Published in final edited form as:

Curr Opin Immunol. 2007 August ; 19(4): 441–447.

Interleukin-12 and tuberculosis: an old story revisited

Andrea M Cooper¹, Alejandra Solache², and Shabaana A. Khader¹

¹The Trudeau Institute, Inc. 154 Algonquin Ave. Saranac Lake, NY 12983

²Millipore, 28820 Single Oak Drive, Temecula, CA 92590

Summary

Our understanding of the role of interleukin (IL)-12 in controlling tuberculosis has expanded due to increased interest in other members of the IL-12 family of cytokines. Recent data show that IL-12, IL-23 and IL-27 have specific roles in the initiation, expansion and control of the cellular response to tuberculosis. Specifically, IL-12, and to a lesser degree IL-23, generate protective cellular responses and promote survival whereas IL-27 moderates the inflammatory response and is required for long-term survival. Paradoxically, IL-27 also limits bacterial control suggesting that a balance between bacterial killing and tissue damage is required for survival. Understanding the balance between IL-12, IL-23 and IL-27 is crucial to development of immune intervention in tuberculosis.

Introduction

The chronic unresolved nature of mycobacterial infection is unusual in disease models and provides a tool with which to dissect the requirements for specific cytokines in initiation, maintenance and control of the cellular immune response. In this review, recent data supporting the importance of IL-12 in controlling human disease will be discussed. This will be followed by discussion of the relative roles of IL-12, IL-23 and IL-27 in the primary, secondary and chronic cellular response to mycobacterial infection.

Recent developments have been made regarding the role of the IL-12 family of cytokines in tuberculosis. Increased interest in these cytokines stems from the fact that absence of IL-12p40 or the IL-12Rβ1 has a greater impact on the incidence of tuberculosis than has the absence of IL-12p35 or IL-12Rβ2 [1,2]. This has led to more intense examination of the role of IL-12p40 dependent cytokines and other IL-12 family cytokines such as IL-27 in the response to mycobacterial infection. This examination will allow for improved vaccine design and immunotherapeutic intervention and also improve understanding of the immune response to other lung pathogens.

The cytokines to be discussed here are: IL-12p40 as a homodimer (IL-12(p40)₂), IL-12p70 (IL-12p40 covalently linked with IL-12p35 subunit (IL-12)), IL-23 (IL-12p40 subunit covalently linked to the IL-23p19 subunit)[3] and IL-27 (a non-covalently linked heterodimer of IL-27p28 and Epstein-Barr virus induced gene 3 (EBI3) [4]. For receptor signaling IL-12 uses an IL-12Rβ1/IL-12Rβ2 complex, IL-23 uses an IL-12Rβ1/IL-23R complex while IL-27 uses a gp130/IL-27Rα complex [3,4].

Correspondence to: Andrea M Cooper.

Email: acooper@trudeauinstitute.org, asolache@chemicon.com, akshabaana@trudeauinstitute.org

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Importance of IL-12 in immunity to Mycobacterial infection in humans

The importance of studying the IL-12 family of cytokines is highlighted by the ongoing discovery of IL-12p40 deficiencies that predispose the host to tuberculosis (Table I). Patients with Mendelian susceptibility to mycobacterial disease (MSMD) are generally healthy but suffer from disease caused by low virulence mycobacteria as well as tuberculosis [2]. Although MSMD is most frequently a result of complete IL-12R β 1, IFN- γ R1 and IL-12p40 deficiency [2], there are potentially other pathways involved [5]. While patients with deficiencies can be treated, this is not always successful [6]. However, recent gene therapy experiments demonstrating reconstitution of IL-12 responsiveness and IFN- γ production in T cells following gene transfer show some promise for future treatment [7].

Recent studies have identified increased susceptibility of individuals with x-linked associated mutations in the NF- κ B essential modulator (NEMO) gene [8]. NEMO is a regulatory subunit of I κ B kinase (IKK) complex that activates the NF- κ B signaling pathway and is important for IL-12 and IFN- γ production following BCG stimulation [9]. Patients with NEMO mutations have selectively impaired CD40-triggered and NF- κ B/c-Rel-mediated induction of IL-12 in mononuclear cells [8]. In addition to MSMD, a recent population screen using single nucleotide polymorphisms (SNP) has identified a SNP associated with increased susceptibility to tuberculosis in both Mexican and Korean populations [10]. Individuals with a GG SNP genotype in the *CCL2* (monocyte chemoattractant protein 1, MCP-1) gene had a 5 fold higher odds of developing tuberculosis than those with the AA genotype. GG patients had higher CCL2 and lower IL-12p40 in their serum and stimulation of GG monocytes with *M. tuberculosis* resulted in higher CCL2 and lower IL-12p40 than in monocytes from AA genotypes. Blocking CCL2 in GG monocytes improved IL-12p40 production and adding MCP-1 to AA monocytes reduced IL-12p40 [10].

