Mycobacterium bovis BCG-Specific Th17 Cells Confer Partial Protection against Mycobacterium tuberculosis Infection in the Absence of Gamma Interferon

Teresa M. Wozniak,1 Bernadette M. Saunders,1,2 Anthony A. Ryan,1 and Warwick J. Britton1,2*

Mycobacterial Research Program, Centenary Institute, Locked Bag No. 6, Newtown, NSW 2042,1 and Discipline of Medicine, Central Clinical School, University of Sydney, Sydney, NSW 2006,2 Australia

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Protective immunity against tuberculosis (TB) requires the integrated response of a network of lymphocytes. Both gamma interferon (IFN-γ)- and interleukin 17 (IL-17)-secreting CD4+ T cells have been identified in subjects with latent TB infection and during experimental Mycobacterium tuberculosis infection, but the contribution of Th17 cells to protective immunity is unclear. To examine their protective effects in vivo, we transferred mycobacterium-specific IL-17- and IFN-γ-secreting CD4+ T cells isolated from M. tuberculosis BCG-immunized IL-12p40−/− and IFN-γ−/− or wild-type mice, respectively, into M. tuberculosis-infected IL-12p40−/− or Rag−/− mice. In the absence of IL-12 and IL-23, neither IL-17-secreting (Th17) nor IFN-γ-secreting (Th1) BCG-specific T cells expanded or provided protection against M. tuberculosis. In Rag−/− recipients with an intact IL-12/IL-23 axis, both Th17 and Th1 cells were activated and induced significant protection against M. tuberculosis. The reduction in the bacterial load following transfer of IFN-γ−/− Th17 cells was associated with significant prolongation of survival compared to recipients of naïve IFN-γ−/− T cells. This effect was at the cost of an increased inflammatory infiltrate characterized by an excess of neutrophils. Therefore, Th17 cells can provide IFN-γ-independent protection against M. tuberculosis, and this effect may contribute to the early control of M. tuberculosis infection.

Protective immunity against Mycobacterium tuberculosis requires the orchestration and integration of innate and adaptive immune responses to generate a robust and long-lived memory T-cell response (17). Understanding the interactions of different types of lymphocytes in response to M. tuberculosis infection or the M. tuberculosis BCG vaccine is important for developing improved vaccines against M. tuberculosis and controlling the tuberculosis (TB) epidemic (29). M. tuberculosis infection stimulates dendritic cells (DCs) to secrete the cytokines interleukin 12 (IL-12) and IL-23, which promote the activation and clonal expansion of antigen-specific T cells in the draining lymph nodes (LN) of the lungs (4, 12). IL-12 and IL-23 are heterodimeric cytokines that have a shared p40 subunit, along with unique p35 and p19 subunits, respectively (18). IL-12 is required for the development of the gamma interferon (IFN-γ) T-cell response, which is essential for resistance to M. tuberculosis in both humans (7) and mice (4). On the other hand, mice lacking IL-12 can control M. tuberculosis infection (12). Although IL-23 is not essential for protection against M. tuberculosis, IL-23 may contribute to protection in the intact host, as plasmid IL-23 is as effective as plasmid IL-12 as an adjuvant to increase the protective response induced by a DNA vaccine (27).

IL-23 has a major role in the expansion of IL-17-producing CD4+ T cells (9, 19). The initial activation of Th17 cells is dependent on IL-6 and TGF-β; however, in models of autoimmune disease, the expansion and proinflammatory effects of Th17 cells require IL-23 (1, 15, 25). Th17 cells secrete multiple cytokines, which induce chemokine production and the recruitment of neutrophils to mucosal sites of bacterial infection (28) and play an essential role in the control of oropharyngeal Candida albicans infection (3). Th17 cells also participate in the early inflammatory response to mycobacterial infection; however, IL-17 production in the lungs is downregulated as IFN-γ T cells emerge (5). The protective potential of Th17 T cells during the early phase of M. tuberculosis infection is unknown.

