Antigen Load Governs the Differential Priming of CD8 T Cells in Response to the Bacille Calmette Guérin Vaccine or Mycobacterium tuberculosis Infection

Anthony A. Ryan, Jonathan K. Nambiar, Teresa M. Wozniak, Ben Roediger, Elena Shklovskaya, Warwick J. Britton, Barbara Fazekas de St. Groth and James A. Triccas

J Immunol 2009; 182:7172-7177; doi: 10.4049/jimmunol.0801694
http://www.jimmunol.org/content/182/11/7172

References
This article cites 40 articles, 24 of which you can access for free at:
http://www.jimmunol.org/content/182/11/7172.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Antigen Load Governs the Differential Priming of CD8 T Cells in Response to the Bacille Calmette Guérin Vaccine or Mycobacterium tuberculosis Infection

Anthony A. Ryan,*‡ Jonathan K. Nambiar,⁎† Teresa M. Wozniak,* Ben Roediger,* Elena Shklovskaya,* Warwick J. Britton,⁎† Barbara Fazekas de St. Groth,* and James A. Triccas2⁎‡

One reason proposed for the failure of Mycobacterium bovis bacille Calmette Guérin (BCG) vaccination to adequately control the spread of tuberculosis is a limited ability of the vaccine to induce effective CD8 T cell responses. However, the relative capacity of the BCG vaccine and virulent Mycobacterium tuberculosis to induce activation of CD8 T cells, and the factors that govern the initial priming of these cells after mycobacterial infection, are poorly characterized. Using a TCR transgenic CD8 T cell transfer model, we demonstrate significant activation of Ag-specific CD8 T cells by BCG, but responses were delayed and of reduced magnitude compared with those following infection with M. tuberculosis. The degree of CD8 T cell activation was critically dependent on the level of antigenic stimulation, as modifying the infectious dose to achieve comparable numbers of BCG or M. tuberculosis in draining lymph nodes led to the same pattern of CD8 T cell responses to both strains. Factors specific to M. tuberculosis infection did not influence the priming of CD8 T cells, as codelivery of M. tuberculosis with BCG did not alter the magnitude of BCG-induced T cell activation. Following transfer to RAG-1−/− recipients, BCG and M. tuberculosis-induced CD8 T cells conferred equivalent levels of protection against M. tuberculosis infection. These findings demonstrate that BCG is able to prime functional CD8 T cells, and suggest that effective delivery of Ag to sites of T cell activation by vaccines may be a key requirement for optimal CD8 T cell responses to control mycobacterial infection. The Journal of Immunology, 2009, 182: 7172–7177.

Rational design of vaccines requires an understanding of the host determinants necessary for resistance to pathogens. An effective vaccine against Mycobacterium tuberculosis should mimic the native immune response to infection, generating a large repertoire of both CD4 and CD8 T cells responding to protective mycobacterial Ags. Although CD4 T cells are known to play a central role in protection against M. tuberculosis (1), there is mounting evidence that CD8 T cells are also important in antimycobacterial immunity. Mice deficient in CD8 T cells succumb rapidly to infection with M. tuberculosis (2), and CD8 T cells are both expanded during M. tuberculosis infection and recruited to sites of bacterial burden (2, 3). Vaccines that elicit CD8 T cells, such as viruses encoding mycobacterial Ags, can induce high levels of protective immunity (4, 5), and subunit vaccines inducing CD8 T cells can protect mice deficient in CD4 T cells from M. tuberculosis (6). The protective effect of CD8 T cells is most apparent late in tuberculosis infection in mice, suggesting this subset may be important in the control of latent infection with M. tuberculosis (7). Therefore an understanding of the factors that facilitate the activation of CD8 T cells is important for the development of effective strategies to control mycobacterial infections. M. tuberculosis is a facultative intracellular pathogen that resides within phagosomes of infected cells, thereby promoting Ag entry into the MHC-class II presentation pathway. M. tuberculosis infection of both humans and mice, however, leads to the generation of pathogen-specific CD8 T cell responses (8). Cross-presentation of mycobacterial Ags by dendritic cells (DCs) may play a role, as evidenced by the demonstration that transfer of apoptotic vesicles from infected macrophages to bystander DCs can stimulate CD8 T cell responses (9, 10). Early reports demonstrated that M. tuberculosis could directly deliver Ag to the class I processing pathway, while M. bovis bacille Calmette Guérin (BCG) was less able to activate CD8 T cells in these studies (11). This suggested egress into the cytoplasm from the endosomal compartment may be a property of M. tuberculosis infection that promotes CD8 T cell activation. Indeed, a recent report suggests that virulent M. tuberculosis and Mycobacterium leprae can translocate into the cytosol of infected DCs, possibly allowing Ags to be directly presented on MHC class I molecules, a property that was not shared by BCG (12). Differences in CD8 T cell activation by different mycobacterial strains may also be influenced by other factors associated with infection. M. tuberculosis infection elicits a substantial inflammatory response, characterized by the release of numerous inflammatory cytokines and chemokines (13). Proinflammatory cytokines play an important role in