Factors impacting IL-12p40 induction

While the human studies are naturally restricted we can more thoroughly investigate the pathways by which mycobacteria induce IL-12p40 using murine models. In this respect the role of Toll-like receptors (TLR) has been studied extensively prompted by the observation that mice lacking the major adaptor protein MyD88, required for TLR signaling, are highly susceptible to *M. tuberculosis* infection [11,12]. While a single TLR has not been shown to be required for resistance a recent study has demonstrated the requirement for TLR9/TLR2 in resistance to *M. tuberculosis* infection [13]. Importantly, while TLR 9 was required for IL-12p40 production in vivo, both TLR9 and TLR2 mediated IL-12p40 production in vitro. While combined TLR9/TLR2 deficiency increased susceptibility to low dose infection, TLR9 deficiency alone was sufficient to increase susceptibility in high dose challenge [13] suggesting that TLR2 cannot compensate when infection is more profound.

It is intriguing to note that while *M. tuberculosis* lipoarabinomannans negatively regulate TLR dependent IL-12p40 production in macrophages [14], the mycobacterial TLR2 agonist, LprA initiates IL-12p40 production [15]. This suggests that there is a balance between induction and inhibition of IL-12p40 production by infected cells. Also to be considered is the possibility that cells other than mononuclear cells can make IL-12p40 as shown recently for mast cells [16].

IL-12p40 and initiation of response

The importance of IL-12p40 is clear from human studies however mechanism is more difficult to define. In this respect recent murine studies have been informative and the relative roles of IL-12 family cytokines in controlling mycobacterial infection have been better defined.

A surprising development has been the identification of IL-12p40 as an agonist for the immune response [17]. Firstly, delivery of IL-12(p40)₂ resulted in restoration of delayed type hypersensitivity responses in IL-12p40 deficient mice infected with BCG [18]. Further investigation showed that chemokine responsiveness and ability of lung DCs to prime naïve T cells were defective in the absence of IL-12p40 and that this response could be restored by IL-12(p40)₂ [19]. This novel ability of by IL-12(p40)₂ to overcome natural resistance of lung to initiation of cellular responses appears related to the relative levels of IL-10 and IL-12p40 induced by mycobacteria as these cytokine cross regulate each other during mycobacterial infection [19](see figure 1). The importance of IL-12p40 in mediating DC migration was confirmed recently by the fact that only IL-12p40 producing DC migrated to the lymph node following subcutaneous delivery of antigen [20].

IL-12 family cytokines in cellular response to mycobacteria

Discovery of IL-23 prompted study of this cytokine in humans and animal models [1,2, 21-24]. These studies, while failing to show a significant role for IL-23 in protection, served to define the relative role of IL-12 and IL-23 in the initiation and maintenance of acquired cellular responses to tuberculosis.

IL-12p40 but not IL-12p35 is absolutely required for the protective IFN- γ responses to *M. tuberculosis* [1] with IL-23 promoting the IFN- γ response in the absence of IL-12p35 [21]. However, this supplementary response is not sufficient for prolonged survival and IL-12p35 deficient mice succumb to infection [1]. IL-12 is needed for prolonged IFN- γ cellular responses [25] and while IL-12 can reconstitute protection against tuberculosis in IL-12p40^{-/-} mice, this is lost when IL-12 is removed [26]. The ability of transferred cells to protect Rag but not IL-12p40^{-/-}Rag mice further supports a need for this cytokine [26]. The failure of humans with IL-12R β 1 deficiency to maintain a Th1 effector memory population suggests that IL-12 is also required to maintain this population in humans [27]. In contrast to IL-12 deficient mice, mice lacking IL-23p19 or mice treated with blocking IL-23 antibody are protected against primary mycobacterial infection [21,23]. Specifically, numbers of Th1, bacterial burden and short-term survival are not compromised [21]. In contrast, the antigen-specific Th17 population is ablated as is the IL-17 mRNA expression in the lung [21,23]. Regardless, delivery of adenovirus expressing IL-23 to WT mice immediately prior to and during infection results in reduced bacterial burden and increased cellular responses [24]. This ability to improve responses suggests that induction of IL-23 following natural pulmonary infection is not optimal. It may therefore be considered a potential therapeutic. Alternatively, limited IL-23 production may be a mechanism whereby complications of chronic inflammation are limited, as IL-23 production is required for sustained symptoms in the murine model of Multiple Sclerosis that utilizes mycobacterial antigen for initiation of disease [28].

