrations in the supernatants rising over the course of the next two days (Fig. 2D). In contrast, anti-CD3/CD28 antibody complexes did not stimulate IL-26 secretion at early time points, with protein release only becoming significantly different from untreated control cells after 24 hours of activation ($p \leq 0.0001$). Furthermore, IL-26 secretion was significantly different between IL-1β and anti-CD3/CD28 stimulation at the 6, 9, 12, and 24 hour time points (Fig. 2D). A dose titration of anti-CD3/CD28 was performed at 4 hours to critically measure the early release of IL-26 in response to TCR activation. At this early timepoint, IL-1β at concentrations as low as 0.032 ng/ml induced significant IL-26 in memory $CD4^+$ T cells ($p = 0.002$). The addition of 25 µl/ml of anti-CD3/CD28, a dose that led to a nearly three-fold increase in IL-26 release on average compared to the highest dose of IL-1β after 48 hours, could only induce one ninth of the amount of IL-26 secreted by IL-1β treated cells at 4 hours [631.4 ± 355.1 pg/ml vs. 70.8 ± 58.0 pg/ml]. These data indicate that suboptimal TCR crosslinking was not responsible for the difference in the kinetics of TCR vs. IL-1β induced IL-26 secretion.

Aside from the difference in the rapid IL-26 secretion from memory $CD4^+$ T cells, we also found a functional difference in cytokine secretion between stimulation with IL-1β and anti-CD3/CD28. While both stimuli induced IL-26 secretion, only anti-CD3/CD28 induced the secretion of other Th17 cytokines, including IL-17A, IL-17F, and IL-22 (Fig. 2C). Therefore, these data demonstrate that IL-1β stimulation of memory $CD4^+$ T cells leads to the rapid release of IL-26 but no other Th17 cell cytokines.

IL-26 is secreted by IL-1RI$^+$ Th17 cells

To determine whether IL-1β directly activated Th17 cells, we isolated the various $CD4^+$ T cell populations from memory $CD4^+$ T cells. Using known surface markers for FACS sorting, we evaluated the effect of IL-1β on Th17 cells ($CD161^+CCR6^+CCR4^+CXCR3^-$), Th1 cells ($CD161^-$

CCR6$^-$CCR4$^-$CXCR3$^+$), and Th2 cells (CD161$^-$CCR6$^-$CCR4$^+$CXCR3$^-$) (Fig. 3A) (Annunziato et al., 2007; Bonecchi et al., 1998; Maggi et al., 2010). We found that IL-1β significantly induced IL-26 secretion from only the Th17 cells (Fig. 3B). By flow cytometry, we determined that the memory Th17 cells were Lin$^+$ and did not express CD127 together with CD117, markers of ILC3 cells (De Grove et al., 2016; Spits et al., 2013). There are several reports of enriched IL-1 receptor expression on Th17 cells (W. W. Lee et al., 2010; Maggi et al., 2010; Sha & Markovic-Plese, 2011), which we hypothesized would enable these cells to respond to exogenous IL-1β, consistent with the finding that IL-1β induction of IL-26 in CD4 T memory cells was abrogated by depletion of IL-1RI$^+$ T cells (fig. S1). To directly determine the response of IL-1RI$^+$ Th17 cells, we sorted IL-1RI$^+$ and IL-1RI$^-$ Th17 cells using FACS (Fig. 3C).. We pre-incubated memory CD4$^+$ T cells with IL-2 overnight before sorting, as IL-2 has been shown to increase the frequency of IL-1RI$^+$ T cells (Mercer, Kozhaya, & Unutmaz, 2010). When stimulated with 100 ng/ml IL-1β or 5 µl/ml anti-CD3/CD28 for 9 hours, IL-1RI$^+$ cells secreted IL-26 whereas IL-1RI$^-$ cells did not (Fig. 3D). This finding reflects a previous report that the potential to upregulate *IL26* mRNA after activation and differentiation under Th17 polarizing conditions was dependent upon IL-1RI expression by the treated naïve cells (W. W. Lee et al., 2010). Both populations secreted IL-17A in response to anti-CD3/CD28, although the IL-1RI$^+$ Th17 cells produced more IL-17A, as previously reported (W. W. Lee et al., 2010) (Fig. 3E). Neither cell population secreted IL-17A in response to IL-1β. These results indicate that within Th17 cells, IL-26 production is restricted to the IL-1RI$^+$ subset whether activated by IL-1β or TCR crosslinking. In contrast, IL-17A secretion required TCR activation.

IL-1RI activation induces a predominant NF-κB dependent gene program in Th17 cells

Having demonstrated that IL-1β stimulation of Th17 cells causes secretion of the antimicrobial protein IL-26, we sought to investigate whether IL-1β induced an antimicrobial

gene expression program in these cells by mRNA sequencing. We treated sorted IL-1RI$^+$ and IL-1RI$^-$ Th17 cells with either IL-1β, anti-CD3/CD28, or media for 9 hours before harvesting mRNA. An unsupervised principal component analysis of the samples clearly showed that stimulation of both IL-1RI$^+$ and IL-1RI$^-$ Th17 cells with anti-CD3/CD28 had a dramatic effect on gene expression and these samples clustered together in a distinct group away from samples treated with IL-1β or media (Fig. 4A). The IL-1β stimulated and media control samples clustered together, suggesting that the gene expression changes induced by IL-1β were less pronounced compared to anti-CD3/CD28. In each cluster, the IL-1RI$^+$ and IL-1RI$^-$ Th17 cells were mixed.

A second unsupervised analysis, hierarchical clustering, revealed a similar pattern to the PCA, with the anti-CD3/CD28 stimulated samples forming a distinct cluster, whereas IL-1β and media treated samples clustered together (Fig. 4B). Within the branch containing IL-1β treated cells and media treated cells, the samples form subclusters based on the blood donor. Finally, the samples were further divided based on cell surface phenotype, that is IL-1RI$^+$ and IL-1RI$^-$ cells. Therefore, for both the PCA and hierarchical clustering, the greatest effect on the Th17 transcriptome was activation with anti-CD3/CD28, with lesser effects seen for IL-1β treatment or the expression of IL-1RI.

When comparing the media treated IL-1RI$^+$ and IL-1RI$^-$ Th17 cells to each other, differential expression analysis revealed 2844 genes whose expression differed with a p-adj ≤ 0.05 between the two populations (Fig. 4C). Consistent with the sorting strategy, IL-1 receptor genes *IL1R1* and *IL1R2* were among the most highly differentially expressed genes by both fold change (FC) and adjusted p-value. Several characteristic Th17 cell genes including *RORC*, *IL23R,* and *LGALS3* were upregulated in IL-1RI$^+$ Th17 cells (Ramesh et al., 2014). Gene expression for the regulatory T cell markers *FOXP3, LRRC32, IL2RA, and CTLA4* were also more highly expressed by these cells (Hori, Nomura, & Sakaguchi, 2003; Sakaguchi,

Sakaguchi, Asano, Itoh, & Toda, 1995; Tran et al., 2009). This finding is consistent with previous reports that IL-1RI$^+$ cells include a population of IL-17 producing regulatory T cells (Raffin, Raimbaud, Valmori, & Ayyoub, 2011; Valmori, Raffin, Raimbaud, & Ayyoub, 2010). TCR repertoire analysis revealed that IL-1RI$^+$ and IL-1RI$^-$ Th17 cells were very similar with respect to TCR usage. Both populations had diverse TCR alpha and beta repertoires with no restriction in terms of V or J usage (fig. S2A). Additionally, there was no preference for specific V-J region combinations (fig. S2B).

In IL-1RI$^+$ Th17 cells, IL-1β stimulation significantly induced the expression of 339 genes with a FC ≥ 1.5 and p-adj ≤ 0.05 compared to media treated cells (Fig. 4D). Functional analysis of these genes with Ingenuity Pathway Analysis (IPA) found that the most significantly enriched canonical pathway was "Th17 cell activation" ($p = 2.51 \times 10^{-17}$), with our dataset containing 21% of the pathway genes. (Table I). These genes encoded effector Th17 cytokines (*IL26, IL17A, IL23A, IL22*), transcription factors (*AHR, HIF1A, BATF*), and signaling molecules (*IRAK2, IRAK3, JAK3*). Of the 19 genes, 15 were more strongly induced by anti-CD3/CD28 vs IL-1β (14/15 were significant, p-adj ≤ 0.05), including *IL17A, IL17F,* and *IL22,* whose expression is increased by approximately 100-fold, 50-fold, and 25-fold, respectively (Table I). Overall, anti-CD3/CD28 induced greater expression of 211 of the 339 genes compared to IL-1β treatment.

Aside from the 'Th17 activation pathway' genes, the transcript most induced by IL-1β in the 320 remaining genes was *CXCL8,* which encodes the protein IL-8 (Fig. 4D). The DESeq2 normalized counts for *CXCL8* were similar for cells treated with IL-1β or anti-CD3/CD28. The level of IL-8 protein secreted by memory CD4$^+$ T cells was roughly equal after stimulation with IL-1β or anti-CD3/CD28, reflecting the similar mRNA expression in IL-1RI$^+$ Th17 cells (Figs. 4D, 4E). Although IL-26 protein was induced 2-fold greater by anti-CD3/CD28 vs IL-1β treatment (Fig. 3D), the *IL26* mRNA induction was 7-fold greater (Fig. 4D). In contrast, IL-17A, IL-17F, and IL-22 proteins were detected following anti-CD3/CD28 but not IL-1β treatment (Fig. 2C, Fig.

3E); as stated, the corresponding mRNAs were induced by greater than 25 fold following anti-CD3/CD28 vs. IL-1β treatment (Fig. 4D). This comparison of mRNA transcript upregulation to protein secretion suggests a threshold for protein induction such that the magnitude of mRNA induction by IL-1β is sufficient to produce IL-26 and IL-8 protein but not IL-17A, IL-17F, and IL-22.

We found using the gene set enrichment analysis tool Enrichr (Kuleshov et al., 2016; Nakatsuji et al., 2008) that genes upregulated by IL-1β in IL-1RI^{+} Th17 cells with a p-adj ≤ 0.05 and a FC ≥ 1.5 were highly significantly enriched for RelA target genes (p-adj = $2.7x10^{-31}$) (Fig. 5A). This is consistent with the canonical IL-1 signaling pathway, in which IL-1 receptor engagement activates MyD88, with subsequent activation of interleukin-1 receptor associated kinases and TNF-receptor associated factor proteins that activate NF-κB phosphorylation and translocation to the nucleus (Weber, Wasiliew, & Kracht, 2010). The transcription factor with the second most significant target gene enrichment in this data set was MYB (p-adj = $3.9x10^{-8}$). In contrast, Enrichr analysis of the upregulated genes in anti-CD3/CD28 treated IL-1RI^{+} Th17 cells revealed enrichment for genes regulated by a variety of transcription factors, including *RELA* (p-adj = $9.5x10^{-22}$), *ETS1* (p-adj = $7.5x10^{-20}$), *VDR* (p-adj = $5.3x10^{-17}$), *GABP* (p-adj = $7.5x10^{-15}$), and *FOXP3* (p-adj = $1.8x10^{-13}$). These data suggest that IL-1β is sufficient to elicit an NF-κB regulated gene expression pattern that leads to IL-26 secretion but, unlike TCR activation, does not robustly stimulate activation of downstream genes under the control of a broad variety of transcription factors.

Given that both IL-1β and TCR stimulation of the IL-1RI^{+} Th17 cells led to induction of an NF-κB transcriptional signature and IL-26 secretion, and for IL-1β treatment the NF-κB downstream gene signature was dominant, we hypothesized that NF-κB played an important regulatory role in IL-26 expression. To test this, we inhibited NF-κB activation with the IκBα phosphorylation inhibitor Bay 11-7082 before activating memory CD4^{+} T cells with IL-1β or anti-

CD3/CD28 antibodies (J. Lee, Rhee, Kim, & Cho, 2012; Pierce et al., 1997). We found that Bay 11-7082 completely abrogated IL-26 secretion from the cells (Fig. 5C). Therefore, we conclude that NF-κB activation is necessary for IL-26 production from T cells.

IL-1β stimulated T cells kill bacteria in an IL-26 dependent manner

The ability of Th17 cells to mount an IL-26 dependent antimicrobial response after TCR activation has been previously demonstrated (Meller et al., 2015; Woetmann et al., 2018), so we thought it important to establish whether activation of memory $CD4^+$ T cells through the alternative IL-1β pathway would lead to a functional antimicrobial response. Memory $CD4^+$ T cells were cultured for 48 hours with or without 20 ng/ml IL-1β and IL-26 secretion in the supernatant was confirmed by ELISA (Fig. 6A). We found that T cell supernatants from IL-1β treated memory $CD4^+$ T cells were directly antimicrobial against *E. coli,* leading to a 69% reduction in CFU formation compared to supernatants from untreated T cells (Fig. 6B). Incubating the supernatants with anti-IL-26 antibodies for one hour prior to the addition of bacteria reduced the antimicrobial ability of the supernatant by 56% while incubation of supernatants with isotype matched control antibodies did not. We found that recombinant IL-1β itself had no effect on bacterial viability (fig. S3A). Bacteria incubated in unconditioned culture media was used as a negative control while incubation in media containing an antibiotic mixture of penicillin (50 U/ml) and streptomycin (50 µg/ml) was used as a positive control. These results establish that IL-1β can activate memory $CD4^+$ T cells to mount an antimicrobial response that is mediated by IL-26 secretion.

Discussion

Given that Th17 cells produce the antimicrobial protein IL-26 that contributes to host defense against bacterial infection, we studied the immune pathways that result in the release of this cytokine in response to the pathogen. Here we establish that *M. leprae* can stimulate memory CD4+ T cells to produce IL-26, but through distinct pathways with different kinetics. *M. leprae* and defined PRR ligands induced rapid and IL-1β dependent IL-26 secretion from PBMCs as early as 3 hours. IL-1β stimulated the secretion of IL-26 from memory $CD4^+$ T cells independent of and more rapidly than by TCR activation. IL-1β did not induce other Th17 cytokines, unlike TCR activation with anti-CD3/CD28 antibody complexes. We identified a subpopulation of Th17 cells that express IL-1RI and are responsible for virtually all of the IL-26 production by Th17 cells in response to either IL-1β or TCR activation. Analysis of the transcriptome of IL-$1RI^+$ Th17 cells indicated IL-1β treatment induced characteristic Th17 genes and revealed that IL-1β also stimulates IL-8 release from IL-$1RI^+$ Th17 cells. IL-1β upregulated an overall gene expression pattern regulated by NF-κB, whereas genes induced by TCR stimulation were regulated by multiple transcription factors. We established that NF-κB regulates IL-26 production in T cells using NF-κB activation inhibitors. Furthermore, IL-1β activated memory $CD4^+$ T cells exhibited antimicrobial activity against *E. coli*, which was blocked by anti-IL-26 neutralizing antibodies. Taken together, these results identify "antimicrobial Th17 cells" that can contribute to innate immunity by their expression of IL-1RI and very rapid response to IL-1β resulting in IL-26 dependent killing of bacteria.

Previously, Th17 cells were shown to secrete IL-26 following activation via the TCR as part of the adaptive immune response (Meller et al., 2015; Wolk et al., 2002). We elucidate a novel IL-1 dependent pathway for IL-26 secretion from memory IL-$1RI^+$ Th17 cells that is independent of and more rapid than TCR activation as part of the innate immune response. The rapidity of the IL-1β pathways compared to TCR triggering enables Th17 cells at the site of

infection to quickly produce an antimicrobial protein even before the response to antigen occurs. Furthermore, we demonstrate that the ability of IL-1β to trigger IL-26 results in a direct antimicrobial activity against a common mucosal pathogen *E. coli*, and that the addition of anti-IL-26 antibodies blocked killing of the bacteria. This did not preclude other antimicrobial mediators, for example we demonstrate that IL-1β induces IL-1RI$^+$ Th17 cells to secrete IL-8, which contains an antimicrobial peptide released following acid hydrolysis (Bjorstad, Fu, Karlsson, Dahlgren, & Bylund, 2005). These data challenge the current paradigm for how Th17 cells respond to infection by demonstrating that human IL-1RI$^+$ Th17 cells can respond rapidly by an IL-1β mediated pathway prior to activation of their known TCR pathway. Thus, we describe a novel T cell subpopulation that can take advantage of both innate and adaptive activation pathways to participate in host defense.

We demonstrate depletion of IL-1RI$^+$ memory CD4$^+$ T cells abrogated the IL-1β induced IL-26 response and that IL-1β stimulates IL-26 secretion from Th17 cells but not from Th1 and Th2 cells. However, we cannot preclude that other IL-1RI expressing T cell populations have the capacity to secrete IL-26 in response to IL-1β. In addition, there is evidence that non-lymphoid cells including alveolar macrophages, fibroblast-like synoviocytes, and vascular smooth muscle cells are capable of producing IL-26 (Che et al., 2014; Corvaisier et al., 2012; Poli et al., 2017). It is therefore possible that cells of the myeloid lineage such as monocytes and dendritic cells contribute directly to the higher level of IL-26 production in IL-1β treated PBMC vs. memory CD4$^+$ T cells. These myeloid cells may also contribute to the production of cytokines which amplify IL-26 secretion. For example, a combination of IL-1β and TNF-alpha enhances IL-26 secretion in smooth muscle cells (Poli et al., 2017).

Our data indicate that IL-1β activation of Th17 cells induced the release of IL-26 and IL-8. The ability of lymphocytes to rapidly respond to exogenous cytokines to produce Th17 cytokines has been observed for other unique lymphoid subpopulations. For example, IL-23

and IL-1β have been shown to induce IL-17 secretion from γδ T cells and IL-17 and IL-22 secretion from ILC3 cells (Cua & Tato, 2010; Hasegawa et al., 2013; Sutton et al., 2009). However, γδ T cells express a restricted range of TCRs and ILCs lack TCRs altogether, precluding their participation in classic memory responses. Analysis of T cell receptor genes in our RNA sequencing data demonstrated that IL-1RI$^+$ Th17 cells expressed a wide range of conventional αβ T cell receptor genes. Our data is distinct in demonstrating that IL-1β triggers conventional αβ T cells to secrete IL-26 and mount an antimicrobial response independent of TCR activation.

IL-1β treatment of memory CD4$^+$ T cells was characterized by the secretion of IL-26 and the predominant induction of NF-κB pathways, whereas in addition, TCR activation triggered the full spectrum of Th17 cytokines and a diverse array of signaling pathways. We demonstrated that IL-1β induction of IL-26 protein secretion was indeed dependent on NF-κB signaling. The IL-26 gene is found on chromosome 12q15 and is flanked by IL-22 and IFN-γ, with this gene order conserved across vertebrates (Collins, Henderson, & Aune, 2012). In agreement with our data, it has been shown that an NF-κB binding site within this locus is involved in the distinct regulation of the IL-26 gene (Collins et al., 2012). It is known that IL-1 signals through MyD88, leading to induction of genes downstream of NF-κB. Activation of Toll-like receptors also proceeds through the same MyD88—NF-κB signaling pathway (Kawasaki & Kawai, 2014; Weber et al., 2010). Thus, IL-1RI$^+$ Th17 cells can take advantage of an archetypal innate signaling pathway to mount a rapid antimicrobial response.

Th17 cells have been previously described as “pathogenic” vs. “non-pathogenic” based on their contribution to tissue injury and their secreted cytokine pattern; pathogenic Th17 cells produce IL-17A and IFN-γ whereas non-pathogenic Th17 cells produce IL-17A and IL-10 (Zielinski et al., 2012). We define “antimicrobial Th17 cells” based upon the expression of IL-1RI, production of IL-26 and the ability to mediate a rapid antimicrobial response against

extracellular bacteria. In addition to their ability to release the antimicrobial protein IL-26, our transcriptome analysis revealed that the IL-1RI$^+$ Th17 cells express Treg markers *FOXP3, LRRC32*, *CTLA4* and *CD25.* It is thought that some of these cells represent intermediates in Th17 development from Treg precursors as they secrete IL-17A, express *FOXP3*, suppress T cell proliferation, and exhibit a central memory phenotype (Raffin et al., 2011; Valmori et al., 2010). Alternatively, CD25 and CTLA4 are features of activated T cells and IL-1RI has been shown to be upregulated by TCR activation, therefore it is possible that IL-1RI$^+$ Th17 isolated *ex vivo* may represent a recently activated group of cells (Linsley et al., 1992; Waldmann, 1989). In this context, IL-1β could trigger a rapid response in IL-1RI$^+$ Th17 cells as well as prolong the antimicrobial response within the slower adaptive immune setting. We note that activation of Th17 cells via the TCR but not IL-1β induces other cytokines such as IL-17A and IL-22, which act to trigger the release of other antimicrobial peptides from bystander cells, perhaps augmenting the effect of IL-26. Nonetheless, we find that IL-1RI$^+$ Th17 cells were the predominant producers of IL-17A in response to anti-CD3/CD28, in agreement with a previous report that also showed and that IL-1β augments cytokine responses from these cells (W. W. Lee et al., 2010).

This work establishes the unusual role of a novel subset of Th17 T cells in contributing to both innate and acquired immunity in the response to microbial infection. Th17 cells have long been linked to host defense against extracellular bacteria, but more recently Th17 cells have been shown to have a role against intracellular bacteria including *M. leprae* (Abdallah, Emam, Attia, Hussein, & Mohamed, 2013; Saini, Ramesh, & Nath, 2013). IL-26 specifically has also been shown to contribute to host defense in leprosy by entering *M. leprae* infected macrophages and reducing of the viability of the intracellular bacteria (Dang et al., 2019). The identification of the factors that regulate the “antimicrobial Th17” compartment and IL-26

provides potential new targets for augmenting the immune response to bacterial infection in humans.

Materials and Methods

Study design

This study was aimed at identifying alternative pathways of IL-26 release from Th17 cells that may contribute to host defense against bacterial infection. Human peripheral blood was obtained from healthy donors at the University of California, Los Angeles (UCLA) and from leprosy donors at the University of Southern California. Researchers were not blinded as to the source of the blood samples. Written informed consent was provided by all donors according to institutional review board protocols of both institutions.

Leprosy PBMC stimulation with *M. leprae* sonicate

Whole blood specimens from leprosy patients were obtained through collaboration with the Hansen's Disease Clinic at Los Angeles County/University of Southern California Medical Center. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood via density centrifugation with Ficoll-Paque solution (GE Healthcare Life Sciences). PBMCs were cultured in RPMI 1640 with 10% fetal calf serum, penicillin (50 U/ml), streptomycin (50 µg/ml), L-glutamine (2mM), and sodium pyruvate (1 mM). $1x10^6$ PBMCs were plated per well in 24 well plates at a density of $1x10^6$ cells/ml and stimulated or not with 10 µg/ml sonicated *M. leprae*. Cell free supernatants were collected after various timepoints for analysis with ELISA kits for IL-26 (Cusabio Biotech), IL-17A, and IFN-γ (DuoSet, R&D Systems)

Stimulation of healthy donor PBMCs with PRR ligands and cytokines

PBMCs were isolated from whole blood of healthy donors who provided written informed consent and were cultured as described above. The cells were stimulated or not with the following PRR ligands (Invivogen): 100 ng/ml Pam3CSK4 (TLR2/1), $1x10^7$ cells/ml heat killed *Listeria monocytogenes* (TLR2), 1 µg/ml high molecular weight poly(I:C) (TLR3), 1 µg/ml low molecular weight poly(I:C) (TLR3), 100 ng/ml lipopolysaccharide (TLR4), 100 ng/ml flagellin (TLR5), 100 ng/ml FSL-1 (TLR2/6), 1 µg/ml imiquimod (TLR7/8), 100 ng/ml ssRNA/LyoVec (TLR8), 1.25 µM dsDNA (TLR9), and 10 ng/ml muramyl dipeptide (NOD2). Cell free supernatants were collected after 16 hours and assayed for cytokine secretion by ELISA. In some experiments, PBMCs were stimulated with a suboptimal dose of 1 ng/ml Pam3CSK4 (TLR2/1L) in the presence or absence of 40 µg/ml of anti-IL-1β blocking antibodies or 40 µg/ml isotype matched control antibody (Invivogen). For cytokine treatment of healthy donor PBMCs, cells were cultured as above with or without 20 ng/ml of recombinant IL-1β, IL-6, IL-23, TGF-β, IL-12, IL-18, IL-4, IL-15, or TNF-α (BioLegend). Cell free supernatants were collected after 48 hours.