1 Microbial Pathogenesis and Immunity Group, Discipline of Infectious Diseases, 2Centenary Institute of Cancer Medicine and Cell Biology, and 3Discipline of Medicine, University of Sydney, Sydney, Australia.

Received for publication May 27, 2008. Accepted for publication March 23, 2009.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This work was supported by the National Health and Medical Research Council of Australia. A.T. is supported by an NHMRC Career Development Award, and B.F. de S.G. is supported by an NHMRC Principal Research Fellowship. A.A.R., B.R., and J.K.N. are supported by Australian Postgraduate Awards and T.M.W. is the recipient of the University of Sydney Faculty of Medicine Postgraduate Scholarship.

Address correspondence and reprint requests to Dr. James A. Triccas, Discipline of Infectious Diseases and Immunology, University of Sydney, Sydney, Australia. E-mail address: janiet@infdis.usyd.edu.au

Copyright © 2009 by The American Association of Immunologists, Inc. 0022-1767/09/$2.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.0801694

†Microbial Pathogenesis and Immunity Group, Discipline of Infectious Diseases, Centenary Institute of Cancer Medicine and Cell Biology, and Discipline of Medicine, University of Sydney, Sydney, Australia.

Published ahead of print on April 18, 2009.

Antigen Load Governs the Differential Priming of CD8 T Cells in Response to the Bacille Calmette Guérin Vaccine or Mycobacterium tuberculosis Infection

Anthony A. Ryan,*‡ Jonathan K. Nambiar,⁎† Teresa M. Wozniak,* Ben Roediger,* Elena Shklovskaya,* Warwick J. Britton,⁎† Barbara Fazekas de St. Groth,* and James A. Triccas2⁎‡

One reason proposed for the failure of Mycobacterium bovis bacille Calmette Guérin (BCG) vaccination to adequately control the spread of tuberculosis is a limited ability of the vaccine to induce effective CD8 T cell responses. However, the relative capacity of the BCG vaccine and virulent Mycobacterium tuberculosis to induce activation of CD8 T cells, and the factors that govern the initial priming of these cells after mycobacterial infection, are poorly characterized. Using a TCR transgenic CD8 T cell transfer model, we demonstrate significant activation of Ag-specific CD8 T cells by BCG, but responses were delayed and of reduced magnitude compared with those following infection with M. tuberculosis. The degree of CD8 T cell activation was critically dependent on the level of antigenic stimulation, as modifying the infectious dose to achieve comparable numbers of BCG or M. tuberculosis in draining lymph nodes led to the same pattern of CD8 T cell responses to both strains. Factors specific to M. tuberculosis infection did not influence the priming of CD8 T cells, as codelivery of M. tuberculosis with BCG did not alter the magnitude of BCG-induced T cell activation. Following transfer to RAG-1−/− recipients, BCG and M. tuberculosis-induced CD8 T cells conferred equivalent levels of protection against M. tuberculosis infection. These findings demonstrate that BCG is able to prime functional CD8 T cells, and suggest that effective delivery of Ag to sites of T cell activation by vaccines may be a key requirement for optimal CD8 T cell responses to control mycobacterial infection. The Journal of Immunology, 2009, 182: 7172–7177.