In stark contrast to the susceptibility of IL-12 deficient mice, in two studies wherein mice were deficient in IL-27 receptor (IL-27R) function due to genetic manipulation of the IL-27R gene (TCCR-KO or WSX-1-KO) showed increased ability to control bacterial burden. While in the WSX-1-KO model, improved bacterial control was linked to increased cellular responses [29], in the TCCR-KO there was a reduction in the amount of IFN- γ per antigen-specific cell within the lung and increased lymphocyte accumulation within lung granulomata [30]. These observations underline the pleiotropic nature of IL-27 and further definition of the role of this cytokine is required.

IL-17 producing cells in mycobacterial disease

The induction of IL-17 cells, while not required for protection, is significant during mycobacterial infection [21,31,32]. *M. tuberculosis* infection induces an antigen-specific IL-17 MHC class II restricted response (Th17) as well as an IL-17-producing $\gamma\delta$ T cell population

[21,31,32]. Mycobacterially-activated DC stimulate and naïve T cells to become Th17 but a sustained Th17 response requires IL-23 [21,28,33] (see figures 1 and 2).

Initiation of Th17 upon mycobacterial infection are undefined although it is likely that that TGF- β but not IL-23 is required [22,28,34-36]. It is likely that IL-23 acts to promote proliferation in newly activated cells as this is reduced in the absence of IL-23 [36] and increased in its presence [22].

IL-12 and IL-23 in vaccine induced responses

To improve upon current vaccines we must determine what factors impact the expression of vaccine-induced responses. Recently, IL-23 was shown to be required to generate a Th17 memory cell population crucial shown for vaccine-induced protection against tuberculosis [36]. In the absence of this Th17 memory population, vaccine-induced accelerated chemokine and IFN- γ response to challenge was lost along with protection [36]. Identification of this memory population provides a target for novel vaccine strategy.

We know that Th1 cells are required for protection against tuberculosis and thus addition of IL-12, IL-23 and IL-27 to vaccine regimens was thought likely to improve vaccines. IL-12 has been studied most extensively and until recently its effect was transient [25,37]. Recently, IL-12 has been encapsulated within microspheres and delivered with a monophosphoryl lipid A, saponin-containing adjuvant (AS01B) and gave improved protection compared to BCG [38]. This improvement correlated with a stronger Th1 response prior to challenge and associated with the long-term availability of IL-12 [38]. Thus, IL-12 acts to promote improved Th1 memory when present for a prolonged period.

DNA vaccines can encode IL-12, IL-23 and IL-27 which can act locally as adjuvants. In this model both IL-12 and IL-23 induce cell proliferation, Th1 polarization and protection upon vaccination ([22,35,39,40], whereas IL-27 has no effect [35]. IL-12 as an adjuvant with DNA vaccine has recently resulted in improved protection relative to BCG in the cynomolgous monkey model [41]. Recent data using gene-deficient mice demonstrated no role for IL-23 in a systemic challenge model following BCG vaccination [23]. This may reflect a redundant role for this cytokine when a live vaccine rather than subunit vaccine is used or it could reflect the lack of requirement for IL-23/IL-17-mediated induction recruitment of Th1 cells following systemic challenge. This issue needs to be addressed.

IL-12 family cytokines in control of chronic disease

Tuberculosis can cause chronic disease therefore the balance between a protective and destructive cellular response is crucial. We propose here that the balance between IL-12, IL-23 and IL-27 is crucial to the maintenance of protective cellular responses with a minimum of tissue involvement (see figure 2). This hypothesis is prompted by two recent reports that highlight the natural tendency of the host to limit the bactericidal response to tuberculosis. In the first, it was shown that mice lacking the enzyme 5-lipoxygenase (5-LO), required for the generation of lipoxins such as lipoxinA₄, have reduced bacterial burdens, increased survival and reduced early inflammation [42]. This is associated with increased IL-12 in the lung within DC and increased local IFN- γ [42]. Despite this early protection, there is the potential that unrestricted responses which kill 5-LO deficient mice infected with *Toxoplasma gondii* [43] may be the eventual cause of death in *M. tuberculosis* infected mice. The importance of the balance between anti-mycobacterial activity and tissue damage was clearer in mice lacking IL-27R signaling. These mice which control bacterial better than wild type mice [29,30] succumb more rapidly to disease [30]. This susceptibility is associated with greater inflammatory damage in the lungs, along with increased TNF and IL-12p40 and was similar to the response in *Leishmania donovani* infected mice [44].

While it is not clear what mediates the susceptibility the IL-27R deficient mice, it is now known that IL-27 limits Th17 responses [45,46] and this has now been shown for tuberculosis (Khader et al unpublished). As IL-17 is associated with inflammatory responses it is possible that IL-17 modulates the granulomatous response. Further, as $\gamma\delta$ T cells are known to produce IL-17 and also to modulate granuloma formation these cells may provide the mechanism by which IL-17 moderates inflammation in tuberculosis [47]. In the absence of IL-23/IL-17 following aerosol *M. tuberculosis* infection, granuloma size and severity of inflammation are modestly increased compared to wild type mice throughout 140 days of infection; this is in contrast to a modest reduction in the extent of fibrin deposition [21]. Th1 responses inhibit the Th17 response in BCG infected mice [33] and IL-12 limits the Th17 response in tuberculosis [21] thus the balance between these cytokines may be crucial to the control of bacteria while maintaining the integrity of the lung tissue (figure 2).