Isolation and stimulation of T cell subsets

T cell subsets were isolated from PBMC with the human T cell isolation kit, human $CD4^+$ T cell isolation kit, human naïve $CD4^+$ T cell isolation kit, and human memory $CD4^+$ T cell enrichment kits (EasySep, StemCell Inc.) and cultured as above. The isolated T cell subsets were routinely of ≥ 97% purity. Isolated $CD3^+$ T cells, $CD4^+$ T cells, naïve $CD4^+$ T cells, and memory $CD4^+$ T cells were stimulated or not with IL-1β, IL-6, or IL-23. Cytokine concentrations and stimulation times varied per experiment, as indicated in the figure legends. For TCR stimulation, varying amounts of Immunocult human T cell activator (StemCell Inc.) were added to the cell culture as indicated in the text and figure legends. Cell free supernatants were collected before analysis of

cytokine secretion with ELISA kits for IL-26 (Cusabio), IL-22, IL-17A, IL-17F, and IL-8 (DuoSet, R&D systems).

Sorting memory CD4$^+$ T cell subsets.

Immunomagnetically isolated memory CD4$^+$ T cells were stained with antibodies against APC- or BV421-CCR6 (BioLegend, clone 29-2L17), PE-CD161 (BioLegend, clone HP-3G10), FITC-CXCR3 (BioLegend, clone G025H7), and PECy7-CCR4 (BioLegend, clone L291H4) and sorted using FACS into Th17 cells (CCR6$^+$CD161$^+$CCR4$^+$CXCR3$^-$), Th1 cells (CCR6$^-$CD161$^-$CCR4$^-$ CXCR3$^+$), and Th2 cells (CCR6$^-$CD161$^-$CCR4$^+$CXCR3$^-$). The gating strategy was to first gate on CCR6$^+$CD161$^+$ (for Th17) or CCR6$^-$CD161$^-$ (for Th1 and Th2), and subsequently gate on CCR4$^+$CXCR3$^-$ (for Th17 and Th2) or CCR4$^-$CXCR3$^+$ (for Th1). In some experiments, memory CD4$^+$ T cells were incubated overnight with 1 nM recombinant human IL-2 and stained additionally with antibodies against APC-IL-1RI (R&D Systems, polyclonal goat anti-human) to sort IL-1RI$^+$ Th17 cells (CCR6$^+$CD161$^+$CCR4$^+$CXCR3$^-$IL-1RI$^+$) and IL-1RI$^-$ Th17 cells (CCR6$^+$CD161$^+$CCR4$^+$CXCR3$^-$IL-1RI$^-$).

Stimulation of sorted memory CD4$^+$ T cell subsets.

Th1, Th2, and Th17 cells were plated in 24 well plates at a density of 3 x 10^5 cells/ml in one ml of RPMI 1640 with 10% fetal calf serum, penicillin (50 U/ml), streptomycin (50 µg/ml), L-glutamine (2mM), and sodium pyruvate (1 mM).). Because of the yield from flow sorting, we plated a lower density of the Th subsets as compared to memory CD4+ T cells. The cells were stimulated with 20 ng/ml IL-1β for 48 hours and cell free supernatants were collected for ELISA. IL-1RI$^+$ and IL-1RI$^-$ Th17 cells were sorted and plated in 200 ul at a density 1 x 10^6 cells/ml in round bottom 96 well plates. In this experiment, we recovered the Th17 subpopulations from an eluted whole blood leukoreduction filter (Trima Accel) in order to increase cell yield. The cells

were stimulated or not with 100 ng/ml IL-1β or 5 µl/ml of Immunocult human T cell activator for 9 hours, as cells were also harvested for RNA sequencing. Cell free supernatants were collected for ELISA analysis.

RNA sequencing of IL-1RI$^+$ and IL-1RI$^-$ Th17 cells

IL-1RI$^+$ and IL-1RI$^-$ Th17 cells were stimulated for 9 hours as described above before cell pellets were lysed with RLT buffer and RNA extracted as per Qiagen RNEasy micro kit instructions (Qiagen). Sequencing libraries were prepared by the Technology Center for Genomics & Bioinformatics at UCLA using Illumina TruSeq Stranded Total RNA Sample Prep kit and sequenced by single end sequencing on an Illumina HiSeq3000.

Analysis of RNA-seq data

The reads were mapped with STAR 2.5.3a to the human genome (Hg38). The counts for each gene were obtained by using --quantMode GeneCounts in STAR (Dobin et al., 2013) commands, and the other parameters during alignment were set to default. Differential expression analyses were carried out using DESeq2 (Love, Huber, & Anders, 2014). Normalized counts were obtained using DESeq2 rlog function with default parameters. Principle component analysis and hierarchical clustering were performed on the normalized counts in R. Functional analysis was performed using Ingenuity Pathway Analysis software (Qiagen). Genes that were significantly upregulated by IL-1β vs media in IL-1RI$^+$ Th17 cells with a FC ≥ 1.5 and p-adj ≤ 0.05 were uploaded and core expression analysis was performed. Upstream transcription factor analysis was performed using the Enrichr website (http://amp.pharm.mssm.edu/Enrichr/).

TCR gene analysis

MiXCR software was used for direct extraction of TCR CDR3 sequences from RNA-Seq datasets. Analyses were performed with "-p rna-seq" option, as recommended for analysis of RNA-seq data (Bolotin et al., 2015). Scripts developed in R (R-Core-Team, 2014) were then used to parse the alignment files and perform aggregate counts of the number of reads per TCR gene segment and TCR gene segment combination (Merleev et al., 2018).

NF-kB signaling pathway inhibition

Immunomagnetically isolated memory $CD4^+$ T cells were cultured as described above and stimulated with 20 ng/ml IL-1β or 0.5 µl/ml Immunocult for 48 hours in the presence or absence of 10 µM Bay 11-7082 (Millipore-Sigma). Cell free supernatants were collected and IL-26 concentrations were measured by ELISA.

Antimicrobial assays

Immunomagnetically purified memory $CD4^+$ T cells were cultured in RPMI 1640 with 10% fetal calf serum, L-glutamine (2mM), and sodium pyruvate (1 mM), with or without 20 ng/ml IL-1β for 48 hours and cell free supernatants were collected. *Escherichia coli* was streaked on LB agar and grown at 37°C overnight. A single colony was picked and inoculated into LB broth, which was incubated overnight at 37°C and 250 rpm shaking. Cell number was determined via OD600. $1x10^2$ bacteria were inoculated into 100 ul of T cell supernatant, with or without 20 µg/ml blocking anti-IL-26 antibodies or isotype control antibodies (R&D systems), or into unconditioned media containing either 20 ng/ml recombinant IL-1β protein, a combination of 50 u/ml penicillin and 50 µg/ml streptomycin, or nothing and incubated overnight at 37°C and 250 rpm shaking. Increasing dilutions of the cultures were plated on LB agar plates and after growth overnight at 37°C viability was assessed by counting colony forming units.

Statistical analysis

Statistical analyses were calculated using GraphPad Prism version 8.0, and p-values ≤ 0.05 were assigned as significant. Each figure legend contains the specific statistical test performed, which were chosen based on the number of comparison groups and normality of the data. For comparisons involving two groups, Paired Student's t-test or one-way ANOVA with Sidak's post-test was performed. For more than two comparison groups, one-way ANOVA with Tukey's post-test, two-way ANOVA with Tukey's post-test, or Friedman's test with Dunn's post-test were performed. Data are represented in figures as mean ± SEM.

Data and materials availability

The RNA-seq data has been uploaded to the Gene Expression Omnibus under accession number GSE127457.

Acknowledgments

We would like to thank R. Teles and D. Elashof for assistance with statistical analysis and helpful discussion; A. Legaspi and A. Choi for tissue culture assistance; A. Garcia, M. Zhou, and I. Williams of the UCLA Jonsson Comprehensive Cancer Center and Center for AIDS Research Flow Cytometry Core Facility for assistance with flow cytometry and cell sorting; and D. Anisman-Posner and A. Bollinger at the UCLA Center for Aids Research Virology Core for access to eluted leukoreduction filters.

Figures

Figure 1

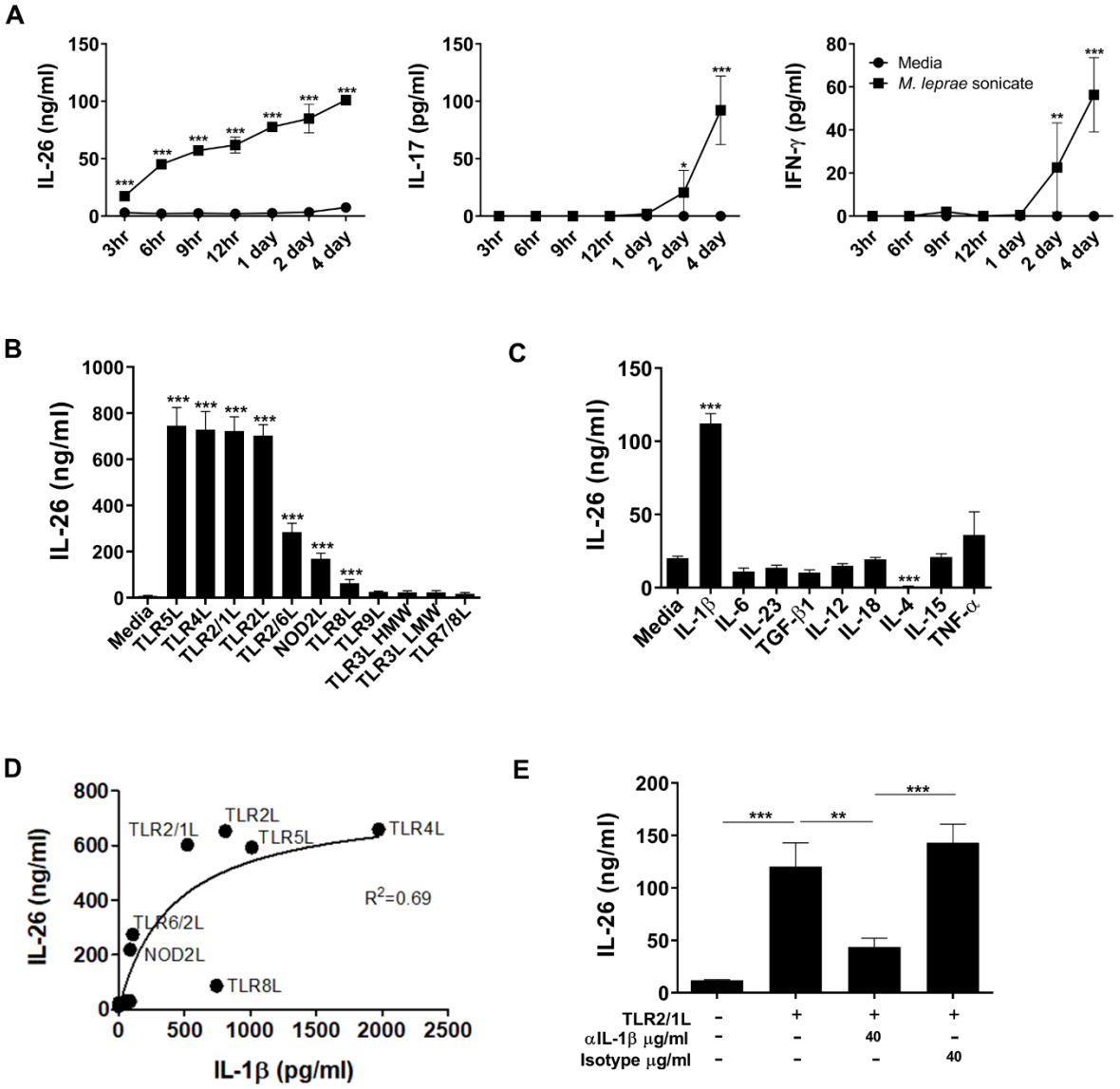

Figure 1. PBMCs rapidly secrete IL-26 in response to innate ligands in an IL-1β dependent manner. (**A**) PBMCs derived from tuberculoid leprosy patients were incubated or not with 10 μg/ml *M. leprae* sonicate for the indicated amount of time before cell free supernatants were collected and cytokine concentrations measured by ELISA, n = 3. (**B**) PBMCs were treated with the indicated PRR ligands for 16 hours before IL-26 concentration in cell free supernatants was measured by ELISA, n = 6. (**C**) PBMCs were treated with 20 ng/ml of

the indicated cytokine for 48 hours before IL-26 concentration was measured in cell free supernatants by ELISA, n = 3. (**D**) The average IL-1β vs IL-26 concentration in supernatants of PRR ligand stimulated PBMCs was plotted with GraphPad Prism 8.0 software and a sigmoidal dose-response curve was fitted to the data, $y= (761.8*x^{1.002})/(416.6+x^{1.002})$, $R^2 = 0.69$. (**E**) PBMCs were stimulated with 1 ng/ml of the TLR2/1 agonist Pam3CSK4 overnight in the presence or absence of blocking anti-IL-1β antibody or isotype matched control antibody, n = 4. (**A-C, E)** Data are represented as mean ± SEM, statistics calculated using two-way ANOVA with Sidak's post-test (A) or one-way ANOVA with Tukey's post-test (B, C, E). **p ≤ 0.05. **p ≤ 0.01. *** p ≤ 0.001*

Figure 2

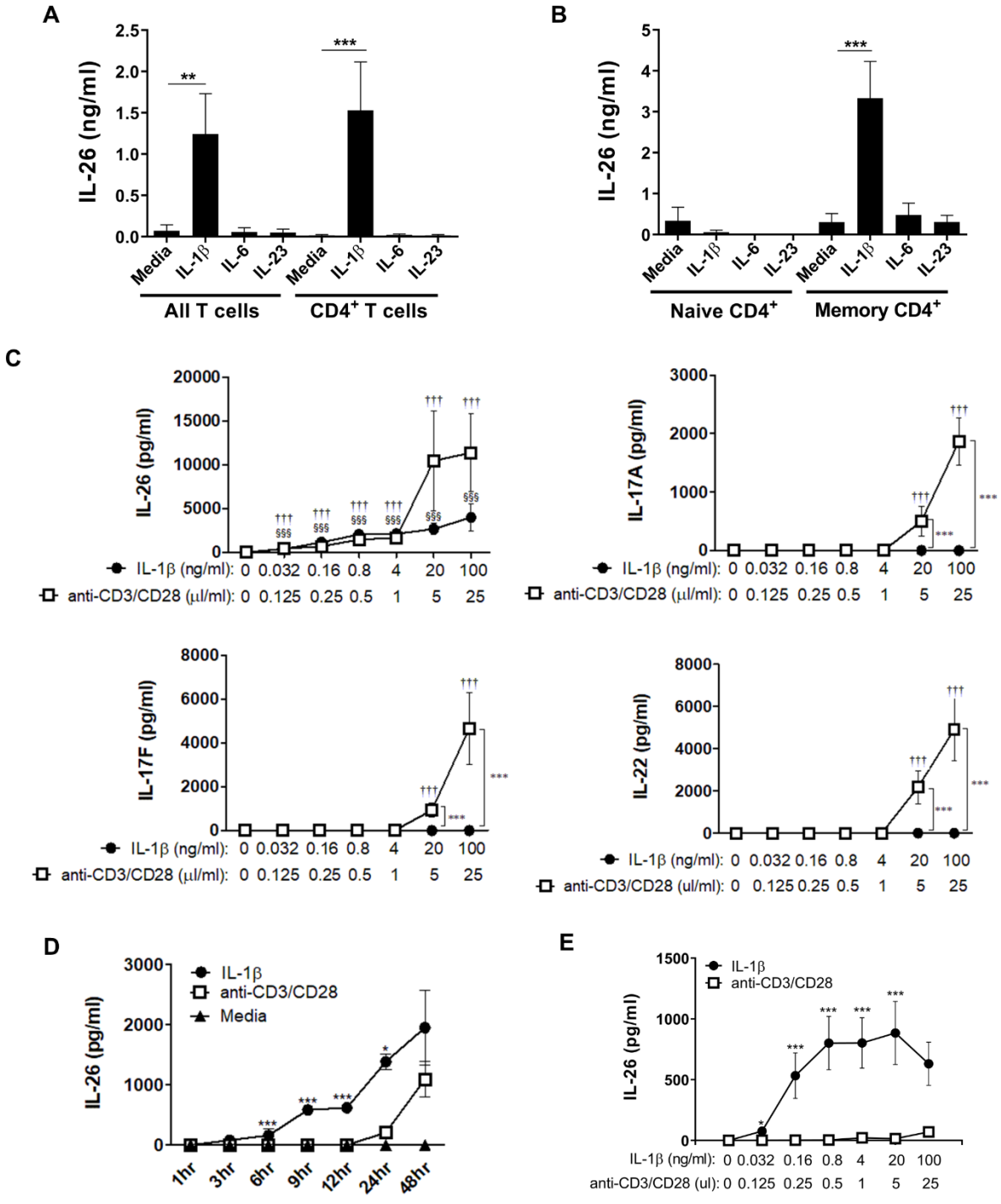

Figure 2. IL-1β induces rapid IL-26 secretion from memory CD4⁺ T cells. (A-B)

Immunomagnetically selected T cell populations were incubated with 20 ng/ml of the indicated

cytokine for 48 hours before cell free supernatants were analyzed by ELISA, n = 8 (A) n = 3 (B). (**C**) Memory $CD4^+$ T cells were incubated with the indicated amounts of IL-1β or anti-CD3/CD28 tetrameric antibody complexes for 48 hours before cell free supernatants were analyzed for IL-26, IL-17A, IL-17F, and IL-22 concentrations by ELISA, n = 4. Data are represented as mean ± SEM, statistics calculated using one-way ANOVA with Tukey's post-test. Levels of significance for three comparisons are indicated as follows: untreated cells to IL-1β treated cells, §; Untreated cells to anti-CD3/CD28 treated cells, †; IL-1β treated cells to anti-CD3/CD28 treated cells, *. (**D**) Memory $CD4^+$ T cells were incubated or not with 20 ng/ml IL-1β or 0.5 µl/ml anti-CD3/CD28 tetrameric antibody complexes and cell free supernatants were analyzed for IL-26 secretion at the indicated time points, n = 3. (**E**) Memory $CD4^+$ T cells were stimulated with the indicated amount of IL-1β or anti-CD3/CD28 for 4 hours before IL-26 concentrations in cell free supernatants were analyzed by ELISA, n = 3. (**A-E**) Data are represented as mean ± SEM, statistics calculated using one-way ANOVA with Tukey's post-test (A, B, C, E) or two-way ANOVA with Tukey's post-test (D). Asterisks denote significance between IL-1β and anti-CD3/CD28 treatment (C, D, E). *$*p \leq 0.05$. **$p \leq 0.01$. *** $p \leq 0.001$*

Figure 3

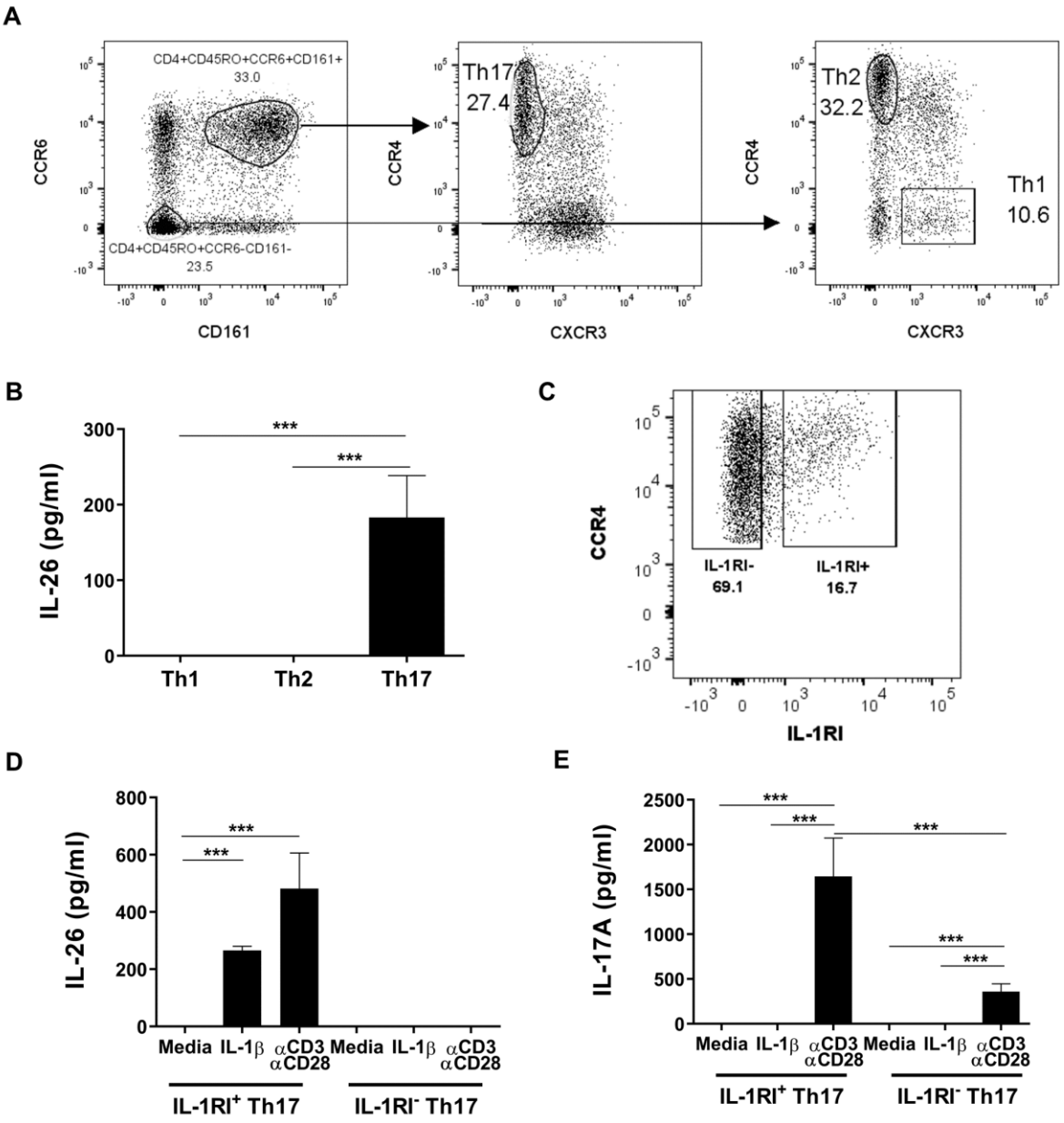

Figure 3. IL-1RI^{+} Th17 cells secrete IL-26 in response to IL-1β. (**A**) Sorting scheme used to isolate helper T cell subsets, a representative donor is shown, n = 3. (**B**) Isolated Th1, Th2, and Th17 cells were stimulated with IL-1β for 48 hours and cell free supernatants were analyzed for IL-26 secretion by ELISA, n = 3. (**C**) A representative sorting scheme for isolating IL-1RI^{+} and

IL-1RI$^-$ Th17 cells, n= 3. (**D, E**) IL-1RI$^+$ and IL-1RI$^-$ Th17 cells were stimulated with 100 ng/ml IL-1β or 5 µl/ml anti-CD3/CD28 for 9 hours before IL-26 (D) and IL-17A (E) was measured in cell free supernatants by ELISA, n = 3. (**B,D**) Data are represented as mean ± SEM, statistics calculated using one-way ANOVA with Tukey's post-test (B, D, E). ***p ≤ 0.01. **** p ≤ 0.001*

Figure 4

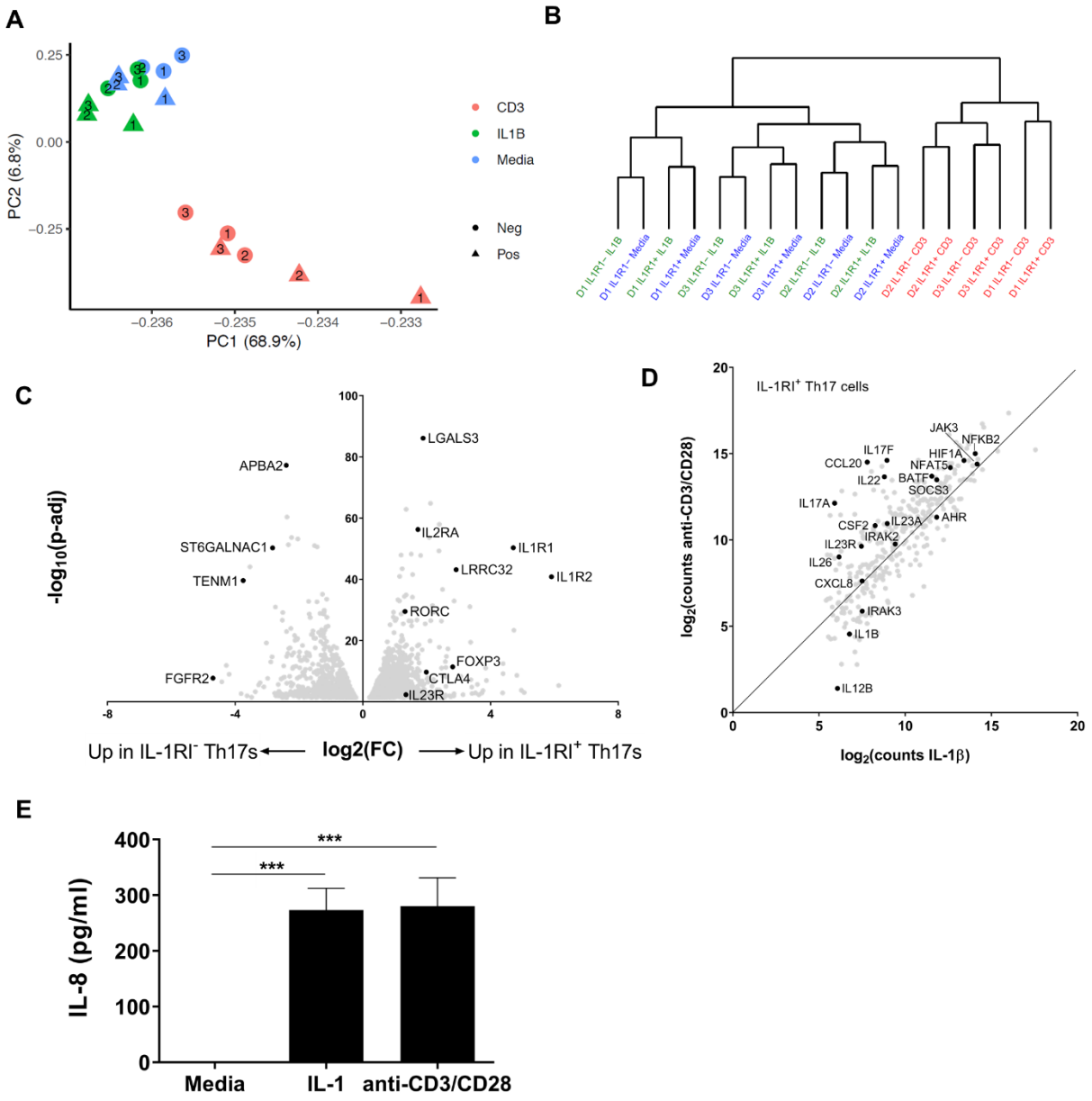

Figure 4. IL-1β upregulates Th17 gene expression program in IL-1RI$^+$ Th17 cells. (**A**) Unsupervised principal component analysis of IL-1RI$^+$ and IL-1RI$^-$ Th17 cells from 3 donors treated with IL-1β, anti-CD3/CD28, or media as a control. Numbers within symbols indicate the donor. (**B**) Hierarchical clustering of the samples. (**C)** Differential gene expression in untreated IL-1RI$^+$ Th17 cells vs. IL-1RI$^-$ Th17 cells is represented in a volcano plot and some of the most

differentially expressed genes are named and highlighted with black dots. (**D**) Genes with a (FC) ≥ 1.5 and p-adj ≤0.05 after treatment of IL-1RI$^+$ Th17 cells with IL-1β vs media are plotted based on their normalized counts after IL-1β or anti-CD3/CD28 treatment. The line y = x was plotted on the graph to show genes with greater relative counts in each condition. Genes in the IPA "Th17 cell activation" canonical pathway and *CXCL8* are highlighted with black dots. (**E)** Memory CD4$^+$ T cells were treated with 100 ng/ml IL-1β and 5 µl/ml anti-CD3/CD28 for 48 hours before supernatants were collected and IL-8 protein concentrations were measured by ELISA, n = 4. Data are represented as mean ± SEM, statistics calculated using one-way ANOVA with Tukey's post-test. *** $p \leq 0.001$.