Rational design of vaccines requires an understanding of the host determinants necessary for resistance to pathogens. An effective vaccine against Mycobacterium tuberculosis should mimic the native immune response to infection, generating a large repertoire of both CD4 and CD8 T cells responding to protective mycobacterial Ags. Although CD4 T cells are known to play a central role in protection against M. tuberculosis (1), there is mounting evidence that CD8 T cells are also important in antimycobacterial immunity. Mice deficient in CD8 T cells succumb rapidly to infection with M. tuberculosis (2), and CD8 T cells are both expanded during M. tuberculosis infection and recruited to sites of bacterial burden (2, 3). Vaccines that elicit CD8 T cells, such as viruses encoding mycobacterial Ags, can induce high levels of protective immunity (4, 5), and subunit vaccines inducing CD8 T cells can protect mice deficient in CD4 T cells from M. tuberculosis (6). The protective effect of CD8 T cells is most apparent late in tuberculosis infection in mice, suggesting this subset may be important in the control of latent infection with M. tuberculosis (7). Therefore an understanding of the factors that facilitate the activation of CD8 T cells is important for the development of effective strategies to control mycobacterial infections. M. tuberculosis is a facultative intracellular pathogen that resides within phagosomes of infected cells, thereby promoting Ag entry into the MHC-class II presentation pathway. M. tuberculosis infection of both humans and mice, however, leads to the generation of pathogen-specific CD8 T cell responses (8). Cross-presentation of mycobacterial Ags by dendritic cells (DCs) may play a role, as evidenced by the demonstration that transfer of apoptotic vesicles from infected macrophages to bystander DCs can stimulate CD8 T cell responses (9, 10). Early reports demonstrated that M. tuberculosis could directly deliver Ag to the class I processing pathway, while M. bovis bacille Calmette Guérin (BCG) was less able to activate CD8 T cells in these studies (11). This suggested egress into the cytoplasm from the endosomal compartment may be a property of M. tuberculosis infection that promotes CD8 T cell activation. Indeed, a recent report suggests that virulent M. tuberculosis and Mycobacterium leprae can translocate into the cytosol of infected DCs, possibly allowing Ags to be directly presented on MHC class I molecules, a property that was not shared by BCG (12). Differences in CD8 T cell activation by different mycobacterial strains may also be influenced by other factors associated with infection. M. tuberculosis infection elicits a substantial inflammatory response, characterized by the release of numerous inflammatory cytokines and chemokines (13). Proinflammatory cytokines play an important role in

1 Microbial Pathogenesis and Immunity Group, Discipline of Infectious Diseases, 2Centenary Institute of Cancer Medicine and Cell Biology, and 3Discipline of Medicine, University of Sydney, Sydney, Australia.

Received for publication May 27, 2008. Accepted for publication March 23, 2009.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This work was supported by the National Health and Medical Research Council of Australia. A.T. is supported by an NHMRC Career Development Award, and B.F. de S.G. is supported by an NHMRC Principal Research Fellowship. A.A.R., B.R., and J.K.N. are supported by Australian Postgraduate Awards and T.M.W. is the recipient of the University of Sydney Faculty of Medicine Postgraduate Scholarship.

Address correspondence and reprint requests to Dr. James A. Triccas, Discipline of Infectious Diseases and Immunology, University of Sydney, Sydney, Australia. E-mail address: janiet@infdis.usyd.edu.au

Copyright © 2009 by The American Association of Immunologists, Inc. 0022-1767/09/$2.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.0801694

†Microbial Pathogenesis and Immunity Group, Discipline of Infectious Diseases, Centenary Institute of Cancer Medicine and Cell Biology, and Discipline of Medicine, University of Sydney, Sydney, Australia.

Published ahead of print on April 18, 2009.
shaping the proliferation and maintenance of CD8 T cells in models of viral and bacterial infection (reviewed in Ref. 14). It is yet to be determined if inflammatory processes or mechanisms specific to M. tuberculosis infection contribute to the initiation of CD8 T cell response to mycobacteria.

To delineate the factors that influence early priming of CD8 T cells upon encounter of mycobacteria by the host immune response, we have compared the ability of the BCG vaccine or virulent M. tuberculosis expressing a recombinant Ag to induce the activation of Ag-specific CD8 T cells after infection of mice. We found that BCG was able to induce specific activation of CD8 T cells, but the response was delayed and of a lesser magnitude when compared with infection with M. tuberculosis. This reduced activation of T cells by BCG was associated with reduced bacterial load in the draining lymph nodes (DLN) at the time of T cell priming. Ag load was the key determinant of CD8 T activation after mycobacterial infection, as delivery of equivalent numbers of BCG and M. tuberculosis to the DLN resulted in equivalent CD8 T cell responses. Further, Ag-independent processes associated with M. tuberculosis infection did not alter the priming of CD8 T cells in response to BCG. BCG-induced CD8 T cells were fully functional, as infection with either BCG or M. tuberculosis induced CD8 T cells with an equivalent protective capacity with 10% oleic acid-albumin-dextrose-catalase enrichment (Difco).