Conclusions

In conclusion, IL-12p40 remains a cornerstone of resistance to mycobacterial infection in experimental models and human populations. The renewed interest in this cytokine and its associated family members has resulted in greatly increased understanding of the cytokine pathways that regulate induction and maintenance of the protective cellular response to tuberculosis. The question requiring further investigation remains, as always: Why does this pathogen, which induces a strong Th1 response, fail to be eradicated from the host? Determining how the balance between IL-12, IL-23 and IL-27 impacts the balance between bacterial killing and tissue damage provides an avenue with which to address this question.

Acknowledgements

This work was supported by the Trudeau Institute, Inc.; a New York Community Trust-Heiser Fund Fellowship to S.A.K. and National Institutes of Health grants AI46530, AI067723, AG028878 to A.M.C.

References

- Cooper AM, Kipnis A, Turner J, Magram J, Ferrante J, Orme IM. Mice lacking bioactive IL-12 can generate protective, antigen-specific cellular responses to mycobacterial infection only if the IL-12 p40 subunit is present. *J Immunol* 2002;168:1322–1327. [PubMed: 11801672]
- Filipe-Santos O, Bustamante J, Chapgier A, Vogt G, de Beaucoudrey L, Feinberg J, Jouanguy E, Boisson-Dupuis S, Fieschi C, Picard C, et al. Inborn errors of IL-12/23- and IFN-gamma-mediated immunity: molecular, cellular, and clinical features. *Semin Immunol* 2006;18:347–361. [PubMed: 16997570]
- Trinchieri G, Pflanz S, Kastelein R. The IL-12 family of heterodimeric cytokines: new players in the regulation of T cell responses. *Immunity* 2003;19:641–644. [PubMed: 14614851]
- Batten M, Ghilardi N. The biology and therapeutic potential of interleukin 27. *J Mol Med*. 2007;10.1007/s00109-007-0164-7
- Bustamante J, Picard C, Fieschi C, Filipe-Santos O, Feinberg J, Perronne C, Chapgier A, de Beaucoudrey L, Vogt G, Sanlaville D, et al. A novel X-linked recessive form of Mendelian susceptibility to mycobacterial disease. *J Med Genet* 2007;44:e65. [PubMed: 17293536]
- Ulrichs T, Fieschi C, Nevicka E, Hahn H, Brezina M, Kaufmann S, Casanova J, Freceirova K. Variable outcome of experimental interferon-gamma therapy of disseminated *Bacillus Calmette-Guerin* infection in two unrelated interleukin-12Rbeta1-deficient Slovakian children. *Eur J Pediatr* 2005;164:166–172. [PubMed: 15633050]
- Bosticardo M, Witte I, Fieschi C, Novelli F, Casanova J, Candotti F. Retroviral-mediated gene transfer restores IL-12 and IL-23 signaling pathways in T cells from IL-12 receptor beta1-deficient patients. *Mol Ther* 2004;9:895–901. [PubMed: 15194056]
- **8. Filipe-Santos O, Bustamante J, Haverkamp M, Vinolo E, Ku C, Puel A, Frucht D, Christel K, von Bernuth H, Jouanguy E, et al. X-linked susceptibility to mycobacteria is caused by mutations in NEMO impairing CD40-dependent IL-12 production. *J Exp Med* 2006;203:1745–1759. [PubMed:

16818673] This study develops the data from ref 9 and demonstrates the molecular consequences of X-linked recessive MSMD mutations in the leucine zipper domain of the NF- κ B essential modulator (NEMO) gene. It also demonstrates a critical role for T cell- and CD40L-triggered, NEMO/NF κ B/c-Rel-mediated induction of IL-12 in protection against mycobacteria in humans.