Recently, we demonstrated that in the absence of the IL-12/IFN-γ axis, BCG-immunized IL-12p40−/− mice developed a robust Th17 T-cell response. This was associated with a significant reduction in the bacterial load following infection with M. tuberculosis (26), suggesting that BCG-specific Th17 cells alone may provide partial protection in the absence of IFN-γ. To investigate this further, we expanded BCG-specific Th17 cells in vitro and examined their protective effects following adoptive transfer into M. tuberculosis-infected immunodeficient mice.

MATERIALS AND METHODS

Mice. Six- to 8-week old female C57BL/6 wild-type (WT) or C57BL/6 Rag−/− (RAG−/−) mice were obtained from the Animal Resources Centre (Perth, WA, Australia). C57BL/6 IL-12p40−/− and C57BL/6 IFN-γ−/− mice were purchased from Jackson Laboratory (Bar Harbor, ME) and were bred in a pathogen-free animal facility. All animal experiments were approved by the Animal Care and Ethics Committee, University of Sydney, Sydney, Australia.

Mycobacteria and immunization. M. tuberculosis H37Rv and M. bovis BCG (Pasteur) were grown in Middlebrook 7H9 broth (Difco Laboratories, Detroit, MI) supplemented with 10% albumin-dextrose-catalase (ADC) (Difco). Middlebrook 7H11 agar supplemented with oleic acid-albumin-dextrose-catalase (OADC) (Difco) was used for growth and enumeration of mycobacteria on solid media. WT, IL-12p40−/−, and IFN-γ−/− mice were immunized by subcutaneous treatment with 107 colony-forming units (CFU) of virulent M. tuberculosis.
s.c. injection with 5 × 10^3 CFU M. bovis BCG at the base of the tail and in both footpads. The mice were infected by intravenous (i.v.) injection with 10^6 CFU M. tuberculosis.

Expansion of BCG-specific T cells. Draining LN from the inguinal, aortic, and popliteal regions were collected from WT, IL-12p40^−/−, and IFN-γ^−/− mice and digested in complete RPMI 1640 medium containing collagenase (10 U/ml) (Becton Dickinson) and DNAse (0.1 mg/ml) (Worthington). Cultures of 10^6 cells/ml were stimulated with BCG lysate prepared by sonication of M. bovis BCG (10 μg/ml) in medium alone or with 10 ng/ml recombinant IL-12 (rIL-12) or rIL-23 (R&D Systems). On day 5, the cultures were replenished with medium containing BCG lysate, rIL-2 (2 ng/ml) (R&D Systems), and rIL-12 or rIL-23. On day 8, intracytoplasmic staining was performed. For adoptive-transfer experiments, single-cell suspensions were cultured for 8 days in complete medium containing BCG lysate and rIL-2 only.

Intracellular cytokine staining. Cytokine production by BCG-stimulated LN cultures was determined by intracytoplasmic staining as previously described (16).

Adoptive transfer of expanded T cells and infection with M. tuberculosis. Recipient WT, IL-12p40^−/−, and RAG^−/− mice (n = 5) were injected i.v. with 2 × 10^6 expanded cells and 1 × 10^8 CFU M. tuberculosis. After 2.5 weeks, the lungs, livers, and spleens of infected mice were plated in 10-fold dilutions on supplemented Middlebrook 7H11 agar. Single-cell suspensions of the mediastinal LN were prepared, stimulated with BCG lysate for 16 h, and intracytoplasmically stained for cytokine expression. In some experiments, recipient RAG^−/− mice were injected intraperitoneally (i.p.) with neutralizing anti-IFN-γ monoclonal antibody (MAb) (XMG 1.2) or control IgG (clone GL113) (500 μg/mouse) on the day of the adoptive transfer and every 3 days thereafter for the duration of the experiment.

Histological analysis. Lungs were perfused and fixed with 10% neutral buffered formalin for 2 weeks before being embedded in paraffin and sectioned at 5 μm. The sections were stained with hematoxylin and eosin. The percentage of lung area with inflammatory involvement was calculated by image analysis (22).

