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

Received 15 December 2009/Returned for modification 29 December 2009/Accepted 25 July 2010

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 provide partial 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 IL-17 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
Recipient WT, IL-12p40/H11002 secreting population. Despite remaining viable for the 8-day proportion of IFN-γ-secreting BCG-specific CD4/H9253/H11001 naïve WT or IL-12p40 specific, as the culture of lymph node and splenic cells from expansion with IL-2 and BCG sonicate); however, no expansion of IFN-γ of BCG-primed T cells from WT mice led to a 30-fold expansion unaffected by the addition of rIL-23 (Fig. 1). Ex vivo expansion IL-12 (0.11%) (Fig. 1). Stimulation of IL-12p40/H11002 (7.8%), but this expansion was inhibited by the presence of Expansion of the mycobacterium-specific Th17 cells occurred with BCG in the presence of IL-23, IL-12, or medium alone.

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/H11002, and RAG/H11002−/− mice (n = 5) were injected i.v. with 2 × 10^6 expanded cells and 1 × 10^6 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.

Statistical analysis. Statistical analysis of the differences between the means of log_{10}-transformed bacterial counts was conducted by analysis of variance (ANOVA) (Statview SAS Institute, Cary, NC). Fisher’s 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 Th17 cells. In the absence of IL-12p40, BCG immunization stimulated antigen-specific CD4+/H11001 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-primed 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+/H11001 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 exansion 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-23 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+/H11001 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+/H11001 T cells secreting IFN-γ expressing T cells expressed a CD62L^{low} CD44^{high} 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+/H11001 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+/H11001 T cells (10.7% of the CD4+/H11001 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+/H11001 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 CD44^{low} 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). The transfer of naïve T cells from nonimmunized WT or IL-12p40−/− mice was also associated with the development of IFN-γ-secreting T cells (Fig. 2A and B). However, bacterial loads were significantly higher in all organs compared to those in mice that received BCG-specific WT Th1 or IL-12p40−/− Th17 T cells. This suggests that the protective effects of the cell populations derived from immunized WT and IL-12p40−/− mice were due to the early expansion of Th1 and Th17 T cells, respectively.

**IL-12/IL-23p40 is essential for the maintenance of BCG-specific Th17 cells in vivo during *M. tuberculosis* infection.** To determine if IL-12p40 was required for the expansion and effects of mycobacterium-specific Th17 cells *in vivo*, BCG-specific IFN-γ or IL-17-secreting cells were adoptively transferred into IL-12p40−/− mice prior to challenge with *M. tuberculosis*. Transfer of expanded BCG-specific T cells from IL-12p40−/− mice into IL-12p40−/− mice resulted in the maintenance of a small but detectable population of IL-17-secreting CD4+ T cells (0.45%) compared to that following the transfer of naïve T cells (0.06%). By comparison, transfer of IFN-γ-secreting Th1 T cells from BCG-immunized WT mice into IL-12p40−/− mice was not associated with the expansion of either IFN-γ or IL-17-secreting CD4+ T cells (data not shown). The transfer of IFN-γ or IL-17-secreting T cells into mice deficient in IL-12p40 failed to control bacterial replication in the spleen, the liver, or the lungs (data not shown). Therefore, although IL-23 was dispensable for the generation of Th17 cells *in vivo* (26) or their expansion *in vitro* (Fig. 1), it was required for the maintenance and survival of Th17 cells during *M. tuberculosis* infection *in vivo*.

**Effect of neutralizing IFN-γ on Th17-mediated protection against *M. tuberculosis*.** To verify that the protective effect of BCG-specific IL-17-secreting T cells was independent of IFN-γ, RAG−/− recipients of BCG-specific IFN-γ- or IL-17-
secretion T cells were infected 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.

**IFN-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 small 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 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 mycobacte-
rium-specific Th17 T cells have an independent protective ef-
fect early in the course of M. tuberculosis infection. Using a
model system to dissect the influence of discrete T-cell popu-
lations 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-

FIG. 3. Th17 cells mediate protection in the absence of IFN-\(\gamma\) during M. tuberculosis infection. Expanded WT or IFN-\(\gamma^{-/-}\) donor cells from BCG-immunized and nonimmunized mice (2 \(\times\) 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 draining LN were harvested, and intracellular IFN-\(\gamma\) (A) and IL-17 (B) secretion was determined in RAG^{-/-} mice that received donor WT or IFN-\(\gamma^{-/-}\) cells. The bacterial loads in the spleen (C) and lungs (D) were measured. The data are the means and SEM for 5 mice and are representative of 2 experiments. The differences between groups were analyzed by analysis of variance (*, \(P < 0.01\); **, \(P < 0.0001\)).

FIG. 4. Th17 cells enhance survival and increase inflammation of RAG^{-/-} recipients in the absence of IFN-\(\gamma\). Expanded WT or IFN-\(\gamma^{-/-}\) donor cells from BCG-immunized and nonimmunized mice (2 \(\times\) 10^6 cells/mouse), in combination with M. tuberculosis H37Rv (10^6 CFU/mouse), were transferred by i.v. injection into RAG^{-/-} mice. (A) The mice were euthanized when signs of ill health were noted. The data represent the times to euthanization of 5 mice per group. Cont, control. (B) At 2.5 weeks, lungs were perfused with 10% neutral buffered formalin, fixed in paraffin, and processed. The data represent the times to euthanization of 5 mice per group. Cont, control. (B) At 2.5 weeks, lungs were perfused with 10% neutral buffered formalin, fixed in paraffin, and processed. The data represent the percent area of involvement of 3 left lung lobes from 4 mice.
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 *vivo*, 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* (26) or the expansion in *vivo* 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.

**ACKNOWLEDGMENTS**

This work was supported by the National Health and Medical Research Council of Australia and the New South Wales Department of Health through its research infrastructure grant to the Centenary Institute.

No conflict of interest was identified.

**REFERENCES**


5. Cruz, A., S. A. Khader, E. Torrado, A. Fraga, J. E. Pearl, J. Pedrosa, A. M.