9. Feinberg J, Fieschi C, Doffinger R, Feinberg M, Leclerc T, Boisson-Dupuis S, Picard C, Bustamante J, Chapgier A, Filipe-Santos O, et al. *Bacillus Calmette Guérin* triggers the IL-12/IFN- γ axis by an IRAK-4- and NEMO-dependent, non-cognate interaction between monocytes, NK, and T lymphocytes. *Eur J Immunol* 2004;34:3276–3284. [PubMed: 15384045]
- **10. Flores-Villanueva P, Ruiz-Morales J, Song C, Flores L, Jo E, Montano M, Barnes P, Selman M, Granados J. A functional promoter polymorphism in monocyte chemoattractant protein-1 is associated with increased susceptibility to pulmonary tuberculosis. *J Exp Med* 2005;202:1649–1658. [PubMed: 16352737] This paper demonstrates an association of a GG SNP phenotype in the CCL2 gene promoter to a 5 fold higher odds of developing tuberculosis than those with the AA genotype. Further, the GG phenotype patients exhibited higher levels of CCL2 and lower IL-12p40 in their serum suggesting that CCL2 can regulate the levels of IL-12p40 during mycobacterial infection in humans.
11. Scanga C, Bafica A, Feng C, Cheever A, Hieny S, Sher A. MyD88-deficient mice display a profound loss in resistance to *Mycobacterium tuberculosis* associated with partially impaired Th1 cytokine and nitric oxide synthase 2 expression. *Infect Immun* 2004;72:2400–2404. [PubMed: 15039368]
12. Fremont C, Yermeev V, Nicolle D, Jacobs M, Quesniaux V, Ryffel B. Fatal *Mycobacterium tuberculosis* infection despite adaptive immune response in the absence of MyD88. *J Clin Invest* 2004;114:1790–1799. [PubMed: 15599404]
- **13. Bafica A, Scanga C, Feng C, Leifer C, Cheever A, Sher A. TLR9 regulates Th1 responses and cooperates with TLR2 in mediating optimal resistance to *Mycobacterium tuberculosis*. *J Exp Med* 2005;202:1715–1724. [PubMed: 16365150] This paper provides the first direct evidence for a role for TLR9 in the immune response to *M. tuberculosis* pulmonary infection. It demonstrates that mycobacterial DNA triggers the production of IL-12p40 via TLR9 and that TLR2 and TLR9 work in cooperation in the induction of Myd-88 dependent IL-12p40 and mediating optimal resistance to *M. tuberculosis* infection.
14. Pathak S, Basu S, Bhattacharyya A, Pathak S, Kundu M, Basu J. *Mycobacterium tuberculosis* lipoarabinomannan-mediated IRAK-M induction negatively regulates Toll-like receptor-dependent interleukin-12 p40 production in macrophages. *J Biol Chem* 2005;280:42794–42800. [PubMed: 16263713]
15. Pecora N, Gehring A, Canaday D, Boom W, Harding C. *Mycobacterium tuberculosis* LprA is a lipoprotein agonist of TLR2 that regulates innate immunity and APC function. *J Immunol* 2006;177:422–429. [PubMed: 16785538]
16. Nakano N, Nishiyama C, Kanada S, Niwa Y, Shimokawa N, Ushio H, Nishiyama M, Okumura K, Ogawa H. Involvement of mast cells in IL-12/23 p40 production is essential for survival from polymicrobial infections. *Blood*. 2007epub
17. Cooper AM, Khader S. IL-12p40: an inherently agonistic cytokine. *Trends Immunol* 2007;28:33–38. [PubMed: 17126601]
18. Holscher C, Atkinson R, Arendse B, Brown N, Myburgh E, Alber G, Brombacher F. A protective and agonistic function of IL-12p40 in mycobacterial infection. *J Immunol* 2001;167:6957–6966. [PubMed: 11739515]
- **19. Khader S, Partida-Sanchez S, Bell G, Jelley-Gibbs D, Swain S, Pearl J, Ghilardi N, deSauvage F, Lund F, Cooper A. Interleukin 12p40 is required for dendritic cell migration and T cell priming after *Mycobacterium tuberculosis* infection. *J Exp Med* 2006;203:1805–1815. [PubMed: 16818672] This paper demonstrates for the first time a role for the homodimeric form of the interleukin IL-12p40 in the migration of lung DCs to the draining lymph node following activation with *Mycobacterium tuberculosis*.
20. Reinhardt R, Hong S, Kang S, Wang Z, Locksley R. Visualization of IL-12/23p40 in vivo reveals immunostimulatory dendritic cell migrants that promote Th1 differentiation. *J Immunol* 2006;177:1618–1627. [PubMed: 16849470]
- *21. Khader S, Pearl J, Sakamoto K, Gilmartin L, Bell G, Jelley-Gibbs D, Ghilardi N, deSauvage F, Cooper A. 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- γ responses if IL-12p70 is available. *J Immunol* 2005;175:788–795. [PubMed: 16002675] This paper demonstrates for the first time that the IL-12p40-dependent, IL-12p70-independent pathway that mediates induction of IFN- γ and resistance to *M.tuberculosis* pulmonary infection is IL-23-dependent. It further demonstrates that if IL-12p70 is present, then IL-23 is dispensable for primary protection against *M.tuberculosis*.