Statistical analysis. Statistical analysis of the differences between the means of the log_{10}-transformed bacterial counts was conducted by analysis of variance (ANOVA) (Statview SAS Institute, Cary, NC). Fishers’ protected least-significant difference ANOVA posthoc test was used for pairwise comparison of data from multiple groups. Survival was calculated on a Kaplan-Meier nonparametric survival plot, and significance was assessed by the log rank-Mantel-Cox test.

RESULTS

IL-23 is not required for the in vitro expansion of BCG-specific CD4^+ T cells. In the absence of IL-12p40, BCG immunization stimulated antigen-specific CD4^+ T cells, which secrete IL-17 (26). To investigate the requirement for IL-12 and IL-23 for the expansion of these IL-17-secreting T cells in vitro, BCG-prime T cells from IL-12p40^−/− mice were stimulated with BCG in the presence of IL-23, IL-12, or medium alone. Expansion of the mycobacterium-specific Th17 cells occurred to an extent similar to that with IL-23 (8.6%) or medium alone (7.8%), but this expansion was inhibited by the presence of IL-12 (0.11%) (Fig. 1). Stimulation of IL-12p40^−/− T cells with BCG in vitro also resulted in the modest expansion of the proportion of IFN-γ-secreting BCG-specific CD4^+ T cells, and this was further increased with the addition of rIL-12 but was unaffected by the addition of rIL-23 (Fig. 1). Ex vivo expansion of BCG-primed T cells from WT mice led to a 30-fold expansion of IFN-γ-secreting T cells (0.03 to 0.91% following ex vivo expansion with IL-2 and BCG sonicate); however, no expansion of IL-17-secreting BCG-specific T cells was seen (0.24 to 0.33% following ex vivo expansion with IL-2 and BCG sonicate). The expansion of cytokine-expressing T cells was antigen specific, as the culture of lymph node and splenic cells from naïve WT or IL-12p40^−/− mice with BCG and IL-12 or IL-17 did not lead to the expansion of either the IL-17- or IFN-γ-secreting population. Despite remaining viable for the 8-day culture period, less than 0.2% of the CD4^+ T cells produced IFN-γ or IL-17 (data not shown).

Therefore, the expansion of activated IL-17-secreting T cells in vitro was independent of IL-23. These BCG-specific CD4^+ and CD8^+ IL-17- and IFN-γ-expressing T cells expressed a CD62L^low CD44high phenotype (data not shown).

BCG-specific Th17 T cells confer partial protection against M. tuberculosis in RAG^−/− mice. To determine the protective effect of these IL-17-secreting cells against M. tuberculosis infection, BCG-specific Th17 cells, derived from BCG-immunized IL-12p40^−/− mice, were expanded in vitro and transferred into M. tuberculosis-infected RAG^−/− mice. After 17 days, the proportion of antigen-specific CD4^+ T cells secreting IL-17 in the recipients of the expanded Th17 cells was 3.09% (Fig. 2B); however, a population of IFN-γ-secreting CD4^+ T cells (10.7% of the CD4^+ T cells) had also emerged (Fig. 2B). To determine the protective effect of these IL-17-secreting cells against M. tuberculosis infection, BCG-specific Th17 cells, derived from BCG-immunized IL-12p40^−/− mice, were expanded in vitro and transferred into M. tuberculosis-infected RAG^−/− mice. After 17 days, the proportion of antigen-specific CD4^+ T cells secreting IL-17 in the recipients of the expanded Th17 cells was 3.09% (Fig. 2B); however, a population of IFN-γ-secreting CD4^+ T cells (10.7% of the CD4^+ T cells) had also emerged (Fig. 2B). In contrast, transfer of BCG-immunized WT T cells into RAG^−/− hosts resulted in 15% of the BCG-specific CD4^+ T cells secreting IFN-γ and only 0.73% secreting IL-17 in response to BCG stimulation (Fig. 2A). The transferred populations of cells did contain a proportion of CD4^+ T cells, and these findings are consistent with the activity of IL-12 in the RAG^−/− mice inducing the activation and differentiation of residual, naïve T cells present in the transferred population and with the effect of IL-23 on the expansion of the polarized BCG-specific Th17 cells following repeat mycobacterial exposure.