LSR-II flow cytometer (BD Biosciences) and data analyzed using FlowJo software (TreeStar). The CFSE profile of dividing cells was analyzed as previously described (19, 20).

Detection of cytokine-producing cells

Single cell suspensions were prepared, resuspended at a concentration of 3 × 10⁹ cells/ml, and either left unstimulated or stimulated overnight with the OVA peptide SIINFEKL (10 μg/ml) and brefeldin A (10 μg/ml). Cell suspensions were washed with FACS buffer (2% FCS, PBS) and surface stained with CD8, CD45.1, or CD45.2 fluorochrome conjugates (BD Pharmingen). Following the surface staining, cells were washed with FACS buffer and permeabilized with Cytofix/Cytoperm buffer (BD Pharmingen). Cells were then washed in FACS buffer and intracellular cytokines were detected with anti-IFN-γ FITC and anti-TNF-PE conjugates (BD Pharmingen).

T cell transfer to RAG-1−/− mice and survival studies

C57BL/6 mice (n = 5) were infected s.c. with 1 × 10⁶ CFU BCG or 5 × 10⁹ CFU M. tuberculosis. Four weeks postinfection, the DLN were harvested and CD8 T cells were purified by positive selection using MACS separation (Miltenyi Biotec). A total of 10⁶ CD8 T cells were injected i.v. into RAG-1−/− mice. Purified CD8 T cells from naive mice were included as controls. One day posttransplant, mice were exposed to M. tuberculosis H37Rv (ATCC 27924) using a Middlebrook airborne infection apparatus (Glas-Col) with an infective dose of ~100 viable bacilli per lung (21). Infected mice were monitored daily and culled if they displayed signs of ill health, including reduced activity, ruffling of fur, and weight loss exceeding 15% of the weight loss of age-matched controls.

Statistical analysis

Statistical analysis of the log₁₀ transformed data was conducted using ANOVA. Fisher’s protected least significant difference ANOVA post hoc test was used for pair-wise comparison of multigroup data sets. For cumulative survival experiments, survival was calculated using a Kaplan-Meier nonparametric survival plot, and significance was assessed by the Mantel-Cox log rank test. Differences with p < 0.05 were considered to be significant.

Results

Differential priming of CD8 T cells in the draining lymph nodes following infection with BCG or M. tuberculosis

To examine in detail the priming of CD8 T cell after mycobacterial infection, we developed recombinant strains of M. tuberculosis or BCG that secrete a truncated form of the c-terminal region of the OVA protein (named M.tb:OVA or BCG:OVA, respectively). Western blotting of cell lysates and supernatants indicated that similar amounts of Ag were expressed by the two strains (data not shown). Mice were adaptively transferred with CFSE-labeled OT-I T cells, which recognize a class I epitope of OVA (22), and subsequently infected with equivalent doses of BCG:OVA or M.tb:OVA. At day 3 postinfection, M.tb:OVA infection had induced OT-I CD8 T cells (defined as CD45.2⁺CD45.1⁺CD8⁺ cells) to undergo rapid cell division and differentiation in the DLN, as assessed by CFSE levels and CD62L staining (Fig. 1A). In contrast, little proliferation of OT-I CD8 T cells was observed in naive mice or those infected with BCG:OVA. In M.tb:OVA-infected mice, ~9-fold more T cells were recruited into cell division on day 3 compared with BCG:OVA (Fig. 1B), and this was reflected in the larger proportion of cells of a CFSElow or CFSEbright phenotype after infection with M.tb:OVA (Fig. 1C). In addition, the total number of OVA-specific CD8 T cells was significantly greater in the DLNs of M.tb:OVA-infected animals at day 3 postinfection (Fig. 1D). By day 7 postinfection, however, the majority of OT-I CD8 T cells expressed a CFSElow differentiated phenotype after BCG:OVA infection (Fig. 1A), although the total number of OT-I CD8 T cells was 10-fold lower than in the M.tb:OVA group (Fig. 1F) and more cells remained CFSEbright after BCG:OVA (Fig. 1C). M.tb:OVA also resulted in a greater proportion and absolute number of OTI CD8 T cells expressing the effector cytokines IFN-γ and TNF in the DLN at day 7 postinfection (Fig. 1, D and E). Evaluation of the

Materials and Methods

Bacterial strains and growth conditions

Mycobacterial strains were grown in Middlebrook 7H9 broth with 10% albumin-dextrose-catalase enrichment (Difco). When required, the antibiotics kanamycin (25 μg/ml) and/or Hygromycin B (50 μg/ml) were added to liquid and/or solid medium for recombinant mycobacterial cultures. Mycobacteria were enumerated on Middlebrook 7H11 agar supplemented with 10% oleic acid-albumin-dextrose-catalase enrichment (Difco).