- *22. Wozniak T, Ryan A, Britton W. Interleukin-23 restores immunity to *Mycobacterium tuberculosis* infection in IL-12p40-deficient mice and is not required for the development of IL-17-secreting T cell responses. *J Immunol* 2006;177:8684–8692. [PubMed: 17142769] This study demonstrates that DNA vaccines expressing either IL-12 or IL-23 can reconstitute proliferation, IFN- γ responses and partially restore protective efficacy in IL-12p40 deficient mice. Further, this study shows that in the absence of IL-12p40 cytokine members there is an expansion of IL-17 producing cells during BCG infection suggesting that IL-23 is not required for the priming of these cells.
23. Chackerian A, Chen S, Brodie S, Mattson J, McClanahan T, Kastelein R, Bowman E. Neutralization or absence of the interleukin-23 pathway does not compromise immunity to mycobacterial infection. *Infect Immun* 2006;74:6092–6099. [PubMed: 16923792]
24. Happel K, Lockhart E, Mason C, Porretta E, Keoshkerian E, Odden A, Nelson S, Ramsay A. Pulmonary interleukin-23 gene delivery increases local T-cell immunity and controls growth of *Mycobacterium tuberculosis* in the lungs. *Infect Immun* 2005;73:5782–5788. [PubMed: 16113296]
25. Stobie L, Gurunathan S, Prussin C, Sacks D, Glaichenhaus N, Wu C-W, Seder R. The role of antigen and IL-12 in sustaining Th1 memory cells *in vivo*: IL-12 is required to maintain memory/effector Th1 cells sufficient to mediate protection to an infectious parasite challenge. *Proc Natl Acad Sci USA* 2000;97:8427–8432. [PubMed: 10890924]
- *26. Feng C, Jankovic D, Kullberg M, Cheever A, Scanga C, Hieny S, Caspar P, Yap G, Sher A. Maintenance of pulmonary Th1 effector function in chronic tuberculosis requires persistent IL-12 production. *J Immunol* 2005;174:4185–4192. [PubMed: 15778379] This study establishes that the continuous presence of IL-12 is required for the maintenance of IFN- γ producing Th1 cells and the control of persistent pulmonary *M.tuberculosis* infection.
27. Cleary A, Tu W, Enright A, Giffon T, Dewaal-Malefyt R, Gutierrez K, Lewis D. Impaired Accumulation and Function of Memory CD4 T Cells in Human IL-12 Receptor beta1 Deficiency. *J Immunol* 2002;170:597–603. [PubMed: 12496448]
- *28. Veldhoen M, Hocking R, Flavell R, Stockinger B. Signals mediated by transforming growth factor- β initiate autoimmune encephalomyelitis, but chronic inflammation is needed to sustain disease. *Nat Immunol* 2006;7:1151–1156. [PubMed: 16998492] This study demonstrates for the first time that mycobacterial antigens stimulate DCs in a way that supports TGF- β -dependent differentiation of Th17 cells without the requirement for exogenous TGF- β . This suggests that the relative amounts of TGF- β or IL-12 induced by mycobacteria will define the differentiation of naïve T cells into a IFN- γ or IL-17 phenotype.
- *29. Holscher C, Holscher A, Ruckerl D, Yoshimoto T, Yoshida H, Mak T, Saris C, Ehlers S. The IL-27 receptor chain WSX-1 differentially regulates antibacterial immunity and survival during experimental tuberculosis. *J Immunol* 2005;174:3534–3544. [PubMed: 15749890] These papers were first to demonstrate the improved control of bacterial growth in the lungs of mice lacking IL-27 receptor signaling.
- *30. Pearl JE, Shabaana AK, Solache A, Gilmartin L, Ghilardi N, deSavage F, Cooper AM. IL-27 signaling compromises control of bacterial growth in mycobacteria-infected mice. *J Immunol* 2004;173:7490–7496. [PubMed: 15585875] These papers were first to demonstrate the improved control of bacterial growth in the lungs of mice lacking IL-27 receptor signaling.
31. Umemura M, Yahagi A, Hamada S, Begum M, Watanabe H, Kawakami K, Suda T, Sudo K, Nakae S, Iwakura Y, et al. IL-17-mediated regulation of innate and acquired immune response against pulmonary *Mycobacterium bovis* Bacille Calmette-Guerin infection. *J Immunol* 2007;178:3786–3796. [PubMed: 17339477]
32. Lockhart E, Green A, Flynn J. IL-17 production is dominated by $\gamma\delta$ T cells rather than CD4 T cells during *Mycobacterium tuberculosis* infection. *J Immunol* 2006;177:4662–4669. [PubMed: 16982905]