The transfer of BCG-specific WT Th1 T cells or IL-
12p40−/− Th17 T-cell populations into RAG−/− recipients conferred equivalent protection against *M. tuberculosis* in the spleen, liver, and lungs (Fig. 2C to E). Donor cells were expanded by culture with BCG sonicate and IL-2 for 8 days prior to transfer into recipient RAG−/− mice. Expanded donor cells (2.5 × 10^6 cells/mouse) from immunized and nonimmunized WT or IL-12p40−/− mice, in combination with *M. tuberculosis* H37Rv (10^4 CFU/mouse), were transferred by i.v. injection into recipient RAG−/− mice. After 2.5 weeks, mesenteric lymph nodes (MLN) were harvested, and intracellular IFN-γ and IL-17 secretion was determined in RAG−/− mice that received donor WT (A) or IL-12p40−/− (B) cells. The bacterial loads in the spleen (C), liver (D), and lungs (E) were measured. The data are the means and standard errors of the mean (SEM) for five mice and are representative of three independent experiments. The differences between groups were analyzed by analysis of variance (**, P < 0.001; ***, P < 0.0001).

**FIG. 2.** Th17 cells mediate protection against *M. tuberculosis* infection of RAG−/− mice. WT and IL-12p40−/− mice were immunized by s.c. injection with *M. bovis* BCG (5 × 10^5 CFU/mouse) at the base of the tail and in the footpads. Draining lymph nodes were collected 3 weeks later from immunized (BCG) and nonimmunized (control) mice. Donor cells were expanded by culture with BCG sonicate and IL-2 for 8 days prior to transfer into recipient RAG−/− mice. Expanded donor cells (2.5 × 10^6 cells/mouse) from immunized and nonimmunized WT or IL-12p40−/− mice were transferred by i.v. injection into recipient RAG−/− mice. After 2.5 weeks, mesenteric lymph nodes (MLN) were harvested, and intracellular IFN-γ and IL-17 secretion was determined in RAG−/− mice that received donor WT (A) or IL-12p40−/− (B) cells. The bacterial loads in the spleen (C), liver (D), and lungs (E) were measured. The data are the means and standard errors of the mean (SEM) for five mice and are representative of three independent experiments. The differences between groups were analyzed by analysis of variance (**, P < 0.001; ***, P < 0.0001).
secretion from Th17 cells was induced with M. tuberculosis and treated with neutralizing anti-IFN-γ or control antibodies. Both the Th1 and Th17 T-cell populations induced a protective effect in RAG−/− mice that received the control antibody, similar to that shown in Fig. 2. Neutralization of IFN-γ in recipients of Th1 T cells resulted in a significant reduction of the protective effect in the spleen, liver, and lungs (Table 1). In recipients of the Th17 cell population, neutralization of IFN-γ was associated with no reduction in the protective effect in the spleen following i.v. M. tuberculosis infection, but there were smaller, significant reductions in the protective effect in the liver and lungs (Table 1). Therefore, the protective effect of the transferred Th17 T-cell population was partially independent of IFN-γ, consistent with the IFN-γ-independent protective effect of BCG vaccination observed in IL-12p40−/− mice (26), but this might have been partially due to the differentiation of naïve T cells into IFN-γ-secreting Th1 T cells during the 17 days of infection. To confirm that Th17 T cells alone could confer protection against M. tuberculosis infection, we examined the effect of BCG-specific Th17 cells derived from IFN-γ−/− mice. INF-γ deficient Th17 T cells protect against M. tuberculosis infection. To confirm that IL-17-secreting Th17 cells have an IFN-γ-independent protective capacity against M. tuberculosis infection, IFN-γ−/− and WT mice were immunized with BCG and cells from the draining LN expanded in vitro and then transferred into RAG−/− mice. The transfer of naïve T cells from WT or IFN-γ−/− mice resulted in a significant reduction in mycobacterial growth in the spleen (both P < 0.01), but not the lungs, compared to untreated control mice (Fig. 3A and B). This was associated with the corresponding emergence of new populations of IFN-γ-secreting T cells in the recipients of transferred WT T cells and IL-17-secreting T cells in the recipients of IFN-γ−/− T cells (Fig. 3C and D). The transfer of BCG-specific Th1 T cells from WT mice and BCG-specific Th17 T cells from IFN-γ−/− mice conferred significantly more protection against M. tuberculosis in the spleen (both P < 0.01) and lungs (both P < 0.01) of the RAG−/− hosts (Fig. 3A and B). There were corresponding significant increases in the proportions of CD4+ T cells secreting IFN-γ in the recipients of WT T cells (mean, 6.10%) and IL-17-secreting T cells in the recipients of IFN-γ−/− T cells (mean, 2.45%) (Fig. 3C and D).