Figure 5

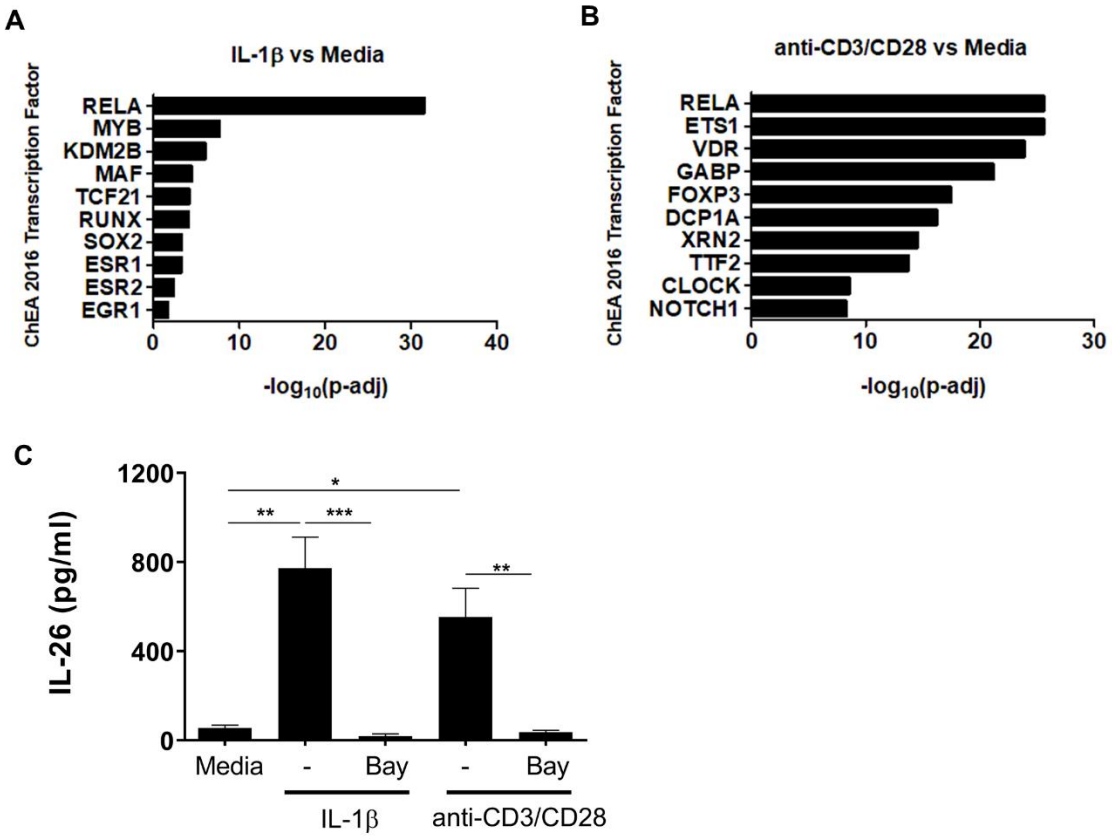

Figure 5. Upstream analysis of RNA-seq dataset reveals IL-26 dependence upon NF-κB. (**A, B**) Genes with a $\log_2$(FC)≥ 0.58 and p-adj ≤0.05 after treatment of IL-1RI$^+$ Th17 cells with IL-1β (A) or anti-CD3/CD28 (B) were analyzed for enrichment of upstream transcription factors using Enrichr and plotted with their -$\log_{10}$(p-adj). **C.** Memory CD4$^+$ T cells were stimulated with either 20 ng/ml IL-1β or 5 µl/ml anti-CD3/CD28 for 24 hours, with or without 1 hour pretreatment with 10µM Bay 11-7082, an NF-κB activation inhibitor. IL-26 secretion into cell supernatants was measured by ELISA, n = 3. Data are represented as mean ± SEM, statistics calculated using one-way ANOVA with Tukey's post-test. **p ≤ 0.05, *** p ≤ 0.001*.

Figure 6

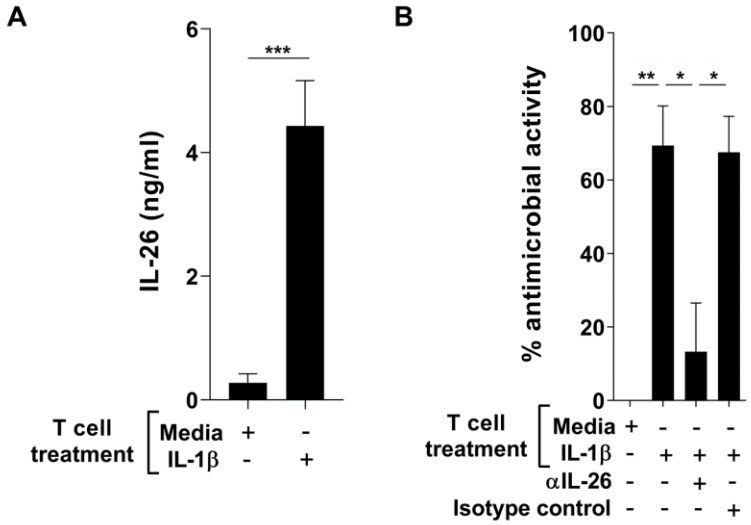

Figure 6. IL-1β stimulated T cell supernatants are antimicrobial. (**A**) IL-26 was measured by ELISA in cell free supernatants collected from memory $CD4^+$ T cells that were stimulated or not with 20 ng/ml IL-1β for 48 hours. **B.** *E. coli* was inoculated into cell culture supernatants from 48 hour IL-1β or media control stimulated T cells, some pre-incubated with 20 µg/ml anti-IL-26 blocking antibody or isotype matched control antibody, and then incubated overnight at 37°C with shaking at 250 rpm. Percent antimicrobial activity was determined by CFU assay of a dilution series plated on LB agar plates, n = 3. **(A, B)** Data are represented as mean ± SEM, statistics calculated using paired Student's t-test (A) or one-way ANOVA with Tukey's post-test (B). **p ≤ 0.05. **p ≤ 0.01.*

Tables

Table I

Gene	DESeq2 FC IL-1β vs media	p-adj IL-1β vs media	DESeq2 FC IL-anti-CD3 CD28 vs IL-1β	p-adj anti-CD3/CD28 vs. IL-1β
AHR	1.6	1.2×10^{-9}	0.8	9.8×10^{-3}
BATF	2.1	4.6×10^{-32}	4.7	4.4×10^{-29}
CCL20	1.6	6.6×10^{-3}	95.8	4.5×10^{-44}
CSF2	25.1	8.4×10^{-5}	12.6	2.5×10^{-4}
HIF1A	1.7	2.8×10^{-15}	2.4	1.3×10^{-17}
IL1B	70.3	3.1×10^{-13}	0.3	1.1×10^{-4}
IL12B	145.6	1.6×10^{-7}	0.01	9.5×10^{-3}
IL17A	5.3	3.2×10^{-4}	105.5	4.2×10^{-13}
IL17F	27.2	3.5×10^{-52}	49.2	2.7×10^{-190}
IL22	21.3	6.8×10^{-50}	25.9	2.5×10^{-7}
IL23A	1.6	1.0×10^{-4}	4.2	1.4×10^{-37}
IL23R	1.8	4.4×10^{-3}	4.8	9.7×10^{-34}
IL26	2.4	1.4×10^{-2}	7.3	2.5×10^{-4}
IRAK2	3.5	1.1×10^{-21}	1.3	2.0×10^{-2}
IRAK3	3.4	3.5×10^{-9}	0.3	1.8×10^{-6}
JAK3	1.8	4.9×10^{-5}	1.2	1.8×10^{-1}
NFAT5	1.5	1.4×10^{-7}	3.1	1.2×10^{-23}
NFKB2	4.5	2.5×10^{-133}	2.0	1.7×10^{-17}
SOCS3	2.3	1.3×10^{-2}	3.6	7.2×10^{-16}

Table I. "Th17 cell activation" pathway genes significantly upregulated by IL-1β treatment of IL-1RI^{+} Th17 cells. The IPA canonical pathway "Th17 cell activation" was the most significantly enriched canonical pathway within our dataset ($p = 2.51 \times 10^{-17}$). This pathway contained 90 genes, 19 of which, or 21%, were contained in our dataset and are displayed in the table.

Supplementary Material:

Figure S1

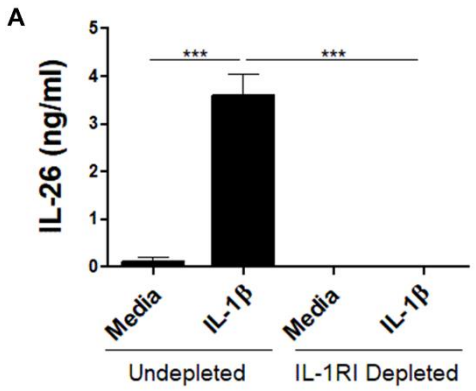

Figure S1. IL-1RI$^+$ cells are necessary for IL-1β induced IL-26 secretion from memory CD4$^+$ T cells. (**A**) Immunomagnetically selected memory CD4$^+$ T cells were stimulated with 20 ng/ml IL-1β for 48hrs before or after depletion of IL-1RI$^+$ cells by FACS, n = 4. Data are represented as mean ± SEM, statistics calculated using one-way ANOVA with Tukey's post-test. **** $p \leq 0.001$.*

Figure S2

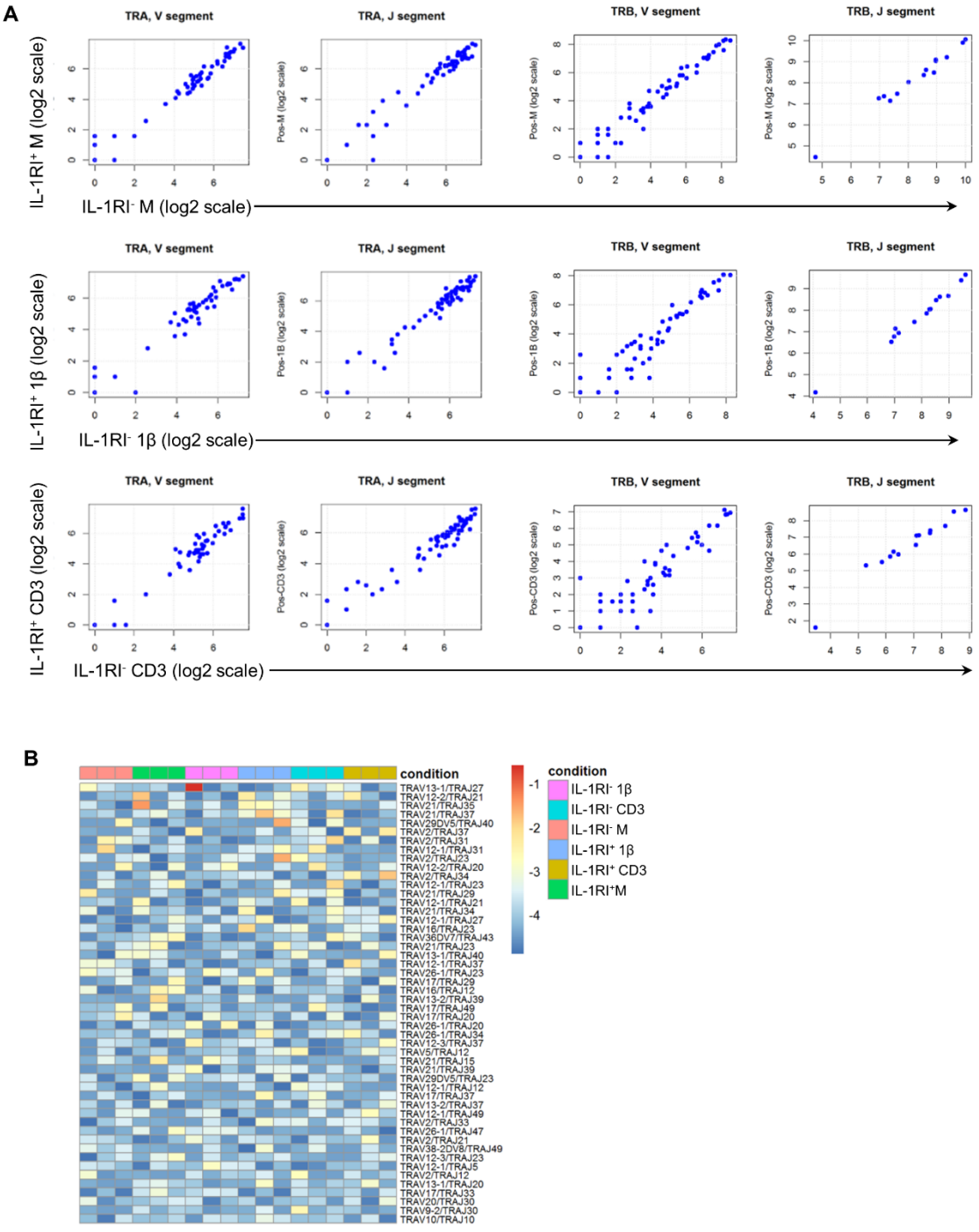

Figure S2. Th17 cells have diverse T cell receptors. (**A**) Relative TCR gene segment abundance for TRA and TRB chains. Each dot represents a specific V or J segment. The coordinate of each dot is the ratio of average number of TCR reads for the specific TCR

segment to average number of TCR V or J segment reads from the same group. (**B**) V/J gene segment recombination Heatmap. Abundance of the 50 most common TRA V/J gene segment combinations are displayed as a heatmap. Each square is colorized based on the ratio of reads number with specific V/J recombination to all reads with any V/J combination. The ratios are log transformed.

Figure S3

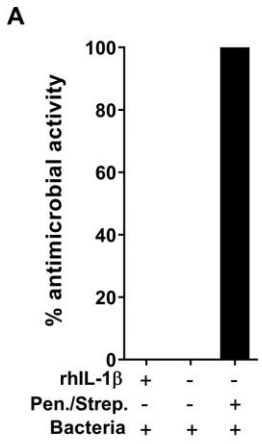

Figure S2. Controls for IL-1β stimulated antimicrobial activity mediated by IL-26. (**A**) **.** *E. coli* was inoculated into unconditioned media containing either 20 ng/ml recombinant IL-1β protein (rIL-1β), a combination of 50 u/ml penicillin and 50 µg/ml streptomycin (Pen./Strep.), or nothing (Bacteria) and incubated overnight at 37°C with shaking at 250 rpm. Percent antimicrobial activity was determined by CFU assay of a dilution series plated on LB agar plates, n = 3. Data are represented as mean ± SEM, statistics calculated using Friedman's test with Dunn's post-test.

References

Abdallah, M., Emam, H., Attia, E., Hussein, J., & Mohamed, N. (2013). Estimation of serum level of interleukin-17 and interleukin-4 in leprosy, towards more understanding of leprosy immunopathogenesis. *Indian Journal of Dermatology, Venereology and Leprology*, *79*(6), 772–776. https://doi.org/10.4103/0378-6323.120723

Acosta-Rodriguez, E. V, Napolitani, G., Lanzavecchia, A., & Sallusto, F. (2007). Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. *Nature Immunology*, *8*(9), 942–949. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17676045

Annunziato, F., Cosmi, L., Santarlasci, V., Maggi, L., Liotta, F., Mazzinghi, B., … Romagnani, S. (2007). Phenotypic and functional features of human Th17 cells. *Journal of Experimental Medicine*, *204*(8), 1849–1861. https://doi.org/10.1084/jem.20070663

Arlehamn, C. L., Seumois, G., Gerasimova, A., Huang, C., Fu, Z., Yue, X., … Peters, B. (2014). Transcriptional profile of tuberculosis antigen-specific T cells reveals novel multifunctional features. *Journal of Immunology*, *193*(6), 2931–2940. https://doi.org/10.4049/jimmunol.1401151

Becattini, S., Latorre, D., Mele, F., Foglierini, M., De Gregorio, C., Cassotta, A., … Sallusto, F. (2015). T cell immunity. Functional heterogeneity of human memory CD4(+) T cell clones primed by pathogens or vaccines. *Science*, *347*(6220), 400–406. https://doi.org/10.1126/science.1260668

Bjorstad, A., Fu, H., Karlsson, A., Dahlgren, C., & Bylund, J. (2005). Interleukin-8-derived peptide has antibacterial activity. *Antimicrobial Agents and Chemotherapy*, *49*(9), 3889–3895. https://doi.org/10.1128/aac.49.9.3889-3895.2005

Bolotin, D. A., Poslavsky, S., Mitrophanov, I., Shugay, M., Mamedov, I. Z., Putintseva, E. V, & Chudakov, D. M. (2015). MiXCR: software for comprehensive adaptive immunity profiling. *Nat Methods*, *12*(5), 380–381. https://doi.org/10.1038/nmeth.3364

Bonecchi, R., Bianchi, G., Bordignon, P. P., D'Ambrosio, D., Lang, R., Borsatti, A., … Sinigaglia, F. (1998). Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *Journal of Experimental Medicine*, *187*, 129–134.

Che, K. F., Tengvall, S., Levanen, B., Silverpil, E., Smith, M. E., Awad, M., … Linden, A. (2014). Interleukin-26 in antibacterial host defense of human lungs. Effects on neutrophil mobilization. *American Journal of Respiratory and Critical Care Medicine*, *190*(9), 1022–1031. https://doi.org/10.1164/rccm.201404-0689OC

Cho, J. S., Pietras, E. M., Garcia, N. C., Ramos, R. I., Farzam, D. M., Monroe, H. R., … Miller, L. S. (2010). IL-17 is essential for host defense against cutaneous Staphylococcus aureus infection in mice. *Journal of Clinical Investigation*, *120*(5), 1762–1773. https://doi.org/10.1172/jci40891

Collins, P. L., Henderson, M. A., & Aune, T. M. (2012). Lineage-specific adjacent IFNG and IL26 genes share a common distal enhancer element. *Genes and Immunity*, *13*(6), 481–488. https://doi.org/10.1038/gene.2012.22

Corvaisier, M., Delneste, Y., Jeanvoine, H., Preisser, L., Blanchard, S., Garo, E., … Jeannin, P. (2012). IL-26 is overexpressed in rheumatoid arthritis and induces proinflammatory cytokine production and Th17 cell generation. *PLoS Biology*, *10*(9), e1001395. https://doi.org/10.1371/journal.pbio.1001395

Cua, D. J., & Tato, C. M. (2010). Innate IL-17-producing cells: the sentinels of the immune system. *Nature Reviews: Immunology*, *10*(7), 479–489. https://doi.org/10.1038/nri2800

Dang, A. T., Teles, R. M. B., Weiss, D. I., Parvatiyar, K., Sarno, E. N., Ochoa, M. T., … Modlin, R. L. (2019). IL-26 contributes to host defense against intracellular bacteria. *Journal of Clinical Investigation*, *130*. https://doi.org/10.1172/JCI99550

De Grove, K. C., Provoost, S., Verhamme, F. M., Bracke, K. R., Joos, G. F., Maes, T., & Brusselle, G. G. (2016). Characterization and Quantification of Innate Lymphoid Cell Subsets in Human Lung. *PloS One*, *11*(1), e0145961. https://doi.org/10.1371/journal.pone.0145961

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., … Gingeras, T. R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*, *29*(1), 15–21. https://doi.org/10.1093/bioinformatics/bts635

Donnelly, R. P., Sheikh, F., Dickensheets, H., Savan, R., Young, H. A., & Walter, M. R. (2010). Interleukin-26: an IL-10-related cytokine produced by Th17 cells. *Cytokine and Growth Factor Reviews*, *21*(5), 393–401. https://doi.org/10.1016/j.cytogfr.2010.09.001

Happel, K. I., Dubin, P. J., Zheng, M., Ghilardi, N., Lockhart, C., Quinton, L. J., … Kolls, J. K. (2005). Divergent roles of IL-23 and IL-12 in host defense against Klebsiella pneumoniae. *Journal of Experimental Medicine*, *202*(6), 761–769. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16157683

Hasegawa, E., Sonoda, K. H., Shichita, T., Morita, R., Sekiya, T., Kimura, A., … Yoshimura, A. (2013). IL-23-independent induction of IL-17 from gammadeltaT cells and innate lymphoid cells promotes experimental intraocular neovascularization. *Journal of Immunology*, *190*(4), 1778–1787. https://doi.org/10.4049/jimmunol.1202495

Hor, S., Pirzer, H., Dumoutier, L., Bauer, F., Wittmann, S., Sticht, H., … Fickenscher, H. (2004). The T-cell lymphokine interleukin-26 targets epithelial cells through the interleukin-20 receptor 1 and interleukin-10 receptor 2 chains. *Journal of Biological Chemistry*, *279*(32), 33343–33351. https://doi.org/10.1074/jbc.M405000200

Hori, S., Nomura, T., & Sakaguchi, S. (2003). Control of regulatory T cell development by the transcription factor Foxp3. *Science*, *299*(5609), 1057–1061. https://doi.org/10.1126/science.1079490

Hsieh, C.-S., Macatonia, S. E., Tripp, C. S., Wolf, S. F., O'Garra, A., & Murphy, K. M. (1993). Development of Th1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science*, *260*, 547–549.