- *33. Cruz A, Khader S, Torrado E, Fraga A, Pearl J, Pedrosa J, Cooper A, Castro A. CE:IFN- γ regulates the induction and expansion of IL-17-producing CD4 T cells during mycobacterial infection. *J Immunol* 2006;177:1416–1420. [PubMed: 16849446] This paper provides evidence that IFN- γ , induced during mycobacterial infection serves to limit the IL-17 response and that this counter-regulation may be critical for limiting the immune-mediated pathology associated with mycobacterial diseases.
34. Veldhoen M, Hocking R, Atkins C, Locksley R, Stockinger B. TGF β in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 2006;24:179–189. [PubMed: 16473830]
- *35. Wozniak T, Ryan A, Triccas J, Britton W. Plasmid interleukin-23 (IL-23), but not plasmid IL-27, enhances the protective efficacy of a DNA vaccine against *Mycobacterium tuberculosis* infection. *Infect Immun* 2006;74:557–565. [PubMed: 16369012] This article demonstrates that the inclusion of plasmids expressing either IL-23 or IL-12, but not those expressing IL-27, in a DNA vaccine stimulate stronger antigen-specific proliferation and Th1 polarization. This suggests that both IL-12 and IL-23 can serve as adjuvants to improve protective efficacy of DNA vaccines in antituberculosis immunity
- **36. Khader S, Bell G, Pearl J, Fountain J, Rangel-Moreno J, Cilley G, Shen F, Eaton S, Gaffen S, Swain S, et al. IL-23 and IL-17 in establishment of protective pulmonary CD4+ T cell responses upon vaccination and during *Mycobacterium tuberculosis* challenge. *Nat Immunol*. 2007in press This paper demonstrates for the first time the requirement for IL-23 in the generation and establishment of a lung-resident IL-17-producing T cell memory population which is important for vaccine-induced protection in a pulmonary *M. tuberculosis* challenge. In the absence of this IL-17-producing population, the vaccine-induced accelerated chemokine and IFN- γ response to challenge is lost and this correlates with the loss of protection.
37. Lindblad E, Elhay M, Silva R, Appelberg R, Andersen P. Adjuvant modulation of immune responses to tuberculosis subunit vaccines. *Infect Immun* 1997;65:623–629. [PubMed: 9009322]
- *38. Ha S, Park S, Kim H, Kim S, Kang H, Lee E, Kwon S, Kim B, Lee S, Kim W, et al. Enhanced immunogenicity and protective efficacy with the use of interleukin-12-encapsulated microspheres plus AS01B in tuberculosis subunit vaccination. *Infect Immun* 2006;74:4954–4959. [PubMed: 16861689] This study uses IL-12 encapsulated within microspheres and delivered in an adjuvant (AS01B) to demonstrate significantly improved protection compared to BCG both in short and long-term studies; this is likely due to the prolonged availability of IL-12.
39. Li H, Li R, Zhong S, Ren H, Zou Y, Chen X, Shi X, Wang M, Long H, Luo Y. The immunogenicity and protective efficacy of Mtb8.4/hIL-12 chimeric gene vaccine. *Vaccine* 2006;24:1315–1323. [PubMed: 16219397]
40. Yoshida S, Tanaka T, Kita Y, Kuwayama S, Kanamaru N, Muraki Y, Hashimoto S, Inoue Y, Sakatani M, Kobayashi E, et al. DNA vaccine using hemagglutinating virus of Japan-liposome encapsulating combination encoding mycobacterial heat shock protein 65 and interleukin-12 confers protection against *Mycobacterium tuberculosis* by T cell activation. *Vaccine* 2006;24:1191–1204. [PubMed: 16216394]
- *41. Okada M, Kita Y, Nakajima T, Kanamaru N, Hashimoto S, Nagasawa T, Kaneda Y, Yoshida S, Nishida Y, Fukamizu R, et al. Evaluation of a novel vaccine (HVJ-liposome/HSP65 DNA+IL-12 DNA) against tuberculosis using the cynomolgus monkey model of TB. *Vaccine*. 2007epub This study demonstrates that in a cynomolgus monkey model, the use of IL-12 as an adjuvant with DNA vaccine results in improved protection relative to BCG alone.
- **42. Bafica A, Scanga C, Serhan C, Machado F, White S, Sher A, Aliberti J. Host control of *Mycobacterium tuberculosis* is regulated by 5-lipoxygenase-dependent lipoxin production. *J Clin Invest* 2005;115:1601–1606. [PubMed: 15931391] This study shows that 5-lipoxygenase (5-LO)-dependent lipoxins, specifically lipoxin A4 (LTA₄) is induced during *M. tuberculosis* pulmonary infection and maintained during chronic experimental tuberculosis. They also show that LTA₄ negatively regulates the Th1 response by limiting IL-12 expression and thereby resistance to infection.
43. Aliberti J, Serhan C, Sher A. Parasite-induced lipoxin A4 is an endogenous regulator of IL-12 production and immunopathology in *Toxoplasma gondii* infection. *J Exp Med* 2002;196:1253–1262. [PubMed: 12417634]