In addition, the transfer of BCG-specific Th17 cells from IFN-γ−/− mice to RAG−/− mice conferred a significant survival advantage compared with M. tuberculosis-infected RAG−/− mice that received T cells from naïve IFN-γ−/− mice (median increase, 11 days; P < 0.007) (Fig. 4A). This was comparable to an increase in survival of over 45 days conferred on RAG mice that received BCG-immunized and nonimmunized WT T cells. Therefore, BCG-specific Th17 cells can constrain the growth of M. tuberculosis and enhance the survival of RAG−/− mice in the absence of IFN-γ-secreting T cells.

To investigate the influence of IL-17-expressing cells on cell recruitment, the inflammatory changes in the lungs of the RAG−/− recipients of the WT and IFN-γ−/− T cells were examined at 2.5 weeks postinfection. RAG−/− recipients of WT (Fig. 5A and B) and IFN-γ−/− (Fig. 5E and F) naïve T cells displayed mild inflammation, leading to mild interstitial pneumonia with a predominantly mononuclear cellular influx (Fig. 5A to D). Lesions were small and diffuse, and the majority of the lung retained the typical lacy alveolar appearance of normal lung tissue. In contrast, RAG−/− recipients of T cells from BCG-immunized mice displayed significantly increased inflammation throughout the lungs, although the pattern of the inflammation differed markedly between mice that received T cells from BCG-immunized WT mice (Fig. 5C and D) and IFN-γ−/− mice (Fig. 5G and H). In RAG−/− recipients of BCG-immunized WT T cells, numerous small to medium-size lesions, composed predominantly of macrophages and lymphocytes, were evident throughout the lungs. In comparison, in the RAG−/− recipients of IL-17-secreting, BCG-immunized IFN-γ−/− T cells, there were fewer, but markedly larger, lesions than in recipients of BCG-immunized WT T cells, resulting in similar proportions of the lungs being infiltrated (Fig. 4B). Importantly, in the recipients of IL-17-secreting cells, the infiltrating leukocytes contained a predominance of neutrophils, with some eosinophils and occasional giant cells. Thus, the transfer of BCG-specific Th17 cells led to a marked influx of neutrophils that was not evident in the mice that received the naïve IFN-γ−/− cells, which had rapidly succumbed to M. tuberculosis infection.

DISCUSSION

IL-17-secreting CD4+ and γδ T cells have been identified during acute M. tuberculosis (11, 14) and BCG (24) infection in mice and in humans with latent TB infection (23). The subsequent production of IFN-γ during mycobacterial infections downregulates the IL-17-secreting T-cell response (11), and
therefore, it has not been possible to determine if mycobacterium-specific Th17 T cells have an independent protective effect early in the course of *M. tuberculosis* infection. Using a model system to dissect the influence of discrete T-cell populations in the absence of potentially confounding effects from other T-cell subsets, this study establishes for the first time that BCG-specific IL-17-secreting T cells, in the absence of IFN-γ, can confer significant protection against systemic *M. tuberculosis* infection. Although this protective effect was observed in immunodeficient mice, it highlights the potential for mycobacterium-specific IL-17-secreting T cells to contribute to the early constraint of *M. tuberculosis* infection prior to the development of the dominant IFN-γ-secreting T-cell response.