Janeway Jr., C. A., & Medzhitov, R. (2002). Innate immune recognition. *Annual Review of Immunology*, *20*, 197–216. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11861602

Jones, C. E., & Chan, K. (2002). Interleukin-17 stimulates the expression of interleukin-8, growth-related oncogene-alpha, and granulocyte-colony-stimulating factor by human airway epithelial cells. *American Journal of Respiratory Cell and Molecular Biology*, *26*(6), 748–753.

Kawaguchi, M., Kokubu, F., Kuga, H., Matsukura, S., Hoshino, H., Ieki, K., … Huang, S. K. (2001). Modulation of bronchial epithelial cells by IL-17. *Journal of Allergy and Clinical Immunology*, *108*(5), 804–809. https://doi.org/10.1067/mai.2001.119027

Kawasaki, T., & Kawai, T. (2014). Toll-like receptor signaling pathways. *Frontiers in Immunology*, *5*, 461. https://doi.org/10.3389/fimmu.2014.00461

Kuleshov, M. V, Jones, M. R., Rouillard, A. D., Fernandez, N. F., Duan, Q., Wang, Z., … Ma'ayan, A. (2016). Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Research*, *44*(W1), W90-7. https://doi.org/10.1093/nar/gkw377

Laan, M., Cui, Z. H., Hoshino, H., Lotvall, J., Sjostrand, M., Gruenert, D. C., … Linden, A. (1999). Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. *Journal of Immunology*, *162*(4), 2347–2352. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9973514

Lee, J., Rhee, M. H., Kim, E., & Cho, J. Y. (2012). BAY 11-7082 is a broad-spectrum inhibitor with anti-inflammatory activity against multiple targets. *Mediators of Inflammation*, *2012*, 416036. https://doi.org/10.1155/2012/416036

Lee, W. W., Kang, S. W., Choi, J., Lee, S. H., Shah, K., Eynon, E. E., … Kang, I. (2010).

Regulating human Th17 cells via differential expression of IL-1 receptor. *Blood*, *115*(3), 530–540. https://doi.org/10.1182/blood-2009-08-236521
Linsley, P. S., Greene, J. L., Tan, P., Bradshaw, J., Ledbetter, J. A., Anasetti, C., & Damle, N. K. (1992). Coexpression and functional cooperation of CTLA-4 and CD28 on activated T lymphocytes. *Journal of Experimental Medicine*, *176*(6), 1595–1604.
Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 550. https://doi.org/10.1186/s13059-014-0550-8
Maggi, L., Santarlasci, V., Capone, M., Peired, A., Frosali, F., Crome, S. Q., … Annunziato, F. (2010). CD161 is a marker of all human IL-17-producing T-cell subsets and is induced by RORC. *European Journal of Immunology*, *40*(8), 2174–2181. https://doi.org/10.1002/eji.200940257
Manel, N., Unutmaz, D., & Littman, D. R. (2008). The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor RORgammat. *Nature Immunology*, *9*(6), 641–649. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18454151
Manetti, R., Parronchi, P., Giudizi, M. G., Piccinni, M.-P., Maggi, E., Trinchieri, G., & Romagnani, S. (1993). Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *Journal of Experimental Medicine*, *177*, 1199–1204.
Meller, S., Di Domizio, J., Voo, K. S., Friedrich, H. C., Chamilos, G., Ganguly, D., … Gilliet, M. (2015). T(H)17 cells promote microbial killing and innate immune sensing of DNA via interleukin 26. *Nature Immunology*, *16*(9), 970–979. https://doi.org/10.1038/ni.3211
Mercer, F., Kozhaya, L., & Unutmaz, D. (2010). Expression and function of TNF and IL-1 receptors on human regulatory T cells. *PloS One*, *5*(1), e8639. https://doi.org/10.1371/journal.pone.0008639
Merleev, A. A., Marusina, A. I., Ma, C., Elder, J. T., Tsoi, L. C., Raychaudhuri, S. P., … Maverakis, E. (2018). Meta-analysis of RNA sequencing datasets reveals an association between TRAJ23, psoriasis, and IL-17A. *JCI Insight*, *3*(13). https://doi.org/10.1172/jci.insight.120682
Milner, J. D., Brenchley, J. M., Laurence, A., Freeman, A. F., Hill, B. J., Elias, K. M., … Douek, D. C. (2008). Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. *Nature*, *452*(7188), 773–776. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18337720
Modlin, R. L., Melanconkaplan, J., Young, S. M. M., Pirmez, C., Kino, H., Convit, J., … Bloom, B. R. (1988). LEARNING FROM LESIONS - PATTERNS OF TISSUE INFLAMMATION IN LEPROSY. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(4), 1213–1217. https://doi.org/10.1073/pnas.85.4.1213
Nakatsuji, T., Liu, Y. T., Huang, C. P., Zouboulis, C. C., Gallo, R. L., & Huang, C. M. (2008). Vaccination targeting a surface sialidase of P. acnes: implication for new treatment of acne vulgaris. *PloS One*, *3*(2), e1551. https://doi.org/10.1371/journal.pone.0001551
Pierce, J. W., Schoenleber, R., Jesmok, G., Best, J., Moore, S. A., Collins, T., & Gerritsen, M. E. (1997). Novel inhibitors of cytokine-induced IkappaBalpha phosphorylation and endothelial cell adhesion molecule expression show anti-inflammatory effects in vivo. *Journal of Biological Chemistry*, *272*(34), 21096–21103.
Poli, C., Augusto, J. F., Dauve, J., Adam, C., Preisser, L., Larochette, V., … Jeannin, P. (2017). IL-26 Confers Proinflammatory Properties to Extracellular DNA. *Journal of Immunology*, *198*(9), 3650–3661. https://doi.org/10.4049/jimmunol.1600594
R-Core-Team. (2014). R: A language and environment for statistical computing. .
Raffin, C., Raimbaud, I., Valmori, D., & Ayyoub, M. (2011). Ex vivo IL-1 receptor type I

expression in human CD4+ T cells identifies an early intermediate in the differentiation of Th17 from FOXP3+ naive regulatory T cells. *Journal of Immunology*, *187*(10), 5196–5202. https://doi.org/10.4049/jimmunol.1101742

Ramesh, R., Kozhaya, L., McKevitt, K., Djuretic, I. M., Carlson, T. J., Quintero, M. A., … Sundrud, M. S. (2014). Pro-inflammatory human Th17 cells selectively express P-glycoprotein and are refractory to glucocorticoids. *Journal of Experimental Medicine*, *211*(1), 89–104. https://doi.org/10.1084/jem.20130301

Saini, C., Ramesh, V., & Nath, I. (2013). CD4+ Th17 cells discriminate clinical types and constitute a third subset of non Th1, Non Th2 T cells in human leprosy. *PLoS Neglected Tropical Diseases*, *7*(7), e2338. https://doi.org/10.1371/journal.pntd.0002338

Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., & Toda, M. (1995). Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *Journal of Immunology*, *155*(3), 1151–1164. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7636184

Seder, R. A., & Paul, W. E. (1994). Acquisition of lymphokine-producing phenotype by CD4+ T cells. *Annual Review of Immunology*, *12*, 635–673.

Sha, Y., & Markovic-Plese, S. (2011). A role of IL-1R1 signaling in the differentiation of Th17 cells and the development of autoimmune diseases. *Self Nonself*, *2*(1), 35–42. https://doi.org/10.4161/self.2.1.15639

Sheikh, F., Baurin, V. V, Lewis-Antes, A., Shah, N. K., Smirnov, S. V, Anantha, S., … Kotenko, S. V. (2004). Cutting edge: IL-26 signals through a novel receptor complex composed of IL-20 receptor 1 and IL-10 receptor 2. *Journal of Immunology*, *172*(4), 2006–2010. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14764663

Spits, H., Artis, D., Colonna, M., Diefenbach, A., Di Santo, J. P., Eberl, G., … Vivier, E. (2013). Innate lymphoid cells--a proposal for uniform nomenclature. *Nature Reviews: Immunology*, *13*(2), 145–149. https://doi.org/10.1038/nri3365

Stenger, S., Hanson, D. A., Teitelbaum, R., Dewan, P., Niazi, K. R., Froelich, C. J., … Modlin, R. L. (1998). An antimicrobial activity of cytolytic T cells mediated by granulysin. *Science*, *282*(5386), 121–125. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9756476

Sutton, C. E., Lalor, S. J., Sweeney, C. M., Brereton, C. F., Lavelle, E. C., & Mills, K. H. (2009). Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity*, *31*(2), 331–341. https://doi.org/10.1016/j.immuni.2009.08.001

Tran, D. Q., Andersson, J., Wang, R., Ramsey, H., Unutmaz, D., & Shevach, E. M. (2009). GARP (LRRC32) is essential for the surface expression of latent TGF-beta on platelets and activated FOXP3+ regulatory T cells. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(32), 13445–13450. https://doi.org/10.1073/pnas.0901944106

Valmori, D., Raffin, C., Raimbaud, I., & Ayyoub, M. (2010). Human RORgammat+ TH17 cells preferentially differentiate from naive FOXP3+Treg in the presence of lineage-specific polarizing factors. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(45), 19402–19407. https://doi.org/10.1073/pnas.1008247107

Waldmann, T. A. (1989). The multi-subunit interleukin-2 receptor. *Annual Review of Biochemistry*, *58*, 875–911. https://doi.org/10.1146/annurev.bi.58.070189.004303

Weber, A., Wasiliew, P., & Kracht, M. (2010). Interleukin-1 (IL-1) pathway. *Sci Signal*, *3*(105), cm1. https://doi.org/10.1126/scisignal.3105cm1

Wilson, N. J., Boniface, K., Chan, J. R., McKenzie, B. S., Blumenschein, W. M., Mattson, J. D., … de Waal, M. R. (2007). Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nature Immunology*, *8*(9), 950–957. Retrieved from

http://www.ncbi.nlm.nih.gov/pubmed/17676044
Woetmann, A., Alhede, M., Dabelsteen, S., Bjarnsholt, T., Rybtke, M., Nastasi, C., … Odum, N. (2018). Interleukin-26 (IL-26) is a novel anti-microbial peptide produced by T cells in response to staphylococcal enterotoxin. *Oncotarget*, *9*(28), 19481–19489. https://doi.org/10.18632/oncotarget.24603
Wolk, K., Kunz, S., Asadullah, K., & Sabat, R. (2002). Cutting edge: immune cells as sources and targets of the IL-10 family members? *Journal of Immunology*, *168*(11), 5397–5402.
Ye, P., Rodriguez, F. H., Kanaly, S., Stocking, K. L., Schurr, J., Schwarzenberger, P., … Kolls, J. K. (2001). Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. *Journal of Experimental Medicine*, *194*(4), 519–527. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11514607
Yoshimoto, T., Takeda, K., Tanaka, T., Ohkusu, K., Kashiwamura, S., Okamura, H., … Nakanishi, K. (1998). IL-12 up-regulates IL-18 receptor expression on T cells, Th1 cells, and B cells: synergism with IL-18 for IFN-gamma production. *Journal of Immunology*, *161*(7), 3400–3407.
Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. *Nature*, *415*(6870), 389–395. https://doi.org/10.1038/415389a
Zielinski, C. E., Mele, F., Aschenbrenner, D., Jarrossay, D., Ronchi, F., Gattorno, M., … Sallusto, F. (2012). Pathogen-induced human TH17 cells produce IFN-gamma or IL-10 and are regulated by IL-1beta. *Nature*, *484*(7395), 514–518. https://doi.org/10.1038/nature10957

CHAPTER 3

IL-26 contributes to host defense against intracellular bacteria

Abstract

Interleukin-26 (IL-26) is an antimicrobial protein that is secreted by Th17 cells with the ability to directly kill extracellular bacteria. To ascertain whether IL-26 contributes to host defense against intracellular bacteria, we studied leprosy, caused by the obligate intracellular pathogen *Mycobacterium leprae*, as a model. Analysis of leprosy skin lesions by gene expression profiling and immunohistology revealed that IL-26 was more strongly expressed in lesions from the self-limited tuberculoid vs. progressive lepromatous patients. IL-26 directly bound to *M. leprae* in axenic culture and reduced bacteria viability. Furthermore, IL-26, when added to human monocyte derived macrophages (MDM) infected with *M. leprae*, entered the infected cell, colocalized with the bacterium, and reduced bacterial viability. In addition, IL-26 induced autophagy via the cytoplasmic DNA receptor STING, as well as fusion of phagosomes containing bacilli with lysosomal compartments. Altogether, our data suggest that the Th17 cytokine IL-26 contributes to host defense against intracellular bacteria.

Introduction

The gene encoding IL-26 was discovered in *Herpesvirus saimiri*-transformed human T cells, having ~25% sequence homology and ~47% amino acid similarity to IL-10 (Knappe, Hor, Wittmann, & Fickenscher, 2000). As such, IL-26 is considered a member of the IL-10 cytokine family, which includes IL-10, IL-19, IL-20, IL-22 and IL-24 (Kotenko, 2002; Stephen-Victor, Fickenscher, & Bayry, 2016). The *IL26* gene is located on chromosome 12q15, between *IFNG* and *IL22*. *IL26* is highly conserved in mammalian species, more weakly similar with non-

mammalian species. However, *IL26* is absent in rodents, despite the presence of both IL-26 receptor genes (Donnelly et al., 2010; Dumoutier, Van Roost, Ameye, Michaux, & Renauld, 2000; Wang, Diaz-Rosales, Martin, & Secombes, 2010). IL-26 contains 171 amino acids, including lysine or arginine 30 residues, forming six highly cationic α-helices (Knappe et al., 2000). Th17 cells are the main producers of IL-26 (Boniface et al., 2010; Wilson et al., 2007), but some Th1 cells (Meller et al., 2015) and NK cells (Hughes et al., 2009) are reported to produce this cytokine. Although monocytes (Guerra-Laso, Raposo-Garcia, Garcia-Garcia, Diez-Tascon, & Rivero-Lezcano, 2015) and macrophages (Che et al., 2014) have been reported to release IL-26, the purity of the cell populations was not clearly stated.

The structure of IL-26 including the α-helices, the amphipathic stretches, the clustering of cationic charges, and the formation of multimers, are hallmarks of naturally-occurring antimicrobial peptides (Ganz, 2003; Zasloff, 2002). This led to the discovery that IL-26 is the only known Th17 cytokine with antimicrobial properties, with the ability to kill extracellular bacteria which largely reside and replicate outside of cells, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* (Meller et al., 2015; Stephen-Victor et al., 2016). Similar to other antimicrobial proteins, IL-26 reduced bacterial viability via direct binding to the bacterial cell wall, leading to pore formation and membrane disruption (Meller et al., 2015).

In addition to its direct antimicrobial activity, IL-26 was shown to signal through the IL-10R2/IL-20R1 heterodimeric receptor, with both subunits simultaneously expressed exclusively and constitutively by epithelial cells (Hor et al., 2004; Sheikh et al., 2004). Despite sharing homology with the anti-inflammatory cytokine IL-10, and a receptor subunit with the IL-10 receptor, the majority of evidence indicates that IL-26 not only initiates inflammation but also propels it (Corvaisier et al., 2012; Miot et al., 2015; Pene et al., 2008). IL-26 was found to induce the expression of IL-10, TNF-α, and IL-8 in epithelial cells, as well as inhibit their

proliferation (Dambacher et al., 2009). IL-26 can also drive inflammation is by acting as a potent chemoattractant for neutrophils (Che et al., 2014), which subsequently can be activated to release inflammatory cytokines, proteases, and antimicrobial peptides (Wilgus, Roy, & McDaniel, 2013). One report indicated that although IL-26 by itself induced cytokine responses in bronchoalveolar lavage (BAL) cells, it inhibited by <25% the release of myeloperoxidase from BAL cells and neutrophils (Che et al., 2014).

In the present study, we investigated whether IL-26 contributes to host defense against an obligate intracellular bacterium by studying human leprosy as a model. The disease, caused by the *Mycobacterium leprae*, forms a spectrum, in which the clinical presentation correlates with the effectiveness of the immune response. In the self-limited tuberculoid (T-lep) form, the bacteria are eliminated; whereas, in the disseminated lepromatous (L-lep) form, bacilli-laden macrophages are prominent, indicating an inability to contain the infection. The results provide evidence that IL-26 may act intracellularly as well as extracellularly to kill microbial pathogens.

Results

IL-26 expression in human leprosy

We mined the gene expression data derived from leprosy lesions (Teles et al., 2013) to determine whether there was differential expression of IL26 mRNA across the spectrum of disease. This analysis revealed that IL-26 mRNA levels were significantly greater in the skin biopsy specimens from T-lep vs. L-lep patients (Figure 7A), with probe intensities of 408 ± 118 in the gene expression data from T-lep lesions versus 33 ± 11 from L-Lep lesions, $p<0.05$. In 7/10 T-lep donors, the expression of IL-26 mRNA was greater than in all the L-lep donors.

The differences in IL-26 expression in leprosy lesions were further investigated by immunohistochemistry with an anti-IL-26 monoclonal antibody. IL-26 protein expression was abundant in T-lep lesions, throughout the granuloma and particularly strong in the vicinity of small lymphoid cells, but also diffusely present near larger mononuclear myeloid cells (Figure

7B). In contrast, IL-26 expression was sparse throughout the L-lep lesions. We also assessed the specificity of the anti-IL-26 monoclonal antibody by adding recombinant monomers of IL-26 protein to the antibody prior to tissue staining. Saturation of the monoclonal antibody by recombinant IL-26 reduced the signal intensity in T-lep lesions, demonstrating specificity of the antibody to the protein (Figure 7C). Anti-CD3 served as a positive control. All isotype controls were consistently negative. Quantification was performed using the online software ImmunoRatio (Tuominen, Ruotoistenmaki, Viitanen, Jumppanen, & Isola, 2010) to measure the IL-26 to nuclear staining ratio, which provides a measure of IL-26 expression. This ratio is not intended as a measure of IL-26-expressing cells as we cannot determine whether IL-26 is being produced or taken up by individual cells. The IL-26 to nuclear staining ratio was approximately ten-fold higher in T-lep lesions compared to L-lep lesions, 56 ± 8.5 vs. 5.2 ± 3.0 ($p<0.05$, Figure 7D).

Confocal laser microscopy was employed to determine the relative localization of IL-26 in relation to T cells and macrophages within leprosy lesions. Again, IL-26 expression was greater in T-lep vs. L-lep lesions, colocalizing more frequently with $CD4^+$ T cells in T-lep vs. L-lep lesions (Figure 8A – 8B), and to a lesser extent with $CD8^+$ T cells (Figure 8C – 8D). The frequency of $CD4^+$ T cells that colocalized with IL-26 was greater in T-lep lesions at $26.7\% \pm 3.7\%$ compared to $3.2\% \pm 0.7\%$ in L-lep lesions, ($p<0.01$, Figure 8B). In addition, the frequency of $CD8^+$ T cells that colocalized with IL-26 was greater in T-lep lesions at $17.5\% \pm 1.2\%$, compared to L-lep lesions which had $7.2\% \pm 1.3\%$ colocalization, ($p<0.05$, Figure 8D). In simultaneously comparing the levels of colocalization of IL-26 with the CD4 and CD8 T cell markers, we found that IL-26 colocalized significantly more with $CD4^+$ than $CD8^+$ T cells, ($p<0.05$, Figure 8E). Additionally, we analyzed the relative location of IL-26 in relation to $CD68^+$ macrophages in leprosy lesions and found that IL-26 was co-expressed with CD68 in both types of lesions (Figure 9A). However, the degree of colocalization between IL-26 and the CD68

marker was also higher in T-lep lesions than L-lep lesions, 16.8% ± 2.1% vs. 8.3% ±1.1% respectively, ($p<0.05$, Figure 9B). There were also areas in T-lep granulomas in which IL-26 colocalized with both $CD4^+$ and $CD68^+$ cells (Figure 9C). In simultaneously comparing the levels of colocalization of IL-26 with the CD4 and CD68 markers, we found that IL-26 colocalized significantly more with $CD4^+$ than $CD68^+$, ($p<0.05$, Figure 9). These data show proximity of IL-26 to $CD4^+$ and $CD68^+$ cells, but again, these data define the microanatomic location of IL-26, but cannot distinguish between production versus uptake of the protein.

IL-26 directly reduces the viability of mycobacteria in axenic conditions

As a first step towards defining the role of IL-26 against intracellular *M. leprae*, we determined whether IL-26 bound to the bacterium. *M. leprae* was incubated with recombinant IL-26 protein in axenic culture. After 6 hours, IL-26 was detected on the surface of some *M. leprae* bacilli (Figure 10A and 10C). Quantification of confocal images confirmed that the majority of bacilli are bound by IL-26, 89.5% ± 0.5%, leaving 9.5% ± 0.51.1% free bacilli (Figure 10B). Analysis of confocal images by Imaris software revealed that the bacilli that were bound to IL-26 had a greater diameter, as measured by transverse width, compared to media controls. The width of the bacilli increased from 0.359 ± 0.012 μm in media treated bacteria to 0.528 ± 0.023 μm in 1 μM or 0.591 ± 0.038 μm in 2 μM of IL-26 treated bacteria ($p<0.01$, Figure 10D). The increased diameter is consistent with osmotic lysis of the bacteria, one mechanism by which antimicrobial peptides mediate an antimicrobial response.

Although *M. leprae* does not grow in culture, it is possible to measure bacterial viability by qPCR using the ratio of *M. leprae* 16S rRNA to repetitive element DNA RLEP as a proxy for transcriptional activity, as previously reported (Liu et al., 2012; Martinez et al., 2009; Teles et al., 2013). We studied the effects of IL-26 in axenic culture, determining whether it further reduced bacterial viability. IL-26 treatment of *M. leprae* for 3 days reduced the viability of the bacteria in

a dose-dependent manner (Figure 11A). The observed antimicrobial activity was diminished when IL-26 was denatured prior to addition to *M. leprae* (Figure 11B). Similarly, IL-26 inhibited the growth of *M. tuberculosis* H37Ra in axenic culture, quantified by a colony forming unit assay (Figure 11C). These findings reveal that IL-26 binds directly to mycobacteria and mediates a reduction in bacteria viability.

IL-26 is taken up by MDM and colocalizes with M. leprae

During *M. leprae* infection, bacilli can be found in a variety of tissues and cell types, but are predominantly located in macrophages (D. S. Ridley, 1971; M. J. Ridley, 1981). Although it has been reported that IL-26 concentrations up to 10 μM did not affect the viability of human primary human immune cells (Meller et al., 2015), we specifically assessed cytotoxic effects of IL-26 on human MDM. When we treated MDM with increasing concentrations of IL-26, we observed some detachment of MDM from the culture plates with the 2 μM IL-26 treatment. At 5 μM IL-26 treatment, the majority of cells were detached and the cells that remained displayed condensed nuclei and positive staining by TUNEL assay, indicative of cellular apoptosis (Figure 12A). Therefore, we stimulated MDM with IL-26 at 2 μM or less in all remaining experiments to ensure optimal cell viability.

Because *M. leprae* is an obligate intracellular organism, IL-26 must gain access to the intracellular compartments in which the bacteria reside in order to exert a direct antimicrobial activity. We therefore evaluated whether IL-26 entered MDM and could be detected in the endosomal pathway in which *M. leprae* resides. Overnight treatment of MDM with Alexa-488-labeled IL-26 resulted in the uptake of IL-26 and colocalization with LAMP1-positive compartments (Figure 12B). The colocalization of Alexa-488-labeled IL-26 with LAMP1 was significantly greater than the addition of Alexa-488 dye itself, increasing from 2.3% ± 0.4% colocalization in dye media alone to 19.6% ± 3.3% in 1 μM and 23% ± 2.3% in 2 μM of IL-26

(p<0.01, Figure 12C). Furthermore, treatment of *M. leprae*-infected MDM with Alexa-488-labeled IL-26 revealed colocalization of intracellular bacilli with IL-26 (Figure 12D). The degree of IL-26 colocalization with *M. leprae* was similar for treatment with the 1 μM and 2 μM concentrations of the cytokine, 11.9% ± 1.5% and 11% ± 0.6%, respectively (Figure 12E). Finally, we assess if pretreatment of MDM with IL-26 affect the levels of infectivity by *M. leprae*. Although pretreating MDM with IL-26 slightly reduced the infection efficiency of *M. leprae*, the differences in percentages of *M. leprae*-infected MDM are not statistically significant (Figure 12F).