Animals

Male B6.SJL/PtpcrC (CD45.1), female C57BL/6 and RAG-1−/− mice aged 6–8 wk were purchased from the Animal Research Centre. OT-I transgenic mice (CD45.2) were a gift of Professor W. Heath (Walter and Eliza Hall Institute, Melbourne, Australia) and were bred in the Centenary Institute animal facility. Mice were maintained in specific pathogen-free conditions in the Centenary Institute animal facility under ethical approval from the Sydney University Animal Ethics committee.

Construction of recombinant mycobacterial strains

Construction of BCG secreting residues 230–359 of the OVA protein (BCG:OVA) has been previously described (15). To construct M. tuberculosis secreting the same OVA fragment, plasmid pLEX785 (17) was transformed into M. tuberculosis Ml103 strain (16). Expression of OVA230–359 was confirmed by Western blotting, using the 9E10 mAb specific for the c-myc epitope tag fused to the C-terminal end of the OVA fragment (17).

CFSE labeling, adoptive transfer, and infection of mice

Single cell suspensions of lymph nodes from male donor OT-I were prepared by collagenase and DNase treatment, and cells labeled with 5 μM 5- (and-6) -CFSE as previously described (18). Five × 10⁶ CFSE-labeled OT-I LN cells were injected i.v. into B6.SJL/PtpcrC host mice. One day posttransfer of CFSE-labeled cells, mice were infected via s.c. injection with varying doses of recombinant mycobacteria. On days 3 and 7 postinfection, the activation of transferred T cells in the DLN (popliteal, inguinal, para-aortic), spleen, and perfused lungs was analyzed by flow cytometry. Single cell suspensions of organs were prepared, incubated with anti-Fe-RHFI/II (clone 2.4G2) and stained with Abs for the following markers using appropriate fluorochromes and concentrations (BD Pharmingen): CD8 or CD4, CD44, CD62L, CD45.1, CD45.2. Samples were acquired using an LSR-II flow cytometer (BD Biosciences) and data analyzed using FlowJo.
number of bacteria present in the DLN after infection indicated there was more \textit{M.} \textit{tuberculosis} than \textit{BCG}:OVA present at both day 3 and 7 postinfection, and this difference was greatest at day 7 (Fig. 1G). Taken together, these results indicate that \textit{BCG} is able to induce the priming of CD8 T cells, although the response invoked by \textit{M. tuberculosis} was more rapid and of a greater magnitude.

**Circulation of activated CD8 T cells following \textit{BCG} or \textit{M. tuberculosis} infection**

We next compared the distribution and phenotype of activated OT-I CD8 T cells following infection with \textit{BCG} or \textit{M. tuberculosis}. No proliferation or recirculation of activated OT-I CD8 T cells was observed at day 3 in either the spleen or lungs of mice infected with the recombinant strains, suggesting the activation of the CD8 T cells was restricted to the DLN at this timepoint (data not shown). At day 7, infection with either \textit{BCG} or \textit{M. tuberculosis} led to migration of activated OT-I CD8 T cells from the DLN to the spleen and lungs (Fig. 2A). Predominately CFSE\textsuperscript{low} cells were present at these sites, consistent with the circulation of these activated cells following initial T cell priming in the DLN. Greater numbers of \textit{OVA}-specific CD8 T cells had circulated to the spleen and lungs after \textit{M. tuberculosis}:OVA infection when compared with infection with \textit{BCG}:OVA (Fig. 2, B and C), most likely due to the increased numbers of activated OT-I CD8 cells generated in the DLN by \textit{M. tuberculosis} (Fig. 1F).
after infection with BCG:OVA (Fig. 2, D and E). More pronounced down-regulation of CD62L on activated OT-I CD8 T cells after M. tuberculosis infection is consistent with an increased differentiation of the T cells after encounter with the pathogen. Similarly, in the spleen and lungs of mice, OVA-specific CD8 T cells activated in response to M.tb:OVA displayed a significantly greater proportion of CD44highCD62Llow cells than those present after BCG:OVA infection (Fig. 2E). These results indicate that infection with either BCG or M. tuberculosis results in CD8 T cell circulation to peripheral sites after activation in the DLN, and this effect is greater after encounter with M. tuberculosis.