There is accumulating evidence for the role of IL-17 during mycobacterial infections. Pulmonary infection with BCG (24) or *M. tuberculosis* (11) stimulated the early secretion of IL-17 within 1 and 14 days, respectively, and this preceded the development of IFN-γ-secreting T cells. The initial source of IL-17 in mice was IFN-γ/T cells in both the lungs and the spleen (14, 24), and subsequently, IL-17-secreting CD4+ T cells infiltrated the lung. During pulmonary BCG infection, IL-17-deficient mice showed reduced IFN-γ T-cell and delayed-type hypersensitivity responses to mycobacterial antigens and impaired granuloma formation in the lungs, suggesting IL-17 was required for the development of optimal Th1 T-cell responses (24). Infection of IL-17−/− mice with *M. tuberculosis* revealed that IL-17 was not essential to control the growth of *M. tuberculosis* during acute infection (11), and it was assumed...
FIG. 5. Th17 and Th1 T cells induce distinct patterns of inflammation in response to *M. tuberculosis* infection. Expanded WT or IFN-γ−/− donor cells from BCG-immunized and nonimmunized mice (2 × 10^6 cells/mouse), in combination with *M. tuberculosis* H37Rv (10^4 CFU/mouse), were transferred by i.v. injection into RAG−/− mice. After 2.5 weeks, the lungs were perfused with 10% neutral buffered formalin, fixed in paraffin, and processed. (A and B) WT naïve T cells injected into RAG−/− recipients. Lesions were discrete and localized and composed predominantly of macrophages and lymphocytes, with mild interstitial pneumonia evident throughout the lung. (C and D) WT-BCG T cells injected into RAG−/− recipients. The lungs showed markedly increased inflammation and larger, often quite diffuse lesions composed predominantly of interspersed macrophages and lymphocytes with occasional neutrophils evident. (E and F) IFN-γ−/− naïve T cells injected into RAG−/− cells. Small lesions were dispersed throughout the lung, with mild interstitial pneumonia evident throughout the lung. The lesions were composed predominantly of macrophages and lymphocytes with occasional neutrophils evident. (G and H) IFN-γ−/− BCG T cells injected into RAG−/− recipients. The lungs showed extensive inflammation, with very large, densely cellular lesions surrounded by tissue with minimal cellular infiltrate. The lesions were composed of macrophages, lymphocytes with marked neutrophils, and some eosinophil infiltrates.
that the emerging IFN-γ-secreting CD4+ and effector CD8+ T cells were sufficient to inhibit mycobacterial replication in the absence of IL-17. More recently, however, it has been suggested that IL-17 is necessary for the maintenance of Th1 T-cell immunity during chronic M. tuberculosis infection, as infected IL-17−/− mice show reduced survival late in the course of infection compared to wild-type mice (J. C. Sodenkamp, I. Forster, W. Muller, S. Ehlers, and C. Holcher, presented at the Keystone Symposia, Tuberculosis: Biology, Pathology and Therapy, Keystone Resort, CO, 2009).

Previously, we demonstrated that BCG immunization of IL-12p40-deficient mice resulted in a small but significant protective effect against M. tuberculosis infection, associated with the emergence of BCG-specific IL-17-secreting T cells (26). When expanded in vitro, both CD4 and CD8 IL-17-secreting T cells demonstrated low levels of L-selectin expression consistent with an activated, effector phenotype. An important feature of effector memory T cells is their ability to migrate into infected tissues, such as the lungs, where they may interact with M. tuberculosis-infected macrophages. IL-23 was not required for the induction in vivo or the expansion in vitro of the BCG-specific IL-17-secreting T cells. This is consistent with the finding that IL-23p19−/− mice produced measurable levels of IL-17 during mycobacterial infection (8). Likewise, IL-17-secreting CD4+ T cells developed during Citrobacter rodentium infection independently of IL-23 (15). Therefore, the critical role of IL-23 may be to enhance the survival and growth of already-differentiated IL-17-secreting CD4+ T cells rather than their differentiation (25).