IL-26 induces autophagy and enhances bacterial traffic to the lysosomes

The process of autophagy is required to overcome the ability of mycobacteria to block phagolysosomal fusion in infected macrophages in order to promote an effective antimicrobial response (Fabri et al., 2011; Hagge, Ray, Krahenbuhl, & Adams, 2004; Sibley, Franzblau, & Krahenbuhl, 1987). Immunofluorescence data indicated a significant increase in the number of autophagosomes in MDM following treatment with IL-26 compared to media control, as determined by quantification of LC3 puncta (Figure 13A). In media alone there were 8.3 ± 1.3 puncta per cell compared to IL-26 treatment in which 57.9 ± 5.5 puncta per cell were detected (p<0.01, Figure 13B). The ability of IL-26 to induce autophagy was confirmed by LC3 I to LC3 II conversion by immunoblotting (Figure 13C). Given that IL-26 has been shown to bind to DNA from dying cells in *in vitro* cultures and traffic this DNA to activate innate cytoplasmic receptors, e.g., STING (Meller et al., 2015; Poli et al., 2017), we investigated whether IL-26 induced autophagy was STING dependent. We found that IL-26 induced autophagy in wild-type THP-1 cells, but was strikingly diminished in THP-1 cells in which STING was ablated by stable knockout. In contrast, rapamycin induced autophagy was STING-independent (Figure 13D). Overall, IL-26 induced autophagy in THP-1 cells, as measured by the number of LC3 puncta per

cell, was blocked by approximately 60% by the deletion of STING (Figure 13E). In contrast, only 25% of rapamycin induced autophagy was STING dependent.

Having demonstrated that IL-26 induces autophagy and colocalizes with *M. leprae* bacilli during infection, we hypothesized that bacteria would localize to autophagosomes following IL-26 stimulation. We observed a significant increase in colocalization of *M. leprae* with the autophagosome marker LC3 and the lysosomal marker LAMP1 (Figure 13F. We observed 5.1% $\pm$ 0.7% colocalization between *M. leprae* and LC3 in media control vs. 18.9% $\pm$ 1.4% and 26% $\pm$ 1.9% colocalization in 1 μM and 2 μM IL-26-treated MDM, respectively ($p<0.05$, Figure 13G). There was similar increase in colocalization of *M. leprae* and the LAMP1 following IL-26 stimulation, with 1.3% $\pm$ 0.2% colocalization between *M. leprae* and LAMP1 in media control vs. 3.3% $\pm$ 0.4% and 6.4% $\pm$ 0.8% colocalization in 1 μM and 2 μM IL-26-treated MDM, respectively ($p<0.01$, Figure 13H). Although treatment with the higher concentration of IL-26 induced more *M. leprae* colocalization with LAMP1, IL-26 colocalization with LAMP1 remained comparable with treatment with either 1 μM and 2 μM IL-26 (Figure 13I, J), consistent with uninfected MDM (Figure 12C). This suggests that the mechanism driving the increased traffic of *M. leprae* to LAMP-1 compartments in infected MDM is not through increased expression of LAMP-1 but potentially through IL-26 induced autophagy.

IL-26 induces antimicrobial activity against intracellular bacteria

Finally, we sought to determine whether IL-26 treatment during infection would result in antimicrobial activity against *M. leprae*. Indeed, IL-26 treatment during *M. leprae* infection significantly reduced the viability of intracellular bacteria compared to media control. The addition of 2 μM of IL-26 to the infected MDM was required to reduce the viability of the intracellular bacteria by approximately 40%, much less than the 10 μM amount need to have similar effects in axenic culture ($p<0.05$, Figure 14A). Antimicrobial activity was dependent on

the native structure of IL-26 because denaturing the protein did not give the same results ($p<0.05$, Figure 14B). We observed a similar antimicrobial activity in MDM against *M. tuberculosis* (H37Ra) ($p<0.05$, Figure 14C). We note that in one study, the addition of IL-26, albeit at lower concentrations (~700-fold lower than given here) to *M. tuberculosis*- infected whole blood did not result in an antimicrobial response; however the authors stated the nature of the whole blood antimicrobial assay made interpretation of this result difficult (Guerra-Laso et al., 2015).

To further investigate the ability of IL-26 to mediate an antimicrobial response against intracellular bacteria, we infected MDM with *S. aureus*, which when taken up by macrophages has been reported to inhibit autophagy and acidification of phagolysosomes (Jubrail et al., 2016; Schnaith et al., 2007; Tranchemontagne, Camire, O'Donnell, Baugh, & Burkholder, 2015). Compared to media control, IL-26 treatment of MDM significantly reduced the viability of intracellular *S. aureus* ($p<0.05$, Figure 14D). Having demonstrated that IL-26 can induce autophagy in MDM, we query whether autophagy was required for antimicrobial activity. We used phosphatidylinositol 3-kinase (PI3K) inhibitor wortmannin to block IL-26-induced autophagy. The addition of wortmannin prior to IL-26 stimulation significantly reduced antimicrobial activity, observed by the increase viability of *M. leprae*, from 53% $\pm$ 7.8% with 2 μM IL-26 treatment to 86.7% $\pm$ 10.1% with wortmannin ($p<0.05$, Figure 14E). In summary, these data demonstrate that IL-26 treatment of infected MDM leads to autophagy, colocalization of IL-26 with the bacilli in LAMP-1 compartments, and an antimicrobial response against intracellular bacteria.

Discussion

The adaptive T cell response kills intracellular pathogens through direct and indirect mechanisms. For example, $CD8^+$ cytotoxic T cells deliver cytotoxic granule contents including

the antimicrobial protein granulysin into infected macrophages leading to a direct reduction in the viability of intracellular pathogens (Ochoa et al., 2001; Stenger, Rosat, Bloom, Krensky, & Modlin, 1999; Walch et al., 2007). $CD4^+$ Th1 cells release IFN-γ that triggers an indirect antimicrobial mechanism through activation of endogenous pathways in macrophages microbicidal pathways. Given that $CD4^+$ Th17 cell production of IL-26 provides a direct antimicrobial pathway against extracellular bacteria (Meller et al., 2015), we explored whether IL-26 can mediate an antimicrobial response against intracellular bacteria. We providence evidence that IL-26 enters bacteria-infected macrophages, induces autophagy and colocalizes with the pathogen, together resulting in an antimicrobial response. These data identify a novel role for IL-26 in host defense against intracellular bacteria.

We investigated the role of IL-26 in leprosy, which provides a unique model given that the disease presents as a spectrum in which the clinical presentation correlates with the immune response to the pathogen. Measuring mRNA and protein at the site of disease, we observed that IL-26 expression was greater in lesions from T-lep vs. L-lep patients, correlating with the group of patients with greatest cell-mediated immunity against the pathogen. IL-26 colocalized with T cells, but also with $CD68^+$ macrophages, indicating proximity to the cell type infected by the leprosy bacillus. The colocalization of IL-26 with $CD68^+$ macrophages was significantly greater in T-lep vs. L-lep lesions, even though there are comparable numbers of macrophages in the different forms of leprosy (Montoya et al., 2009). The greater expression of IL-26 in T-lep lesions correlates with the lower bacterial load in this form of leprosy (Job, Jayakumar, & Aschhoff, 1999; van Voorhis et al., 1982). In contrast, in L-lep lesions, the relatively lower expression of IL-26 is associated with a higher bacterial load. The lack of IL-26 may permit bacterial growth, and/or the bacilli may potentially inhibit production and secretion of IL-26. For example, expression of IL-4, IL-10 and Type I IFN have been reported to be higher in L-lep compared T-lep lesions, and these cytokines are known to inhibit anti-mycobacterial

pathways (Edfeldt et al., 2010; Krutzik et al., 2003; Schenk et al., 2012; Sieling et al., 1993; Teles et al., 2013). Accordingly, the Type I IFN gene expression profile is also enhanced in the blood of patients with active tuberculosis (Berry et al., 2010; Maertzdorf et al., 2012), which may contribute to the lower amount of IL-26 release following *M. tuberculosis* infection of a monocyte-enriched population from tuberculosis patients compared with healthy controls (Guerra-Laso et al., 2015).

IL-26 may contribute to an antimicrobial response against intracellular bacteria in infected macrophages through two mechanisms. First, IL-26 has direct antimicrobial activity against intracellular mycobacteria as shown in axenic culture and co-localizes with *M. leprae* in infected MDMs. Second, we found that IL-26 induces autophagy in MDM, which was required for its antimicrobial response against intracellular *M. leprae*. Since mycobacteria are known to inhibit phagolysosomal fusion required for intracellular killing (Frehel & Rastogi, 1987) and *S. aureus* is known to subvert acidification of phagolysosomes (Jubrail et al., 2016; Schnaith et al., 2007; Tranchemontagne et al., 2015), IL-26 may stimulate/activate infected cells to overcome this inhibition, facilitating the antimicrobial response. Additionally, the higher expression of IL-26 in T-lep lesions also correlates with the higher number of autophagosomes detected in this form of leprosy (Silva et al., 2017). Although autophagy is required for killing of intracellular mycobacteria; however some intracellular bacteria evade autophagy-dependent killing.

We found that IL-26 induced autophagy was dependent on the cytoplasmic DNA receptor STING, consistent with the demonstrated ability of IL-26 to bind to DNA from dying cells in the *in vitro* cultures and traffic this DNA to activate STING (Meller et al., 2015; Poli et al., 2017) and the ability of STING activation to trigger autophagy in mycobacteria infected macrophages (Watson, Manzanillo, & Cox, 2012). Although the IL-26 receptor, a heterodimer composed of IL-10R2 and IL-20R1, is expressed exclusively and constitutively by epithelial cells (Hor et al., 2004; Sheikh et al., 2004), human monocytes/macrophages express IL-10R2 but not

IL-20R1. One report demonstrated that IL-26 induction of IL-6 from monocytes was blocked by approximately 50% using a polyclonal goat anti-IL-10R2 antibody, thereby triggering IL-10R2 alone in or in combination with an undetermined co-receptor (Corvaisier et al., 2012). It is therefore possible that IL-10R2 in myeloid cells is involved in uptake and/or signaling of IL-26 to induce autophagy.

Given that IL-26 is produced by Th17 cells, our data, along with previous studies, suggest the possibility that Th17 cells contribute to host defense in leprosy. Serum IL-17 was lowest in L-lep patients compared to all other forms of leprosy (Abdallah, Emam, Attia, Hussein, & Mohamed, 2013). Th17 cells were more frequent both in the lesions and in *M. leprae* stimulated PBMC in T-lep vs. L-lep patients (Saini, Ramesh, & Nath, 2013). Furthermore, SNPs in *IL17F* were associated with leprosy susceptibility (Chaitanya et al., 2014). The related bacterium, *M. tuberculosis*, induces IL-23, a key cytokine in Th17 polarization by DC (Gerosa et al., 2008), as well as Th17 cells (Khader et al., 2005). Yet, IL-17R$^{-/-}$ (Freches et al., 2013) and IL-23$^{-/-}$ (Khader et al., 2005) mice were more susceptible to *M. tuberculosis* infection. In mouse models of TB, IL-17 was required for generation of IFN-γ-producing Th1 cells (Umemura et al., 2007), granuloma formation, inflammation (Khader et al., 2005; Umemura et al., 2007) and vaccine-induced immunity (Khader et al., 2007). However, the functional studies of Th17 cells in tuberculosis utilize mouse models, and as mentioned, IL-26 is not present in the mouse genome, such that it will be important to determine if Th17 cells contribute to host defense though other mechanisms than via IL-26 release.

In addition to mycobacteria, our data indicate that IL-26 has an antimicrobial effect on another cutaneous pathogen, *S. aureus*, when taken up by macrophages. Previous studies reported that defective Th17 responses in STAT3 deficient patients have been associated with increased susceptibility to *S. aureus* and *S. pyogenes* infections (de Beaucoudrey et al., 2008; Ma et al., 2008) indicating that this T cell subset plays a major role in the defense against

extracellular bacterial infections, potentially due to reduced STAT3 dependent activation by IL-26 (You et al., 2013).

The current immunologic premise holds that Th1 cells defend against intracellular pathogens, whereas Th17 cells are required to defend against extracellular bacteria. In previous work we demonstrated that Th1 cell release of IFN-γ activates macrophages to kill intracellular mycobacteria (Fabri et al., 2011; Teles et al., 2013), while human Th17 cells release IL-26 which kills extracellular bacteria in axenic (i.e. cell-free) cultures (Meller et al., 2015). Nevertheless, vaccines which induce only Th1 cells are not sufficient to engender protection against intracellular mycobacteria (Tameris et al., 2013). Our data provide evidence that the Th17 cytokine IL-26 can contribute to host defense against intracellular bacteria, identifying one mechanism by which Th17 cells contribute to host defense against such pathogens.

Methods

Microarray data analysis

The reported gene expression profiles of mRNAs derived from skin biopsy specimens of 16 leprosy patients (T-lep, n = 10; L-lep, n = 6) as determined using Affymetrix Human U133 Plus 2.0 microarrays were mined to investigate IL-26 mRNA expression (Bleharski et al., 2003; Teles et al., 2013). Gene expression files containing array data are available under the accession GSE17763 in the Gene Expression Omnibus (GEO) database.

Generation of monocyte-derived macrophages

Monocyte-derived macrophages (MDM) were derived from whole blood from healthy donors, obtained with informed consent at UCLA. PBMC were isolated using Ficoll (GE Healthcare) gradient centrifugation. $CD14^+$ cells were positively selected from PBMC using CD14 magnetic beads (Miltenyi, 130-050-201) and cultured in the presence of M-CSF (50 ng/ml) (R&D

Systems) and used at day 5-6. Routinely, we achieve >90% purity of monocytes by CD14 immunomagnetic selection.

Viability of M. leprae by PCR

Cells were stimulated with indicated concentrations of IL-26 for 30 minutes, then infected with *M. leprae*. For autophagy inhibition, MDM were treated with 500 nM of wortmannin prior to IL-26 stimulation. mRNA was isolated from cells using TRIzol reagent (*Invitrogen Life Technologies)* according to the manufacturers recommended protocol. The viability of intracellular *M. leprae* was determined by qPCR and quantified as previously described (Corvaisier et al., 2012; Teles et al., 2013; Tuominen et al., 2010). Briefly, comparison of the bacterial DNA to the mammalian 36B4 levels was used to monitor infectivity between all the conditions in the assay as well as PCR quality. The 16S rRNA and genomic DNA values were calculated using the $\Delta\Delta$CT analysis, with the bacterial DNA value serving as the housekeeping gene.

mLep 16s RNA FWD – GCATGTCTTGTGGTGGAAAGC

mLep 16s RNA REV – CACCCCACCAACAAGCTGAT

mLep RLEP DNA FWD – GCAGCAGTATCGTGTTAGTGAA

mLep RLEP DNA REV – CGCTAGAAGGTTGCCGTAT

H36B4 FWD – CCACGCTGCTGAACATGCT

H36B4 REV – TCGAACACCTGCTGGATGAC

Mycobacterium tuberculosis axenic cultures

Recombinant IL-26 monomers (R&D Systems) were diluted in 10 mM sodium phosphate, pH 7.2. Aliquots of 2.5 x 10^5 *M. tuberculosis* (H37Ra) bacteria were added to 100 ul of Middlebrook 7H9 medium (BD Biosciences) containing recombinant IL-26 monomers (R&D Systems) or 20

ug/ml (final concentration) rifampicin (Sigma-Aldrich). Cultures were incubated at 37 °C for 72 hours, at which time 900 ul of 10 mM sodium phosphate, pH 7.2 was added to bring to 1 ml volume. From the 1 ml suspension, 10 ul was plated on Middlebrook 7H10 agar plates (Teknova) for 3 weeks and CFU were enumerated.

Infection of MDM and quantification of M. tuberculosis CFU

Human MDM were pretreated with IL-26 (R&D Systems) or 1 ug/ml (final concentration) rifampicin (Sigma-Aldrich) in 10% Omega FCS for 30 minutes and infected with *Mycobacterium tuberculosis* (H37Ra) at a multiplicity of infection (MOI) of 5. The infected cells were harvested after 4 days, pelleted and lysed with 100 µl of 0.2% saponin in 1X PBS on ice for 20 minutes. PBS 1X, 900 µl, was added to bring the cell lysate to 1 ml final volume. From the 1 ml suspension, 1 µl stock was plated on Middlebrook 7H10 agar plates (Teknova) for 3 weeks and CFU were enumerated.

IL-26 culture with Mycobacterium leprae

Recombinant IL-26 monomers (R&D Systems) were diluted in 10 mM sodium phosphate, pH 7.2, and *labelled using the Alexa Fluor ® 488 Microscale Protein Labeling Kit (Molecular Probes, Invitrogen).* Aliquots of 1 x 10^6 *M. leprae* bacteria, labeled by PKH-26, were incubated with A488-IL-26 in 50 ul volume of 10 mM sodium phosphate, pH 7.2 for 6 hours, then mounted on microscopy slides with Prolong Gold (*Invitrogen Life Technologies)*. Images were examined using a Leica microscope (Leica, Heidelberg, Germany). The diameter of *M. leprae* bacilli were quantified by Imaris software.

Infection of MDM and quantification of Mycobacterium leprae viability

Mycobacteria leprae was grown in the footpad of nu/nu mice as described previously (Lahiri, Randhawa, & Krahenbuhl, 2005), and was provided by the National Hansen's Disease Program. Human MDM were pretreated with IL-26 (R&D Systems) in 10% Omega FCS for 30 minutes and infected with *M. leprae* at a multiplicity of infection (MOI) of 5. The infected cells were harvested after 4 days. RNA and DNA were isolated from infected cells by TRIzol (*Invitrogen Life Technologies*) method. cDNA was made from RNA. DNA was treated with RNase to removed residual RNA. The viability of intracellular *M. leprae* was determined by qPCR and quantified as previously described (Martinez et al., 2009; Teles et al., 2013). Briefly, the levels of bacterial 16S rRNA and the genomic DNA element RLEP of *M. leprae* were measured by qPCR. The 16S rRNA and RLEP DNA values were determined by using the ΔΔCT analysis, with the DNA value serving as the housekeeping gene. The ratio of RNA to DNA was calculated for each replicate and the percent of bacterial viability was calculated relative to the respective media control. The efficiency of infection was determined by confocal microscopy. Approximately 50-70% of cells were infected. Cell viability following washes and infection was >95% of the total cells. To denature recombinant IL-26, the protein was treated with 5% beta-mercaptoethanol (Invitrogen) and heated at 95°C for 10 minutes.

Infection of MDM and quantification of intracellular S. aureus CFU

S. aureus strain DU5938 (Hla− Hlb− Hlg−) (Nilsson, Hartford, Foster, & Tarkowski, 1999) were grown to the mid-log phase at 37°C with shaking (150 rpm) in brain-heart infusion (BHI) medium, collected by centrifugation for 10 minutes at 5,000 X g. For cellular infection, the bacterial suspension was diluted with sterile 1X PBS. The number of viable bacteria was determined by serial dilution and plating onto BHI agar plates (Schindler et al., 2012). Human MDM (2×10^6 cells) were pretreated with IL-26 for 4 hours, washed, and infected with *S. aureus* at an MOI of 5 for 4 hours in RPMI with 10% FCS antibiotic-free media. Next, 100 µg/ml of

gentamycin was added to the wells for 20 minutes to kill extracellular bacteria. Cells were then washed to further remove any remaining extracellular bacteria. Infected cells were cultured overnight at 37°C in a 4% CO_2 incubator. To measure intracellular killing of bacteria, cells were pelleted and lysed with 100 µl of 0.2% saponin in 1X PBS on ice for 20 minutes. PBS 1X, 900 µl, was added to bring the cell lysate to 1 ml final volume, of which 1 µl was plated and CFU determined.

Patients and clinical specimens

Patients with leprosy were classified according to the criteria of Ridley and Jopling (D. S. Ridley & Jopling, 1966). The designation of tuberculoid leprosy (T-lep) included patients that were classified clinically as borderline tuberculoid, “BT”, and the designation of lepromatous leprosy (L-lep) only included patients classified as “LL”. All T-lep and L-lep skin biopsy specimens were taken at the time of diagnosis, prior to initiating treatment. Specimens were embedded in OCT medium (Ames, Elkhart, IN), snap frozen in liquid nitrogen and stored at -80°C.

Tissue immunoperoxidase labeling

Frozen tissue sections were blocked with normal horse serum before incubation with monoclonal antibodies (mAbs) for IL-26 (clone 2A8, IgG2a, Sigma-Aldrich) and CD3 (clone UCHT1, IgG1k, BD Biosciences) for 2 hours, followed by incubation with biotinylated horse anti-mouse IgG for 90 minutes. To test antibody specificity, IL-26 antibody was incubated with IL-26 monomers (R&D Systems) for 15 minutes prior to incubation on tissue sections. Slides were counterstained with hematoxylin and mounted in crystal mounting medium (Biomeda) and were visualized using the ABC Elite system (Vector Laboratories). Skin sections were examined using a Leica microscope (Leica, Heidelberg, Germany). Ratios were calculated by ImmunoRatio© online software, (Jorma Isola & Vilppu Tuominen – Institute of Biomedical

Technology, University of Tampere) (Tuominen et al., 2010), an automated image analysis application which calculates the percent diaminobenzidine (DAB)-stained nuclear area per total area.

Cell culture immunofluorescence labeling

Recombinant IL-26 monomers (R&D Systems) were labelled using the Alexa Fluor ® 488 Microscale Protein Labeling Kit (Molecular Probes, Invitrogen). MDM were treated with labelled rIL-26 monomers in RPMI media with 10% FCS for 30 minutes. As a control, MDM were treated with the *Alexa Fluor ® 488* dye media used for the labelling reaction. Treated MDM were next infected with live PKH26 labeled -*M. leprae* overnight. Cells were then washed, and fixed for 30 minutes with 4% PFA, and washed again. Next, cells were permeabilized with 0.25% saponin for 20 minutes, blocked with serum for 30 minutes, and stained with primary antibodies for CD68 (clone Y1/82A, IgG2b, Biolegend), LAMP1 (clone H4A3, IgG1, Biolegend), or LC3 (clone 4E12, IgG1, MBL International) for 2 hours. Following washing, cells were stained with secondary antibodies (*Molecular Probes, Invitrogen)* for 90 minutes, washed, and mounted with Prolong Gold with DAPI (*Invitrogen Life Technologies)*. For the quantification of autophagy, the percentages of LC3 punctated cells were evaluated using florescence microscopy. Approximately 100 cells, over six different random fields of view, were scored for each condition of each experiment. Rapamycin was used as a positive control for autophagy induction at 300 nM. Immunofluorescence of cell cultures was examined using a Leica-TCS-SP MP inverted single confocal laser-scanning and a two-photon laser microscope (Leica, Heidelberg, Germany) at the Advanced Microscopy/Spectroscopy Laboratory Macro-Scale Imaging Laboratory, California NanoSystems Institute, University of California at Los Angeles. Colocalization quantifications were performed using ImageJ software by splitting final image

overlay into individual single channel, and selecting 'Colocalization Threshold' option to calculate percentage of pixel overlap between channels.

Culture and treatment of wild type and STING knockout THP-1 cells.

Wild type and STING knockout THP-1 Dual reporter cell lines were purchased from Invivogen (San Diego, CA). The STING knockout THP-1 Dual cells were created by stable knockout of STING in wild type THP-1 Dual cells. The cells were cultured as according to the manufacture's protocol. For all experiments, both wild type and STING knockout THP-1 cells were passaged no more than 5 times. WT and STING knockout cells were plated at a density of 0.5×10^6 cells/ml on 8 well glass slides and were made adherent by treating with 400 ng/ml PMA for 48 hours. The cells were subsequently treated or not with 2uM rIL-26 monomers for 24 hours. 300 nM rapamycin was added to some wells for 3 hours to serve as a positive control for autophagy induction. Immunofluorescence labelling for LC3 puncta detection was performed as described previously in the *Cell culture immunofluorescence labeling* section, with the number of LC3 puncta per cell quantification was performed using ImageJ software.

Tissue immunofluorescence labeling

Immunofluorescence was performed by serially incubating cryostat tissue sections with anti-human mAbs of different isotypes for 2 hours (IL-26 – clone 2A8, IgG2a, Sigma-Aldrich; CD4 – clone A16A1, IgG2b, Biolegend; CD8 – clone HIT8a, IgG1, Biolegend; CD68 – clone KP1, IgG1, Abcam), washed three times with 1X PBS, followed by incubation with isotype-specific fluorochrome (A488, A568, A647)-labeled goat anti-mouse immunoglobulin antibodies (Molecular Probes, Invitrogen) for 90 minutes. Negative controls were stained with matching isotype antibodies. Nuclei were stained with DAPI (4',6'-diamidino-2-phenylindole) (Invitrogen Life Technologies). Immunofluorescence of skin sections was examined using a Leica-TCS-SP

MP inverted single confocal laser-scanning and a two-photon laser microscope (Leica, Heidelberg, Germany) at the Advanced Microscopy/Spectroscopy Laboratory Macro-Scale Imaging Laboratory, California NanoSystems Institute, University of California at Los Angeles. Colocalization quantifications were performed using ImageJ software.