44. Rosas L, Satoskar A, Roth K, Keiser T, Barbi J, Hunter C, de Sauvage F, Satoskar A. Interleukin-27R (WSX-1/T-cell cytokine receptor) gene-deficient mice display enhanced resistance to *Leishmania donovani* infection but develop severe liver immunopathology. *Am J Path* 2006;168:158–169. [PubMed: 16400019]
- **45. Batten M, Li J, Yi S, Kljavin N, Danilenko D, Lucas S, Lee J, de Sauvage F, Ghilardi N. Interleukin 27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17-producing T cells. *Nat Immunol* 2006;7:929–936. [PubMed: 16906167]
- **46. Stumhofer J, Laurence A, Wilson E, Huang E, Tato C, Johnson L, Villarino A, Huang Q, Yoshimura A, Sehy D, et al. Interleukin 27 negatively regulates the development of interleukin 17-producing T helper cells during chronic inflammation of the central nervous system. *Nat Immunol* 2006;7:937–945. [PubMed: 16906166] These two articles provide evidence that IL-27 negatively regulates the development of Th17 type cells and thereby limits tissue damaging inflammation. These studies show that this is mediated through STAT1 but is independent of IFN- γ .
47. D'Souza CD, Cooper AM, Frank AA, Mazzaccaro RJ, Bloom BR, Orme IM. An anti-inflammatory role for $\gamma\delta$ T lymphocytes in acquired immunity to *Mycobacterium tuberculosis*. *J Immunol* 1997;158:1217–1221. [PubMed: 9013962]

Figure 1. IL-12 family cytokines in cellular response to *M.tuberculosis* infection

Macrophages and DCs are exposed to *M. tuberculosis* following aerosol infection of the lung. *M. tuberculosis* activated lung DCs produce IL-12p40, become responsive to the chemokines CCL19 and CCL21 and carry the antigen from the lung to the draining lymph node. IL-23 produced by activated DCs during the DC-T cell interaction promotes proliferation of antigen-specific CD4 T cells. IL-12 (and to a lesser extent IL-27 and IL-23) produced by activated DCs promotes polarization of naïve CD4 T cells into a Th1 phenotype while TGF- β and IL-6 promote polarization of Th17 cells. Both Th1 and Th17 cells migrate and populate the lung compartment in response to inflammation-induced chemokine gradients. The arrival of the IFN- γ producing Th1 cells correlates with the activation of infected macrophages in the lung and bacterial control. The absence of the IL-17 producing Th17 cells does not impact bacterial burden but does alter granuloma formation. $\gamma\delta$ T cells also produce IL-17 in the lung of mycobacterially-infected mice.

Figure 2. Regulation of cellular responses by IL-12 cytokine family members during *M. tuberculosis* infection

During chronic tuberculosis, IL-12 is required for the long-term maintenance of Th1 CD4 T cells in the lung, while IL-23 is required for the persistence and maintenance Th17 cells and IL-17. IFN- γ produced by Th1 cells negatively regulates Th17 while IL-27 promotes production of IFN- γ by Th1 cells and limits inflammation. Lipoxin negatively regulates IL-12 production during infection and may limit the inflammatory response in chronic disease. IL-23/IL-17 from Th17 and/or $\gamma\delta$ T cells modulates the inflammatory response possibly by recruitment of neutrophils.

Table 1

Newly identified gene deficiencies associated with altered IL-12p40 levels and susceptibility to tuberculosis.

Human			
Gene		Function	Reference
NF- κ B essential modulator (NEMO)	Increased X-linked susceptibility to mycobacterial infections in patients carrying a mutation in the leucine zipper domain of the <i>NEMO</i> gene	NEMO is a regulatory component of IKK complex. It regulates T-cell dependent and CD40L triggered IL-12 production and IFN- γ production to mediate protection against mycobacterial infections in humans.	[8,9]
Chemokine (C-C motif) ligand 2 (CCL2) Aka: Monocyte Chemoattractant protein-1 (MCP-1)	Increased susceptibility to tuberculosis was associated with a Single Nucleotide Polymorphism in the <i>CCL2</i> gene promoter in humans.	Higher level of CCL2 in the susceptible phenotype correlates with lower expression of IL-12p40. Blocking CCL2 raises IL-12p40. Suggests that CCL2 negatively regulates IL-12p40 expression during tuberculosis	[10]
Animal			
Toll like receptor 2 and Toll like receptor 9 (TLR2/TLR9)	TLR2/TLR9 double deficient mice exhibit increased susceptibility to <i>M. tuberculosis</i> infection.	DNA from <i>M. tuberculosis</i> triggers IL-12p40 from DCs through TLR9 signaling and regulates level of IFN- γ production during infection.	[13]
5-lipoxygenase (5-LO)-dependent lipoxin A4 (LXA ₄)	Enhanced protection against <i>M. tuberculosis</i> in 5-LO deficient mice	LXA ₄ negatively regulates IL-12 expression and regulates IFN- γ responses in vivo during chronic mycobacterial infection.	[42]