The partial control of M. tuberculosis infection conferred by BCG-specific IL-17-secreting T cells in the absence of IFN-γ was associated with increased leukocyte recruitment, including a marked neutrophil influx. The transfer of either BCG-primed IFN-γ-secreting or IL-17-secreting T cells into RAG−/− mice resulted in increased inflammation in the lungs during M. tuberculosis infection compared to recipients of nonprimed T cells, and there were major differences in the patterns of the inflammatory infiltrates. Recipients of WT IFN-γ-secreting T cells showed a mixed lymphocyte and macrophage infiltrate in the lungs, while recipients of IL-17-secreting T cells showed an increased number of neutrophils (Fig. 5G and H). IL-17 promotes chemokine production from leukocytes and epithelial cells, leading to the recruitment of neutrophils to the sites of extracellular bacterial infections (21), and this effect was evident in M. tuberculosis infection in the absence of a balancing IFN-γ T-cell response. Neutrophils, are recruited early in the course of M. tuberculosis infection in WT mice, and neutropenic mice display reduced IFN-γ and inducible nitric oxide synthase (iNOS) production following M. tuberculosis infection, with a corresponding increase in the bacterial burden (20). The IL-17-producing T cells themselves produce other proinflammatory cytokines, including IL-17F, IL-22 (13), and tumor necrosis factor (TNF) (26), and IL-17 stimulates TNF and IL-1β production from macrophages (10). The resulting TNF can partially activate mycobacterium-infected macrophages, even in the absence of IFN-γ (2). The increased cellular infiltrate driven by IL-17 may also include increased numbers of endogenous NK cells, which produce IFN-γ and TNF and contribute to control of M. tuberculosis infection in an IL-12p40-dependent fashion (6). The short-term protective effect provided by the IL-17-secreting cells occurred at the cost of increased tissue damage characterized by a marked neutrophil infiltrate. Although, the transfer of BCG-specific IL-17-secreting T cells increased the survival of M. tuberculosis-infected immunodeficient mice, this survival advantage was significantly less than that provided by BCG-specific IFN-γ-secreting T cells. Nevertheless, the short-term protective effect of Th17 cells may provide a temporary benefit in WT mice, while the full range of adaptive T-cell responses are engaged.

IL-17 is produced by both CD4+ T cells and γδ T cells during the human immune response to M. tuberculosis. In healthy adults exposed to mycobacteria, over 20% of BCG-specific CD4+ T cells produced either IL-17 or IL-22, and these memory T cells were distinct from each other and from IFN-γ-secreting CD4+ T cells (23). There was a balance between the Th17 and Th1 T-cell responses, as IFN-γ inhibited the production of IL-17, but not IL-22, from BCG-stimulated T cells, and during active TB disease, the frequency of IL-17-producing CD4+ T cells was decreased. In a separate study, the proportion of IL-17-secreting γδ T cells was increased in TB patients compared to BCG vaccinees; however, stimulation with M. tuberculosis in vitro resulted in an increase in IFN-γ-secreting γδ T cells with no change in the proportion of IL-17-secreting γδ T cells (21). Overall these studies suggest that in humans, Th17 cells may be maintained during the memory response to M. tuberculosis infection but are downregulated during active TB infection by the upregulated IFN-γ T-cell response to the increased antigen load.

In summary, this study shows that an IL-17-secreting T-cell response to mycobacteria emerges in vivo in the absence of IFN-γ. The resulting BCG-specific IL-17-secreting cells did confer a significant protective effect against M. tuberculosis in T-cell-deficient mice; however, in the absence of a balancing IFN-γ-secreting T-cell response, this effect was associated with increased neutrophil infiltrate and damage to the infected organs. This supports a role for IL-17-secreting CD4+ T cells, as well as γδ T cells, in the integrated cellular response to mycobacterial infections.

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