Immunofluorescence colocalization analysis

The colocalization threshold plugin by ImageJ was used for all colocalization analysis related to confocal images. Briefly, this plugin compares two images for correlated pixel intensities, a positive correlation between two images indicates that the signal in one channel (green) is observed at the same time as the signal in the other channel (red). The plugin performs a converging search for the image thresholds and reports various metrics on the resulting thresholded images. We used the % volume metric. Colocalization colors are Red + Green = Yellow; Red + Cyan = Magenta; Red + Green + Cyan = White.

TUNEL assay

To evaluate cell death by apoptosis, we utilized Roche *In Situ Cell Death Detection Kit, Fluorescein according to manufacturer's protocol.* Briefly, human MDM were differentiated on glass slides and treated with IL-26 or overnight. Cells were then washed, fixed, and labeled TUNEL reaction mixture. Nuclei were stained with DAPI (4',6'-diamidino-2-phenylindole). Immunofluorescence was examined using a Leica-TCS-SP MP inverted single confocal laser-scanning and a two-photon laser microscope (Leica, Heidelberg, Germany) at the Advanced Microscopy/Spectroscopy Laboratory Macro-Scale Imaging Laboratory, California NanoSystems Institute, University of California at Los Angeles.

Immunoblotting analysis

MDM, $2X10^6$ cells per condition, were collected in NP-40 lysis buffer (50 mM Tris, pH 7.4, 50 mM NaCl, 5 mM EDTA,50 mM NaF, 1 mM Na3VO4, 1% Nonidet P40 (NP40),0.02% NaN3 and 1 mM PMSF) containing complete protease inhibitors (Roche Applied Science, Germany). Total protein from cell lysates were quantified using Bradford Assay. Lysates were separated on a 7.5% nondenaturing gel, followed by immunoblot analysis with anti-HSP90 and anti-LC3 with detection by enhanced chemiluminescence (Pierce Biotechnology, Rockford, Illinois, USA).

Statistics

Statistics reported are of the entire series of experiments and described as mean ± the standard error mean. GraphPad Prism 6 software was used for graphing and statistical analysis. For comparison between three or more groups, we utilized repeated measures one-way ANOVA, with the Greenhouse-Geisser correction, along with Tukey's multiple comparisons test, with individual variances computed for each comparison. The two-tailed student's t-test was used for all other two-group analyses. For the WT and STING $^{-/-}$ experiments we utilized repeated measures two-way ANOVA, along with Tukey's multiple comparisons test. For all experiments, a P value less than 0.05 was considered to be significant.

Study approval

This study was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the local Institutional Review Boards. All donors provided written informed consent for the collection of peripheral blood and skin specimens for subsequent scientific analysis. All leprosy patients were recruited with approval from the Institutional Review Board of University of Southern California School of Medicine and the Institutional Ethics Committee of Oswald Cruz Foundation, as well as the University of California, Los Angeles.

Author contributions: R.L.M, A.T.D, R.M.B.T and D.I.W designed the experiments, interpreted the data, and drafted the manuscript. B.R.B assisted with the conceptual framework and writing. A.T.D performed most of the experiments. D.I.W., K.P. and G.C performed the autophagy immunoblotting experiment. R.M.B.T. performed microarray analysis and quantified confocal imaging. E.N.S and M.T.O provided the clinical samples.

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Figure 7

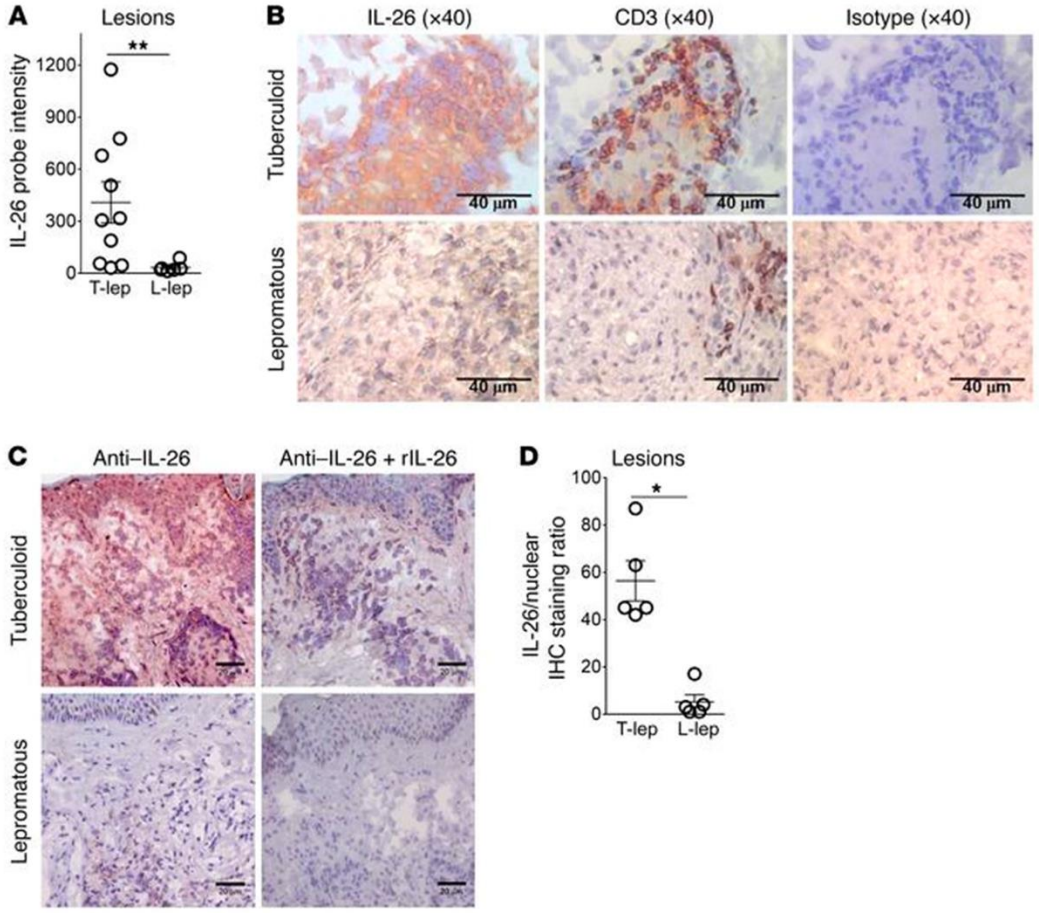

Figure 7. IL-26 in leprosy lesions. (**A**) IL-26 mRNA probe intensity of skin leprosy lesions quantified by microarray gene expression. (**B**) IL-26 expression in leprosy lesions (T-lep and L-lep); one representative labeled section is shown out of at least four individuals. Scale bars equal 40 μm. (**C**) IL-26 expression following saturation of IL-26 antibody with recombinant IL-26, demonstrating antibody specificity; one representative labeled section is shown out of three individuals. Scale bars equal 20 μm. (**D**) Ratio of IL-26 and nuclear staining quantified by ImmunoRatio. Data are represented as mean ± SEM, n = 4. *$P < 0.05$, two-tailed student's t-test.

Figure 8

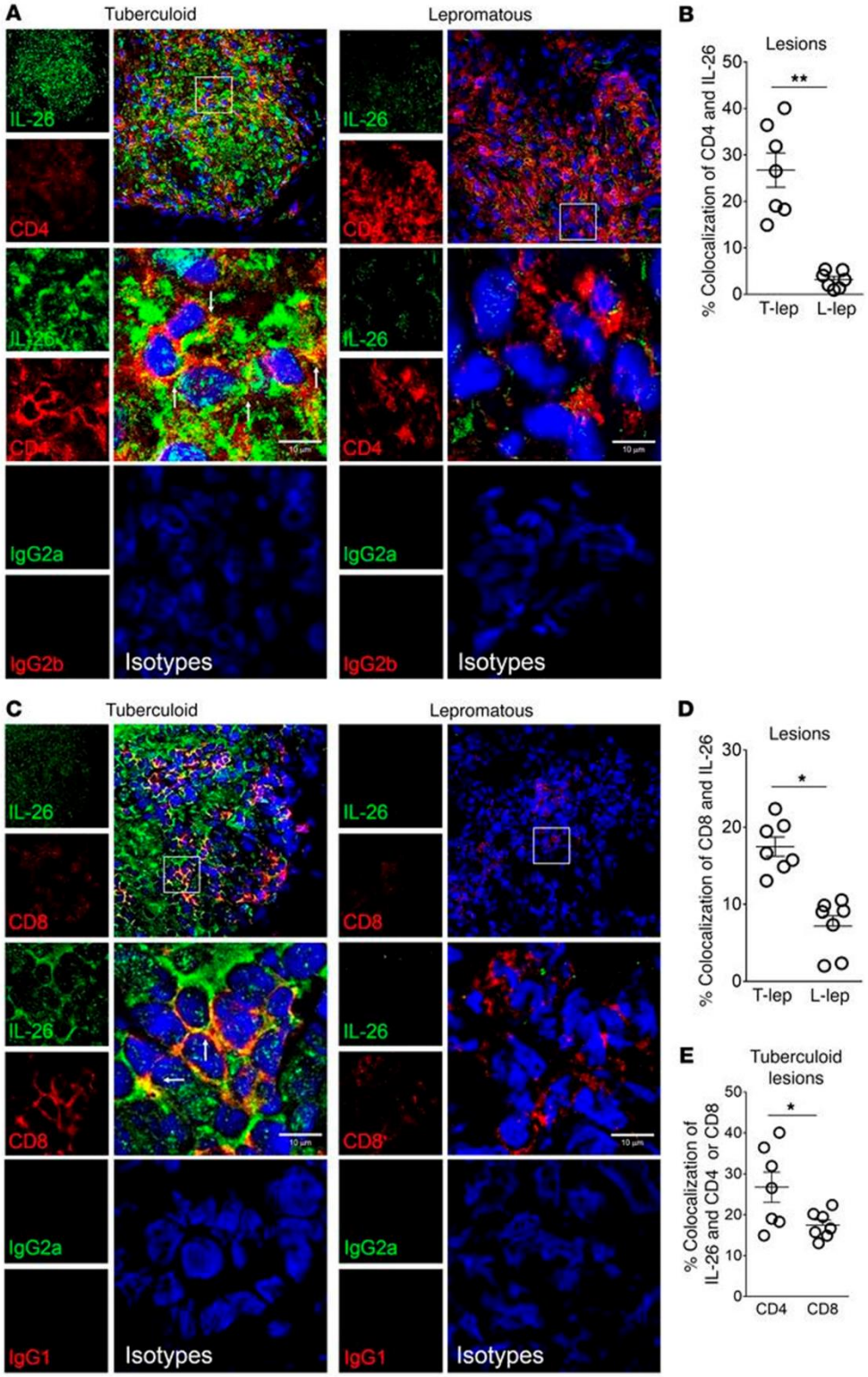
A
Tuberculoid
Lepromatous
IL-26
CD4
IgG2a
IgG2b
Isotypes
B
Lesions
% Colocalization of CD4 and IL-26
T-lep
L-lep
C
CD8
IgG1
D
% Colocalization of CD8 and IL-26
E
Tuberculoid lesions
% Colocalization of IL-26 and CD4 or CD8
CD4
CD8

Figure 8. IL-26 colocalization with CD4 and CD8. (**A**) Immunofluorescence of IL-26 (green), CD4 (red), and nuclei (DAPI-blue) in T-lep and L-lep lesions. Data are representative of four individual samples. (**B**) Colocalization of (A) by ImageJ software. Data are represented as mean ± SEM, n = 4. $^{**}P < 0.01$. (**C**)Immunofluorescence of IL-26 (green), CD8 (red), and nuclei (DAPI-blue) in T-lep and L-lep lesions. (**D**) Colocalization of (C) by ImageJ software. Data are represented as mean ± SEM, n = 4. $^{*}P < 0.05$. White arrows indicate colocalization of green and red (yielding yellow). Scale bars equal 10 μm. (**E**) Comparison of CD4 or CD8 colocalization with IL-26 in T-lep using ImageJ software. Data are represented as mean ± SEM, n = 4. $^{*}P < 0.05$. Two-tailed student's t-test.

Figure 9

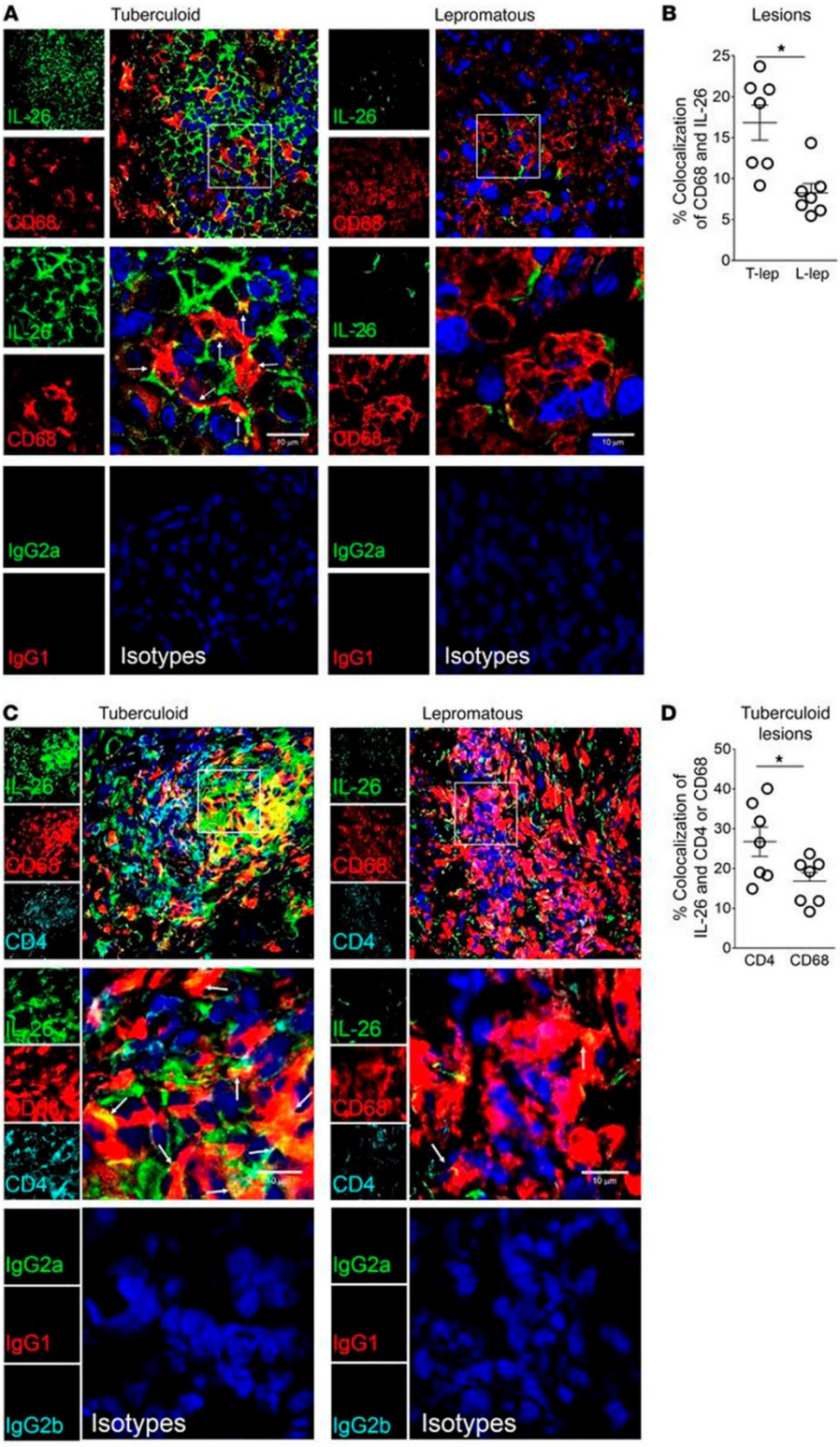
A
Tuberculoid
Lepromatous
IL-26
CD68
IgG2a
IgG1
Isotypes
B
Lesions
% Colocalization of CD68 and IL-26
T-lep
L-lep
C
CD4
IgG2b
D
Tuberculoid lesions
% Colocalization of IL-26 and CD4 or CD68

Figure 9. IL-26 colocalization with CD68+ macrophages in leprosy lesions. (**A**) Immunofluorescence of IL-26 (green), CD68 (red), and nuclei (DAPI-blue) in T-lep and L-lep lesions. Data are representative of five individual samples. (**B**) Colocalization of IL-26 (green) and CD68 (red) in T-lep and L-lep lesions using ImageJ software. Data are represented as mean ± SEM, n = 5. *$P < 0.05$, two-tailed student's t-test. (**C**) Immunofluorescence of IL-26 (green), CD68 (red), CD4 (cyan), and nuclei (DAPI-blue) in T-lep and L-lep lesions. Data shown are representative of three individual samples. White arrows indicate colocalization of green and red (yielding yellow). Scale bars equal 10 μm. (**D**) Comparison of CD4 or CD68 colocalization with IL-26 in T-lep using ImageJ software. Data are represented as mean ± SEM, n = 4. *$P < 0.05$. Two-tailed student's t-test.

Figure 10

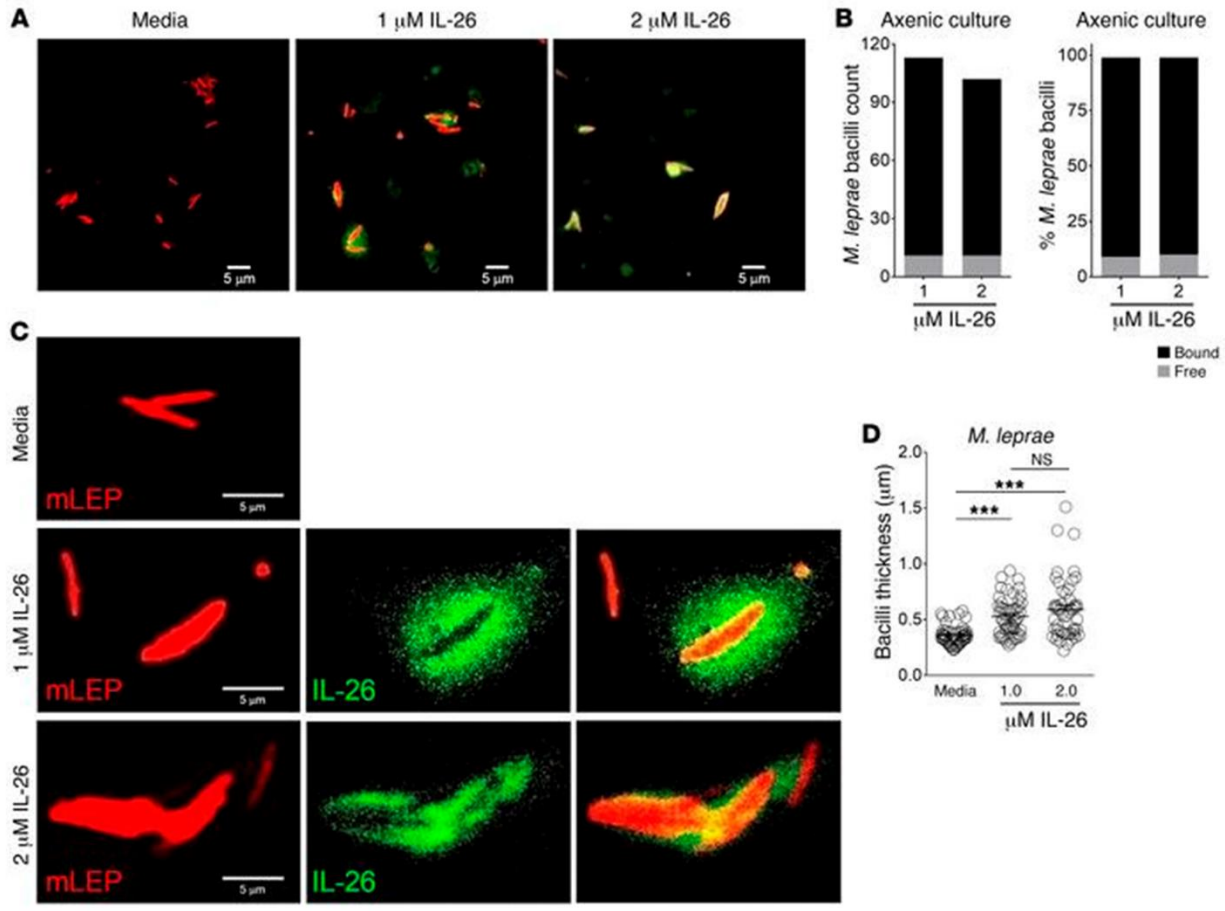

Figure 10. IL-26 binds directly to *M. leprae* bacilli. (**A** and **C**) Confocal microscopy images of Alexa-488-IL-26 (green) cultured with *M. leprae* (red) for 6 hours. Data shown are representative of four independent experiments. (**B**) Quantification of number of free bacilli or IL-26-bound bacilli (left). Percentage of free or IL-26-bound bacilli of total bacilli counts (right). (**D**) Measurement of *M. leprae* bacilli thickness from confocal microscopy images of (C). Media contained *Alexa Fluor ® 488* dye as control. Data are represented as mean ± SEM, n = 50 bacilli for each condition. Data shown are representative of four independent experiments. ****P* < 0.001. Repeated measures one-way ANOVA. Scale bars equal 5 μm.

Figure 11

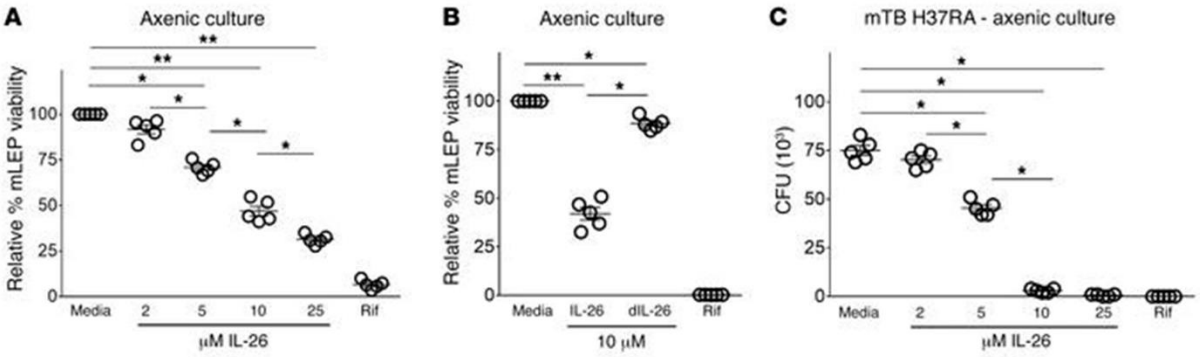

Figure 11. IL-26 has direct antimicrobial activity against mycobacteria. (**A**) *M. leprae* was cultured with increasing concentrations of IL-26 for 4 days. Rifampicin was used as positive control. Viability of *M. leprae* was calculated by the ratio of bacterial 16S rRNA and RLEP DNA detected by qPCR. Relative viability was determined by comparing treatment ratio to media ratio. Data are represented as mean ± SEM, n = 5. (**B**) *M. leprae* was cultured with 10 μM of native IL-26 or denatured IL-26 for 4 days. Rifampicin was used as positive control. Viability of *M. leprae* was calculated by the ratio of bacterial 16S rRNA and RLEP DNA detected by qPCR. Relative viability was determined by comparing treatment ratio to media ratio. Data are represented as mean ± SEM, n = 5. (**C**) *M. tuberculosis* (H37Ra) was cultured with increasing concentrations of IL-26 for 4 days. Rifampicin was used as positive control. Colony forming unit assay was performed and quantified after 3 weeks. Data are represented as mean ± SEM, n = 5. **P* < 0.05. ***P* < 0.01. Repeated measures one-way ANOVA.

Figure 12

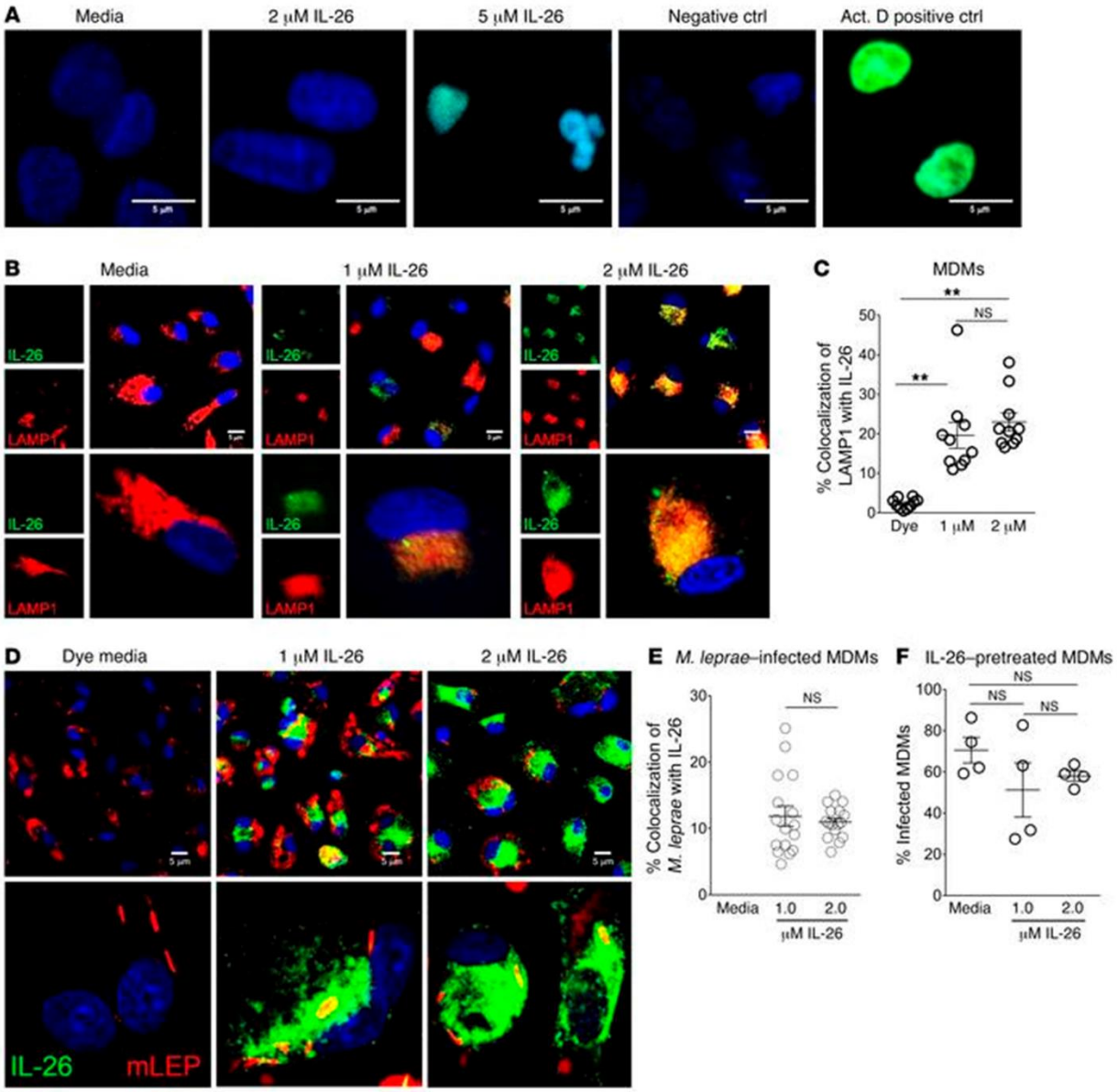

Figure 12. IL-26 is taken up by MDM and colocalizes with *M. leprae*. (**A**) Human MDM were treated with IL-26 overnight. Cells were washed, fixed, and apoptosis was determined using TUNEL (green) assay. Nuclei (DAPI-blue). Data shown are representative of five individual donors. (**B**) Human MDM were treated with Alexa-488-IL-26 (green) overnight. Cells were washed, fixed, and immunolabeled with anti-LAMP1 antibody (red). Nuclei (DAPI-blue).

Data shown are representative of five individual donors. (**C**) Colocalization of LAMP1 (red) and IL-26 (green) were quantified with ImageJ. Data are representative as mean percentage colocalization ± SEM, n ≥ 50 cells from three donors. ***P* < 0.01. (**D**) Human MDM were treated with Alexa-488-IL-26 (green) for 30 minutes and infected with *M. leprae* (red) overnight. Cells were washed and fixed. Nuclei (DAPI-blue). Media contained *Alexa Fluor ® 488* dye as control. Data shown are representative of four individual donors. (**E**) Colocalization of *M. leprae* (red) and IL-26 (green) were quantified by ImageJ. Data are representative as mean percentage colocalization ± SEM, n ≥ 40 cells from four donors. (**F**) Quantification of MDM infected by *M. leprae* following 30 minutes treatment with IL-26. Data are representative as mean percentage ± SEM, n = 4. Repeated measures one-way ANOVA. Scale bars equal 5 μm.

Figure 13

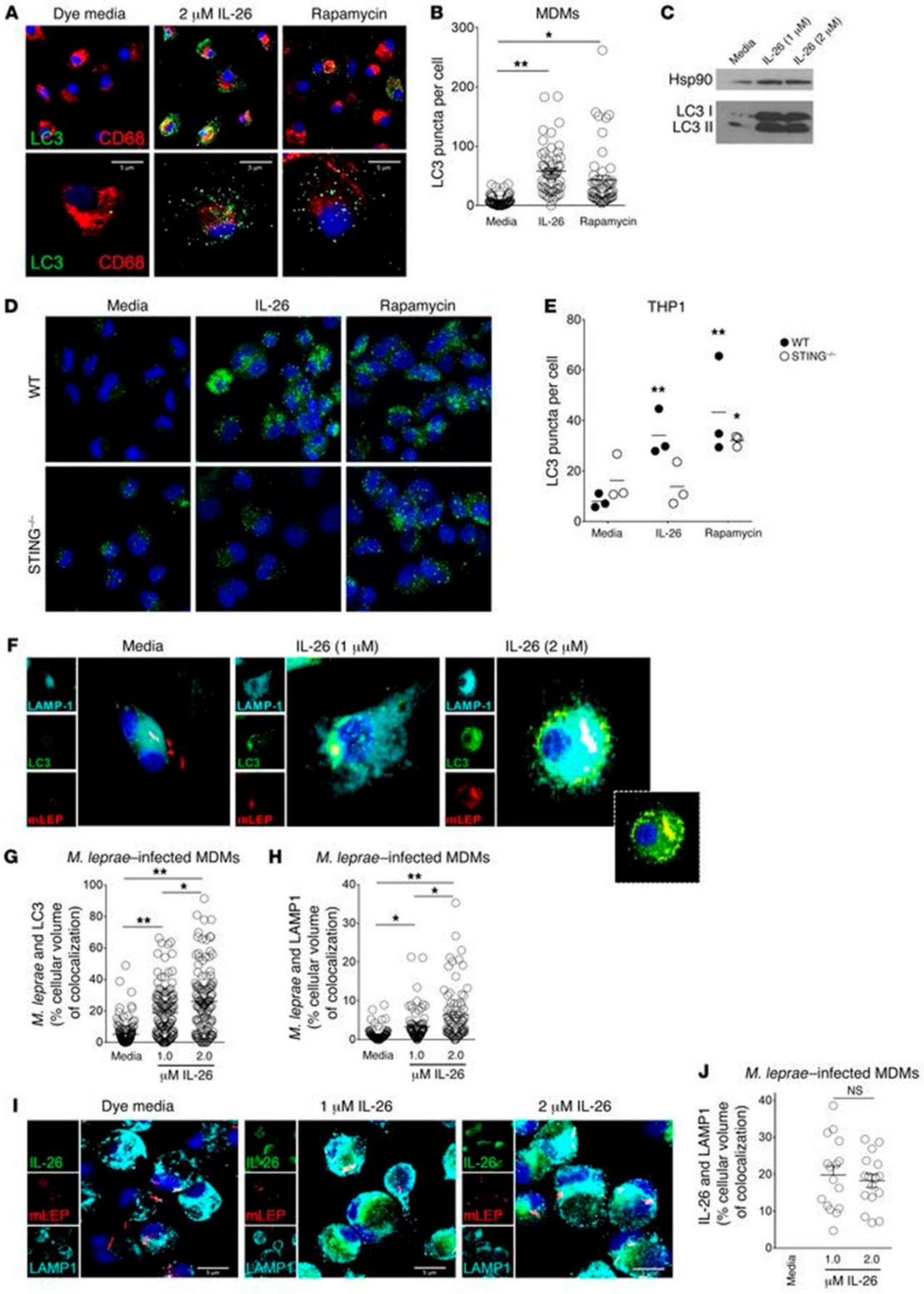
A
Dye media
2 μM IL-26
Rapamycin
LC3
CD68
B
MDMs
LC3 puncta per cell
Media
IL-26
Rapamycin
C
Media
IL-26 (1 μM)
IL-26 (2 μM)
Hsp90
LC3 I
LC3 II
D
Media
IL-26
Rapamycin
WT
STING−/−
E
THP1
LC3 puncta per cell
WT
STING−/−
F
Media
IL-26 (1 μM)
IL-26 (2 μM)
LAMP-1
LC3
mLEP
G
M. leprae–infected MDMs
M. leprae and LC3 (% cellular volume of colocalization)
μM IL-26
H
M. leprae–infected MDMs
M. leprae and LAMP1 (% cellular volume of colocalization)
μM IL-26
I
Dye media
1 μM IL-26
2 μM IL-26
IL-26
mLEP
LAMP1
J
M. leprae–infected MDMs
NS
IL-26 and LAMP1 (% cellular volume of colocalization)
μM IL-26

Figure 13. IL-26 induces autophagy and enhances bacterial traffic to the lysosomes. (**A**) MDM were cultured with IL-26 or media overnight, and immunolabeled with anti-LC3 antibody (green) and anti-CD68 antibody (red). Nuclei (DAPI-blue). (**B**) LC3 puncta per cell ± SEM, n ≥ 50 cells from four donors. (**C**) LC3 I to LC3 II conversion was detected by immunoblotting. Hsp90 was used as internal control. (**D**) PMA-treated THP-1 cells were treated with IL-26 (2μM) for 24h and rapamycin (300nM) for 6h, and immunolabeled with LC3 antibody (green). Nuclei (DAPI-blue). Data shows one representative of three independent experiments for both wild type (WT) and STING$^{-/-}$ THP-1 cells. Magnification 63X. (**E**) LC3 puncta per cell ± SEM, n ≥ 50 cells from three donors for both WT and STING$^{-/-}$ THP-1 cells. (F) Human MDM were treated with IL-26 for 30 minutes and infected with *M. leprae* (red) overnight. Cells were washed and fixed, and immunolabeled with anti-LC3 (green) and LAMP-1 (cyan). Nuclei (DAPI-blue). Data shown are representative of four individual donors. Inset image is of LC3 (green) and *M. leprae* (red) overlay, without LAMP1 (cyan). (**G**) Colocalization of LC3 (green) and *M. leprae* (red), and (**H**) Colocalization of LAMP1 (cyan) and *M. leprae* (red) were quantified by ImageJ. Data are represented as mean percentage of cellular volume of colocalization ± SEM n ≥ 30 cells from four donors. (**I**) Human MDM were treated with Alexa-488-IL-26 (green) for 30 minutes and infected with *M. leprae* (red) overnight. Cells were washed and immunolabeled with anti-LAMP1 (cyan). Nuclei (DAPI-blue). Media contained Alexa Fluor ® 488 dye as control. Data shown are representative of four individual donors. (**J**) Colocalization of IL-26 (green) and LAMP-1 (cyan) were quantified by ImageJ. Data are represented as mean percentage of cellular volume of colocalization ± SEM, n ≥ 40 cells from four donors. *$P < 0.05$. **$P < 0.01$. Repeated measures one-way ANOVA. Scale bars equal 5 μm.

Figure 14

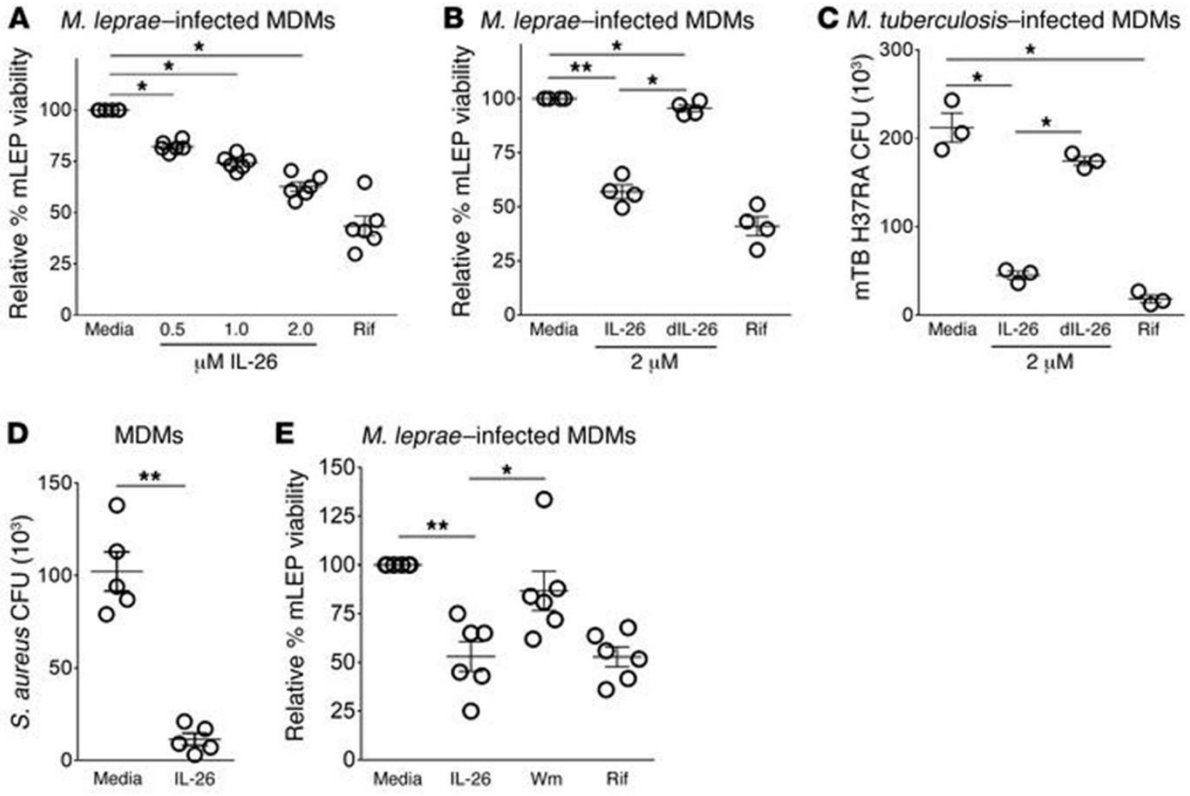

Figure 14. IL-26 induces antimicrobial activity against intracellular bacteria. (**A**) MDM were treated with IL-26 for 30 minutes then infected with *M. leprae* for 4 days. Rifampicin (RIF) was used as positive control. Viability of *M. leprae* was measured by qPCR, n = 6. (**B**) MDM were treated with IL-26 or denatured IL-26 then infected with *M. leprae*, and bacterial viability measured as in (A), n = 4. (**C**) MDM were treated with IL-26 or denatured IL-26, then infected with *M. tuberculosis* (H37Ra) as in (A). Viability of intracellular *M. tuberculosis* was determined by CFU assay, n = 3. (**D**) MDM were treated with IL-26 for 4 hours, washed, infected with *S. aureus* for 4 hours, the extracellular bacteria removed, then after overnight culture, the viability of intracellular *S. aureus* was determined by CFU assay, n = 5. (**E**) MDM were treated with IL-26 with or without wortmannin, then infected with *M. leprae*, and bacterial viability measured as

in (A), n = 6. (A-E) *P < 0.05. **P < 0.01. All data are represented as mean ± SEM. Repeated measures one-way ANOVA.

References

Abdallah, M., Emam, H., Attia, E., Hussein, J., & Mohamed, N. (2013). Estimation of serum level of interleukin-17 and interleukin-4 in leprosy, towards more understanding of leprosy immunopathogenesis. *Indian Journal of Dermatology, Venereology and Leprology*, *79*(6), 772–776. https://doi.org/10.4103/0378-6323.120723

Berry, M. P., Graham, C. M., McNab, F. W., Xu, Z., Bloch, S. A., Oni, T., … O'Garra, A. (2010). An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature*, *466*(7309), 973–977. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20725040

Bleharski, J. R., Li, H. Y., Meinken, C., Graeber, T. G., Ochoa, M. T., Yamamura, M., … Modlin, R. L. (2003). Use of genetic profiling in leprosy to discriminate clinical forms of the disease. *Science*, *301*(5639), 1527–1530. https://doi.org/DOI 10.1126/science.1087785

Boniface, K., Blumenschein, W. M., Brovont-Porth, K., McGeachy, M. J., Basham, B., Desai, B., … de Waal Malefyt, R. (2010). Human Th17 cells comprise heterogeneous subsets including IFN-gamma-producing cells with distinct properties from the Th1 lineage. *Journal of Immunology*, *185*(1), 679–687. https://doi.org/10.4049/jimmunol.1000366

Chaitanya, V. S., Jadhav, R. S., Lavania, M., Singh, M., Valluri, V., & Sengupta, U. (2014). Interleukin-17F single-nucleotide polymorphism (7488T>C) and its association with susceptibility to leprosy. *International Journal of Immunogenetics*, *41*(2), 131–137. https://doi.org/10.1111/iji.12097

Che, K. F., Tengvall, S., Levanen, B., Silverpil, E., Smith, M. E., Awad, M., … Linden, A. (2014). Interleukin-26 in antibacterial host defense of human lungs. Effects on neutrophil mobilization. *American Journal of Respiratory and Critical Care Medicine*, *190*(9), 1022–1031. https://doi.org/10.1164/rccm.201404-0689OC

Corvaisier, M., Delneste, Y., Jeanvoine, H., Preisser, L., Blanchard, S., Garo, E., … Jeannin, P. (2012). IL-26 is overexpressed in rheumatoid arthritis and induces proinflammatory cytokine production and Th17 cell generation. *PLoS Biology*, *10*(9), e1001395. https://doi.org/10.1371/journal.pbio.1001395

Dambacher, J., Beigel, F., Zitzmann, K., De Toni, E. N., Goke, B., Diepolder, H. M., … Brand, S. (2009). The role of the novel Th17 cytokine IL-26 in intestinal inflammation. *Gut*, *58*(9), 1207–1217. https://doi.org/10.1136/gut.2007.130112

de Beaucoudrey, L., Puel, A., Filipe-Santos, O., Cobat, A., Ghandil, P., Chrabieh, M., … Casanova, J. L. (2008). Mutations in STAT3 and IL12RB1 impair the development of human IL-17-producing T cells. *Journal of Experimental Medicine*, *205*(7), 1543–1550. https://doi.org/10.1084/jem.20080321

Donnelly, R. P., Sheikh, F., Dickensheets, H., Savan, R., Young, H. A., & Walter, M. R. (2010). Interleukin-26: an IL-10-related cytokine produced by Th17 cells. *Cytokine and Growth Factor Reviews*, *21*(5), 393–401. https://doi.org/10.1016/j.cytogfr.2010.09.001

Dumoutier, L., Van Roost, E., Ameye, G., Michaux, L., & Renauld, J. C. (2000). IL-TIF/IL-22: genomic organization and mapping of the human and mouse genes. *Genes and Immunity*, *1*(8), 488–494. https://doi.org/10.1038/sj.gene.6363716

Edfeldt, K., Liu, P. T., Chun, R., Fabri, M., Schenk, M., Wheelwright, M., … Modlin, R. L. (2010). T-cell cytokines differentially control human monocyte antimicrobial responses by regulating vitamin D metabolism. *Proceedings of the National Academy of Sciences of the*

United States of America, *107*(52), 22593–22598. https://doi.org/10.1073/pnas.1011624108

Fabri, M., Stenger, S., Shin, D. M., Yuk, J. M., Liu, P. T., Realegeno, S., … Modlin, R. L. (2011). Vitamin D Is Required for IFN-gamma-Mediated Antimicrobial Activity of Human Macrophages. *Science Translational Medicine*, *3*(104), 104ra102. https://doi.org/ARTN 104ra102DOI 10.1126/scitranslmed.3003045

Freches, D., Korf, H., Denis, O., Havaux, X., Huygen, K., & Romano, M. (2013). Mice genetically inactivated in interleukin-17A receptor are defective in long-term control of Mycobacterium tuberculosis infection. *Immunology*, *140*(2), 220–231. https://doi.org/10.1111/imm.12130

Frehel, C., & Rastogi, N. (1987). Mycobacterium leprae surface components intervene in the early phagosome-lysosome fusion inhibition event. *Infection and Immunity*, *55*(12), 2916–2921.

Ganz, T. (2003). Defensins: antimicrobial peptides of innate immunity. *Nature Reviews: Immunology*, *3*(9), 710–720. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12949495

Gerosa, F., Baldani-Guerra, B., Lyakh, L. A., Batoni, G., Esin, S., Winkler-Pickett, R. T., … Trinchieri, G. (2008). Differential regulation of interleukin 12 and interleukin 23 production in human dendritic cells. *Journal of Experimental Medicine*, *205*(6), 1447–1461. https://doi.org/10.1084/jem.20071450

Guerra-Laso, J. M., Raposo-Garcia, S., Garcia-Garcia, S., Diez-Tascon, C., & Rivero-Lezcano, O. M. (2015). Microarray analysis of Mycobacterium tuberculosis-infected monocytes reveals IL26 as a new candidate gene for tuberculosis susceptibility. *Immunology*, *144*(2), 291–301. https://doi.org/10.1111/imm.12371

Hagge, D. A., Ray, N. A., Krahenbuhl, J. L., & Adams, L. B. (2004). An in vitro model for the lepromatous leprosy granuloma: fate of Mycobacterium leprae from target macrophages after interaction with normal and activated effector macrophages. *Journal of Immunology*, *172*(12), 7771–7779.

Hor, S., Pirzer, H., Dumoutier, L., Bauer, F., Wittmann, S., Sticht, H., … Fickenscher, H. (2004). The T-cell lymphokine interleukin-26 targets epithelial cells through the interleukin-20 receptor 1 and interleukin-10 receptor 2 chains. *Journal of Biological Chemistry*, *279*(32), 33343–33351. https://doi.org/10.1074/jbc.M405000200

Hughes, T., Becknell, B., McClory, S., Briercheck, E., Freud, A. G., Zhang, X., … Caligiuri, M. A. (2009). Stage 3 immature human natural killer cells found in secondary lymphoid tissue constitutively and selectively express the TH 17 cytokine interleukin-22. *Blood*, *113*(17), 4008–4010. https://doi.org/10.1182/blood-2008-12-192443

Job, C. K., Jayakumar, J., & Aschhoff, M. (1999). “Large numbers” of Mycobacterium leprae are discharged from the intact skin of lepromatous patients; a preliminary report. *International Journal of Leprosy and Other Mycobacterial Diseases*, *67*(2), 164–167.

Jubrail, J., Morris, P., Bewley, M. A., Stoneham, S., Johnston, S. A., Foster, S. J., … Dockrell, D. H. (2016). Inability to sustain intraphagolysosomal killing of Staphylococcus aureus predisposes to bacterial persistence in macrophages. *Cellular Microbiology*, *18*(1), 80–96. https://doi.org/10.1111/cmi.12485

Khader, S. A., Bell, G. K., Pearl, J. E., Fountain, J. J., Rangel-Moreno, J., Cilley, G. E., … Cooper, A. M. (2007). IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during Mycobacterium tuberculosis challenge. *Nature Immunology*, *8*(4), 369–377. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17351619

Khader, S. A., Pearl, J. E., Sakamoto, K., Gilmartin, L., Bell, G. K., Jelley-Gibbs, D. M., … Cooper, A. M. (2005). IL-23 compensates for the absence of IL-12p70 and is essential for

the IL-17 response during tuberculosis but is dispensable for protection and antigen-specific IFN-gamma responses if IL-12p70 is available. *Journal of Immunology*, *175*(2), 788–795. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16002675

Knappe, A., Hor, S., Wittmann, S., & Fickenscher, H. (2000). Induction of a novel cellular homolog of interleukin-10, AK155, by transformation of T lymphocytes with herpesvirus saimiri. *Journal of Virology*, *74*(8), 3881–3887. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10729163

Kotenko, S. V. (2002). The family of IL-10-related cytokines and their receptors: related, but to what extent? *Cytokine and Growth Factor Reviews*, *13*(3), 223–240.

Krutzik, S. R., Ochoa, M. T., Sieling, P. A., Uematsu, S., Ng, Y. W., Legaspi, A., … Modlin, R. L. (2003). Activation and regulation of Toll-like receptors 2 and 1 in human leprosy. *Nature Medicine*, *9*(5), 525–532. https://doi.org/10.1038/nm864

Lahiri, R., Randhawa, B., & Krahenbuhl, J. (2005). Application of a viability-staining method for Mycobacterium leprae derived from the athymic (nu/nu) mouse foot pad. *Journal of Medical Microbiology*, *54*(Pt 3), 235–242. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15713606

Liu, P. T., Wheelwright, M., Teles, R., Komisopoulou, E., Edfeldt, K., Ferguson, B., … Modlin, R. L. (2012). MicroRNA-21 targets the vitamin D-dependent antimicrobial pathway in leprosy. *Nature Medicine*, *18*(2), 267–273. https://doi.org/10.1038/nm.2584

Ma, C. S., Chew, G. Y., Simpson, N., Priyadarshi, A., Wong, M., Grimbacher, B., … Cook, M. C. (2008). Deficiency of Th17 cells in hyper IgE syndrome due to mutations in STAT3. *Journal of Experimental Medicine*, *205*(7), 1551–1557. https://doi.org/10.1084/jem.20080218

Maertzdorf, J., Weiner III, J., Mollenkopf, H. J., Network, T., Bauer, T., Prasse, A., … Kaufmann, S. H. (2012). Common patterns and disease-related signatures in tuberculosis and sarcoidosis. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(20), 7853–7858. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22547807

Martinez, A. N., Lahiri, R., Pittman, T. L., Scollard, D., Truman, R., Moraes, M. O., & Williams, D. L. (2009). Molecular determination of Mycobacterium leprae viability by use of real-time PCR. *Journal of Clinical Microbiology*, *47*(7), 2124–2130. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19439537

Meller, S., Di Domizio, J., Voo, K. S., Friedrich, H. C., Chamilos, G., Ganguly, D., … Gilliet, M. (2015). T(H)17 cells promote microbial killing and innate immune sensing of DNA via interleukin 26. *Nature Immunology*, *16*(9), 970–979. https://doi.org/10.1038/ni.3211

Miot, C., Beaumont, E., Duluc, D., Le Guillou-Guillemette, H., Preisser, L., Garo, E., … Jeannin, P. (2015). IL-26 is overexpressed in chronically HCV-infected patients and enhances TRAIL-mediated cytotoxicity and interferon production by human NK cells. *Gut*, *64*(9), 1466–1475. https://doi.org/10.1136/gutjnl-2013-306604

Montoya, D., Cruz, D., Teles, R. M., Lee, D. J., Ochoa, M. T., Krutzik, S. R., … Modlin, R. L. (2009). Divergence of macrophage phagocytic and antimicrobial programs in leprosy. *Cell Host Microbe*, *6*(4), 343–353. https://doi.org/10.1016/j.chom.2009.09.002

Nilsson, I. M., Hartford, O., Foster, T., & Tarkowski, A. (1999). Alpha-toxin and gamma-toxin jointly promote Staphylococcus aureus virulence in murine septic arthritis. *Infection and Immunity*, *67*(3), 1045–1049.

Ochoa, M. T., Stenger, S., Sieling, P. A., Thoma-Uszynski, S., Sabet, S., Cho, S. G., … Modlin, R. L. (2001). T-cell release of granulysin contributes to host defense in leprosy. *Nature Medicine*, *7*(2), 174–179. https://doi.org/10.1038/84620

Pene, J., Chevalier, S., Preisser, L., Venereau, E., Guilleux, M. H., Ghannam, S., … Gascan, H. (2008). Chronically inflamed human tissues are infiltrated by highly differentiated Th17 lymphocytes. *Journal of Immunology*, *180*(11), 7423–7430. Retrieved from

http://www.ncbi.nlm.nih.gov/pubmed/18490742
Poli, C., Augusto, J. F., Dauve, J., Adam, C., Preisser, L., Larochette, V., … Jeannin, P. (2017). IL-26 Confers Proinflammatory Properties to Extracellular DNA. *Journal of Immunology*, *198*(9), 3650–3661. https://doi.org/10.4049/jimmunol.1600594
Ridley, D. S. (1971). Pathology and bacteriology of early lesions in leprosy. *International Journal of Leprosy and Other Mycobacterial Diseases*, *39*(2), 216–224.
Ridley, D. S., & Jopling, W. H. (1966). Classification of leprosy according to immunity. A five-group system. *Int J Lepr*, *34*, 255–273.
Ridley, M. J. (1981). The mononuclear cell series in leprosy: an ultrastructural report. *Leprosy Review*, *52*, 35–50.
Saini, C., Ramesh, V., & Nath, I. (2013). CD4+ Th17 cells discriminate clinical types and constitute a third subset of non Th1, Non Th2 T cells in human leprosy. *PLoS Neglected Tropical Diseases*, *7*(7), e2338. https://doi.org/10.1371/journal.pntd.0002338
Schenk, M., Krutzik, S. R., Sieling, P. A., Lee, D. J., Teles, R. M., Ochoa, M. T., … Modlin, R. L. (2012). NOD2 triggers an interleukin-32-dependent human dendritic cell program in leprosy. *Nature Medicine*, *18*(4), 555–563. https://doi.org/10.1038/nm.2650
Schindler, D., Gutierrez, M. G., Beineke, A., Rauter, Y., Rohde, M., Foster, S., … Medina, E. (2012). Dendritic cells are central coordinators of the host immune response to Staphylococcus aureus bloodstream infection. *American Journal of Pathology*, *181*(4), 1327–1337. https://doi.org/10.1016/j.ajpath.2012.06.039
Schnaith, A., Kashkar, H., Leggio, S. A., Addicks, K., Kronke, M., & Krut, O. (2007). Staphylococcus aureus subvert autophagy for induction of caspase-independent host cell death. *Journal of Biological Chemistry*, *282*(4), 2695–2706. https://doi.org/10.1074/jbc.M609784200
Sheikh, F., Baurin, V. V, Lewis-Antes, A., Shah, N. K., Smirnov, S. V, Anantha, S., … Kotenko, S. V. (2004). Cutting edge: IL-26 signals through a novel receptor complex composed of IL-20 receptor 1 and IL-10 receptor 2. *Journal of Immunology*, *172*(4), 2006–2010. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14764663
Sibley, L. D., Franzblau, S. G., & Krahenbuhl, J. L. (1987). Intracellular fate of Mycobacterium leprae in normal and activated mouse macrophages. *Infection and Immunity*, *55*(3), 680–685.
Sieling, P. A., Abrams, J. S., Yamamura, M., Salgame, P., Bloom, B. R., Rea, T. H., & Modlin, R. L. (1993). Immunosuppressive roles for interleukin-10 and interleukin-4 in human infection: In vitro modulation of T cell responses in leprosy. *Journal of Immunology*, *150*, 5501–5510.
Silva, B. J., Barbosa, M. G., Andrade, P. R., Ferreira, H., Nery, J. A., Corte-Real, S., … Pinheiro, R. O. (2017). Autophagy Is an Innate Mechanism Associated with Leprosy Polarization. *PLoS Pathogens*, *13*(1), e1006103. https://doi.org/10.1371/journal.ppat.1006103
Stenger, S., Rosat, J. P., Bloom, B. R., Krensky, A. M., & Modlin, R. L. (1999). Granulysin: a lethal weapon of cytolytic T cells. *Immunology Today*, *20*(9), 390–394. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10462738
Stephen-Victor, E., Fickenscher, H., & Bayry, J. (2016). IL-26: An Emerging Proinflammatory Member of the IL-10 Cytokine Family with Multifaceted Actions in Antiviral, Antimicrobial, and Autoimmune Responses. *PLoS Pathogens*, *12*(6), e1005624. https://doi.org/10.1371/journal.ppat.1005624
Tameris, M. D., Hatherill, M., Landry, B. S., Scriba, T. J., Snowden, M. A., Lockhart, S., … McShane, H. (2013). Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. *Lancet*, *381*(9871), 1021–1028. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23391465

Teles, R. M., Graeber, T. G., Krutzik, S. R., Montoya, D., Schenk, M., Lee, D. J., … Modlin, R. L. (2013). Type I interferon suppresses type II interferon-triggered human anti-mycobacterial responses. *Science*, *339*(6126), 1448–1453. https://doi.org/10.1126/science.1233665

Tranchemontagne, Z. R., Camire, R. B., O'Donnell, V. J., Baugh, J., & Burkholder, K. M. (2015). Staphylococcus aureus Strain USA300 Perturbs Acquisition of Lysosomal Enzymes and Requires Phagosomal Acidification for Survival inside Macrophages. *Infection and Immunity*, *84*(1), 241–253. https://doi.org/10.1128/iai.00704-15

Tuominen, V. J., Ruotoistenmaki, S., Viitanen, A., Jumppanen, M., & Isola, J. (2010). ImmunoRatio: a publicly available web application for quantitative image analysis of estrogen receptor (ER), progesterone receptor (PR), and Ki-67. *Breast Cancer Research*, *12*(4), R56. https://doi.org/10.1186/bcr2615

Umemura, M., Yahagi, A., Hamada, S., Begum, M. D., Watanabe, H., Kawakami, K., … Matsuzaki, G. (2007). IL-17-mediated regulation of innate and acquired immune response against pulmonary Mycobacterium bovis bacille Calmette-Guerin infection. *Journal of Immunology*, *178*(6), 3786–3796. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17339477

van Voorhis, W. C., Kaplan, G., Sarno, E. N., Horwitz, M. A., Steinman, R. M., Levis, W. R., … Cohn, Z. A. (1982). The cutaneous infiltrates of leprosy: cellular characteristics and the predominant T-cell phenotypes. *New England Journal of Medicine*, *307*, 1593–1597.

Walch, M., Latinovic-Golic, S., Velic, A., Sundstrom, H., Dumrese, C., Wagner, C. A., … Ziegler, U. (2007). Perforin enhances the granulysin-induced lysis of Listeria innocua in human dendritic cells. *BMC Immunology*, *8*, 14. https://doi.org/10.1186/1471-2172-8-14

Wang, T., Diaz-Rosales, P., Martin, S. A., & Secombes, C. J. (2010). Cloning of a novel interleukin (IL)-20-like gene in rainbow trout Oncorhynchus mykiss gives an insight into the evolution of the IL-10 family. *Developmental and Comparative Immunology*, *34*(2), 158–167. https://doi.org/10.1016/j.dci.2009.09.003

Watson, R. O., Manzanillo, P. S., & Cox, J. S. (2012). Extracellular M. tuberculosis DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. *Cell*, *150*(4), 803–815. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22901810

Wilgus, T. A., Roy, S., & McDaniel, J. C. (2013). Neutrophils and Wound Repair: Positive Actions and Negative Reactions. *Adv Wound Care (New Rochelle)*, *2*(7), 379–388. https://doi.org/10.1089/wound.2012.0383

Wilson, N. J., Boniface, K., Chan, J. R., McKenzie, B. S., Blumenschein, W. M., Mattson, J. D., … de Waal, M. R. (2007). Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nature Immunology*, *8*(9), 950–957. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17676044

You, W., Tang, Q., Zhang, C., Wu, J., Gu, C., Wu, Z., & Li, X. (2013). IL-26 promotes the proliferation and survival of human gastric cancer cells by regulating the balance of STAT1 and STAT3 activation. *PloS One*, *8*(5), e63588. https://doi.org/10.1371/journal.pone.0063588

Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. *Nature*, *415*(6870), 389–395. https://doi.org/10.1038/415389a

CHAPTER 4

SUMMARY

The goal of the studies presented above was to further understand the mechanisms by which Th17 cells can protect against intracellular bacterial infection, specifically through the production of the antimicrobial protein IL-26. While it was previously held that Th1 cells are the major players in cell mediated immunity against mycobacterial species, it has become clear that Th1 cells cannot act alone to ensure protective immunity (Tameris et al., 2013). The first study presented in Chapter 2, highlights a unique method by which IL-26 can be secreted by Th17 cells. We show that, even in the absence of T cell receptor activation, IL-1β alone can induce secretion of IL-26 from PBMC, memory $CD4^+$ T cells, and Th17 cells that express the IL-1 receptor IL-1RI. The research presented in Chapter 3 demonstrates that IL-26 is antimicrobial against *M. leprae,* both in liquid culture and, more importantly, within infected macrophages. Antimicrobial activity was partially dependent upon the ability of IL-26 to induce autophagy in the macrophage, an important process by which cells can induce lysosomal fusion with phagosomes occupied by mycobacteria.

The ability of Th17 cells to secrete IL-26 in response to IL-1β is a remarkable discovery for a number of reasons. First of all, it demonstrates activation of T cells, which are an important cell type of adaptive immunity, in a manner that does not depend on antigen specificity. One of the hallmarks of adaptive immunity is specificity, and therefore this finding represents a fundamentally incongruous process in the paradigm of acquired immunity (Janeway, Travers, Walport, & Shlomchik, 2001). Essentially, this is a method by which adaptive immune cells can participate in the innate immune response. Non-specific activation of lymphocyte subsets has been shown before, but typically for specialized cell types such as γδ T cells, innate lymphoid

cells, and natural-killer like T cells (Cua & Tato, 2010; Hasegawa et al., 2013; Kawano et al., 1997; Sutton et al., 2009). These cell types, despite deriving from a lymphoid progenitor, typically function during the innate immune response, and can be activated either by cytokines or by specific pathogen associated small molecules recognized by semi-invariant T cell receptors. The novelty of our finding therefore lies partly in the conventionality of the Th17 cells that respond to IL-1β and produce IL-26, given that RNA sequencing revealed diverse αβ T cell receptor expression by these cells. These Th17 cells are therefore not restricted to a specific range of antigens, but rather can theoretically respond to a variety of antigens as well as non-specifically to IL-1β. Furthermore, the secretion of IL-26 by conventional T cells in response to IL-1β is also notable due to the direct antimicrobial ability of IL-26. Activation of γδ T cells, innate lymphoid cells, and natural-killer like T cells leads to the secretion of inflammatory and immunomodulatory cytokines such as IFN-γ, IL-4, IL-23, and IL-1β, none of which can directly inhibit bacterial growth. In addition, IL-8 secretion in response to IL-1β may also give rise to antimicrobial peptides, given that the IL-8 protein is acid hydrolysable into antimicrobial peptide fragments (Bjorstad, Fu, Karlsson, Dahlgren, & Bylund, 2005). Additionally, our work is notable for revealing that IL-1β triggers $CD4^+$ memory T cells to secrete IL-26 faster than TCR triggering, even with high concentrations of anti-CD3/CD28 antibodies that lead to similar amounts of IL-26 secretion at later time points. Thus, not only could mucosal resident T cells respond nonspecifically to activation with IL-1β, but also recruited antigen specific T cells could secrete an initial fast IL-26 even as their T cell receptor signaling response is unfolding. Lastly, we defined an antimicrobial subset of Th17 cells that express $IL\text{-}1RI^+$, which was necessary for IL-26 secretion by the cells. While IL-1β did not induce secretion of other Th17 cytokines from these cells, it did induce transcription of those cytokines and many other typical Th17 genes. IL-1β has been shown to enhance anti-CD3/CD28 induced IL-17A secretion, perhaps as a result of this enhanced cytokine transcription (Lee et al., 2010). These $IL\text{-}1RI^+$ Th17 cells are therefore

able to produce antimicrobial markers. Therefore, we present a novel, rapid, and antimicrobial pathway for Th17 activation and IL-26 secretion.

Having characterized a novel method by which IL-26 could be released from Th17 cells, and in light of evidence that Th17 cells are differentially represented in the spectrum of leprosy disease, we next investigated the role of IL-26 specifically in host defense against leprosy. Our finding is unique in the fact that it establishes an antimicrobial role for IL-26 against intracellular bacteria. We demonstrated that IL-26 was directly cytotoxic against *M. leprae* and *M. tuberculosis* H37Ra in axenic culture and, importantly, that IL-26 treatment of infected macrophages led to reduced bacterial viability. We were able to demonstrate that IL-26 colocalizes with *M. leprae* and the lysosomal marker LAMP-1 in MDM, indicating its ability to access the intracellular compartments in which *M. leprae* reside. Furthermore, we determined that IL-26 enhanced the fusion of lysosomes with phagosomes, as well as induced STING-dependent autophagy within MDM. Notably, we reveal that IL-26 is more strongly expressed in tuberculoid leprosy lesions vs lepromatous leprosy lesions, lending *in vivo* support to the idea that IL-26 is playing an active role in host defense against *M. leprae*.

There remain many more questions to be answered regarding the projects presented in the body of this dissertation. Regarding the IL-1RI$^+$ Th17 cells, we have not yet demonstrated whether or not these cells are directly present at the site of infection in leprosy lesions. While Th17 cells are present in leprosy granulomas, we found that IL-1RI$^+$ Th17 cells represented only a small percentage of all Th17 cells. However, after 9 hours of stimulation with IL-1β or anti-CD3/CD28 we also demonstrated that the IL-1RI$^+$ Th17 cells were the only Th17 cells capable of secreting measurable amounts of IL-26. While we cannot exclude the possibility of other T cell or non-T cell sources of IL-26 in the leprosy lesions, we believe it is a distinct possibility that the some of the abundant IL-26 detected in tuberculoid leprosy lesions is derived from IL-1RI$^+$ Th17 cells. The origin of these cells is also not completely understood, especially given the fact

that they express regulatory T cell markers. Additionally, whether IL-1RI expression by Th17 cells is stable or not is unknown. Th17 cells are known to exhibit remarkable plasticity and change their cytokine secretion pattern, in some cases even ceasing to produce IL-17 (Annunziato et al., 2007; Cosmi et al., 2011, 2010; Maggi et al., 2014). Further experiments in the lab are necessary to answer these questions.

Many gaps in knowledge remain regarding the antimicrobial effects of IL-26 in infected macrophages as well. For example, the mechanism by which IL-26 enters the infected cell is unknown. It has been speculated that the structure of IL-26 has common features with an engineered DNA delivery peptide called KALA, including large positive area, two amphipathic helices, and an IPM anchor (Poli et al., 2017). It is proposed that KALA, and other similar cell permeable peptides, are mainly taken up by cells via endocytosis (Copolovici, Langel, Eriste, & Langel, 2014). Alternative explanations could be that activation of the IL-26 receptor or TLR9 by IL-26-DNA complexes triggers uptake of IL-26 by the cells. Additionally, we find that IL-26 induced autophagy was STING dependent, however the exact mechanism of STING activation as a result of IL-26 treatment is unclear. Typically, cytosolic DNA is sensed through the enzyme cGAS, which catalyzes the production of cGAMP, which subsequently activates STING (Collins et al., 2015; Mankan et al., 2014). Whether IL-26-DNA complexes activate cGAS is unknown, nor is how IL-26 binding affects the recognition of DNA by cGAS. STING activation can also proceed independently of cGAS by associating with the DNA binding protein IFI6, although this has only been shown in the context of nuclear DNA damage (Dunphy et al., 2018).

The elucidation of novel determinants of effective cell mediated immunity against mycobacteria is essential for the development of potential therapeutic and preventative medicines. The current multi-drug treatment regimen is very successful for *M. leprae*, while in the case of *M. tuberculosis* there is a growing issue of drug resistance. In order to prevent further drug resistance and spread of mycobacterial diseases, an effective vaccine must be

developed. While many have attempted to make a tuberculosis vaccine, none so far have made it through clinical trials for FDA approval (Zhu, Dockrell, Ottenhoff, Evans, & Zhang, 2018). A well made vaccine should induce the cells and molecules necessary for protective immunity against mycobacteria, which the work presented above helps demonstrate that Th17 cells and IL-26 may play a key role in host defense against mycobacterial disease.

References

Annunziato, F., Cosmi, L., Santarlasci, V., Maggi, L., Liotta, F., Mazzinghi, B., … Romagnani, S. (2007). Phenotypic and functional features of human Th17 cells. *Journal of Experimental Medicine*, *204*(8), 1849–1861. https://doi.org/10.1084/jem.20070663

Bjorstad, A., Fu, H., Karlsson, A., Dahlgren, C., & Bylund, J. (2005). Interleukin-8-derived peptide has antibacterial activity. *Antimicrobial Agents and Chemotherapy*, *49*(9), 3889–3895. https://doi.org/10.1128/aac.49.9.3889-3895.2005

Collins, A. C., Cai, H., Li, T., Franco, L. H., Li, X. D., Nair, V. R., … Shiloh, M. U. (2015). Cyclic GMP-AMP Synthase Is an Innate Immune DNA Sensor for Mycobacterium tuberculosis. *Cell Host Microbe*, *17*(6), 820–828. https://doi.org/10.1016/j.chom.2015.05.005

Copolovici, D. M., Langel, K., Eriste, E., & Langel, Ü. (2014). Cell-Penetrating Peptides: Design, Synthesis, and Applications. *ACS Nano*, *8*(3), 1972–1994. https://doi.org/10.1021/nn4057269

Cosmi, L., Cimaz, R., Maggi, L., Santarlasci, V., Capone, M., Borriello, F., … Annunziato, F. (2011). Evidence of the transient nature of the Th17 phenotype of CD4+CD161+ T cells in the synovial fluid of patients with juvenile idiopathic arthritis. *Arthritis & Rheumatism*, *63*(8), 2504–2515. https://doi.org/10.1002/art.30332

Cosmi, L., Maggi, L., Santarlasci, V., Capone, M., Cardilicchia, E., Frosali, F., … Annunziato, F. (2010). Identification of a novel subset of human circulating memory CD4+ T cells that produce both IL-17A and IL-4. *Journal of Allergy and Clinical Immunology*, *125*(1), 222-230.e4. https://doi.org/10.1016/J.JACI.2009.10.012

Cua, D. J., & Tato, C. M. (2010). Innate IL-17-producing cells: the sentinels of the immune system. *Nature Reviews: Immunology*, *10*(7), 479–489. https://doi.org/10.1038/nri2800

Dunphy, G., Flannery, S. M., Almine, J. F., Connolly, D. J., Paulus, C., Jønsson, K. L., … Unterholzner, L. (2018). Non-canonical Activation of the DNA Sensing Adaptor STING by ATM and IFI16 Mediates NF-κB Signaling after Nuclear DNA Damage. *Molecular Cell*, *71*(5), 745-760.e5. https://doi.org/10.1016/j.molcel.2018.07.034

Hasegawa, E., Sonoda, K. H., Shichita, T., Morita, R., Sekiya, T., Kimura, A., … Yoshimura, A. (2013). IL-23-independent induction of IL-17 from gammadeltaT cells and innate lymphoid cells promotes experimental intraocular neovascularization. *Journal of Immunology*, *190*(4), 1778–1787. https://doi.org/10.4049/jimmunol.1202495

Janeway, C. A., Travers, P., Walport, M., & Shlomchik, M. J. (2001). Adaptive immunity to infection. In *Immunobiology 5 : the immune system in health and disease*.

Kawano, T., Cui, J., Koezuka, Y., Toura, I., Kaneko, Y., Motoki, K., … Taniguchi, M. (1997). CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides. *Science*, *278*, 1626–1629.

Lee, W. W., Kang, S. W., Choi, J., Lee, S. H., Shah, K., Eynon, E. E., … Kang, I. (2010).

Regulating human Th17 cells via differential expression of IL-1 receptor. *Blood*, *115*(3), 530–540. https://doi.org/10.1182/blood-2009-08-236521

Maggi, L., Cimaz, R., Capone, M., Santarlasci, V., Querci, V., Simonini, G., … Cosmi, L. (2014). Brief Report: Etanercept Inhibits the Tumor Necrosis Factor α-Driven Shift of Th17 Lymphocytes Toward a Nonclassic Th1 Phenotype in Juvenile Idiopathic Arthritis. *Arthritis & Rheumatology*, *66*(5), 1372–1377. https://doi.org/10.1002/art.38355

Mankan, A. K., Schmidt, T., Chauhan, D., Goldeck, M., Honing, K., Gaidt, M., … Hornung, V. (2014). Cytosolic RNA:DNA hybrids activate the cGAS-STING axis. *EMBO Journal*, *33*(24), 2937–2946. https://doi.org/10.15252/embj.201488726

Poli, C., Augusto, J. F., Dauve, J., Adam, C., Preisser, L., Larochette, V., … Jeannin, P. (2017). IL-26 Confers Proinflammatory Properties to Extracellular DNA. *Journal of Immunology*, *198*(9), 3650–3661. https://doi.org/10.4049/jimmunol.1600594

Sutton, C. E., Lalor, S. J., Sweeney, C. M., Brereton, C. F., Lavelle, E. C., & Mills, K. H. (2009). Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity*, *31*(2), 331–341. https://doi.org/10.1016/j.immuni.2009.08.001

Tameris, M. D., Hatherill, M., Landry, B. S., Scriba, T. J., Snowden, M. A., Lockhart, S., … McShane, H. (2013). Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. *Lancet*, *381*(9871), 1021–1028. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23391465

Zhu, B., Dockrell, H. M., Ottenhoff, T. H. M., Evans, T. G., & Zhang, Y. (2018). Tuberculosis vaccines: Opportunities and challenges. *Respirology*, *23*(4), 359–368. https://doi.org/10.1111/resp.13245