The magnitude of CD8 T cell responses is governed by Ag load in the DLN at the time of T cell priming

We considered two possible causes for the differential priming of CD8 T cell responses by BCG and M. tuberculosis. First, increased Ag load at sites of T cell activation after M. tuberculosis infection may account for the differences observed. Alternatively, factors specific to M. tuberculosis may influence the response, such as mechanisms to facilitate entry to the MHC-I Ag-processing pathway (12), or the induction of inflammatory responses that are associated with M. tuberculosis infection (13). We first addressed the issue of Ag dose, as results from Fig. IA suggested an association between CD8 T cell activation and bacterial load. We delivered differing doses of BCG:OVA and M.tb:OVA to arrive at equivalent numbers of bacteria present in the DLN at day 7 postinfection. We determined that infection with 1 × 10^7 CFU of BCG:OVA and 5 × 10^7 M.tb:OVA led to ~1 × 10^8 CFU of each strain in the DLN at day 7 (Fig. 3A). After infection of mice, which had previously received CFSE-labeled OT-I CD8 T cells, with these modified doses of M.tb:OVA or BCG:OVA we observed that the proliferation of OT-I CD8 T cells in the DLN was identical between the two groups, both in terms of the division profile for each strain (Fig. 3B) and the total number of OT-I CD8 cells that were recovered from the DLN (Fig. 3C). In addition, a comparable proportion (Fig. 3D) and number (Fig. 3E) of IFN-γ^+TNF^+ CD8 OT-I cells were detected after infection with the modified doses of M.tb:OVA and BCG:OVA.

To determine whether factors relating specifically to M. tuberculosis infection may influence the priming of CD8 T cell responses, we codelivered wild-type M. tuberculosis (not expressing OVA) and BCG:OVA and assessed the impact on the induction of CD8 T cell responses directed against BCG. Codelivery of M. tuberculosis with BCG:OVA did not enhance the priming of specific CD8 T cells, as an equivalent response was observed in mice infected with BCG:OVA alone (Fig. 4A). Infection with an equivalent dose of M.tb:OVA led to greater proliferation of OT-I CD8 cells (Fig. 4B) and a markedly greater number of these cells in the DLN (Fig. 4C). Together, these data suggest that the differential Ag load in the DLN after infection with M. tuberculosis or BCG, rather than factors specific to M. tuberculosis infection, is the key determinant accounting for the differential priming of CD8 T cell responses in our model.

CD8 T cells from BCG- or M. tuberculosis-infected mice confer comparable survival against aerosol M. tuberculosis challenge

To determine whether the equivalent priming of OT-I CD8 T cells observed following high dose BCG or low dose M. tuberculosis infection...
infection translated into comparable abilities of activated CD8 T cells to protect against virulent *M. tuberculosis* challenge. CD8 T cells from *M. tuberculosis*- or BCG-infected mice were purified and adaptively transferred into T cell-deficient RAG-1–/– recipients. Mice were subsequently infected via the aerosol route with *M. tuberculosis* and survival of immunocompromised mice was monitored. RAG-1–/– mice that received CD8 T cells from naive donors or had received no T cells survived to ~50 days after *M. tuberculosis* challenge (Fig. 5). Transfer of CD8 T cells from mice immunized with BCG or *M. tuberculosis* resulted in RAG-1–/– deficient mice surviving significantly longer than control mice, with mean survival times of 125 and 130 days respectively (Fig. 5). No statistically significant difference was observed between mice that had received BCG- or *M. tuberculosis*-activated CD8 T cells. These results suggest that BCG is competent at generating protective CD8 T cell responses, and delivery of equivalent doses of BCG or *M. tuberculosis* to the DLN induced CD8 T cells with an equivalent functional capacity to protect against infection.

**Discussion**

The BCG vaccine has been unable to adequately control the spread of tuberculosis, however the immunological basis for the limited protective efficacy of the vaccine is unknown. In this report, we have addressed the long-held view that BCG is a poor inducer of CD8 T cells, which may limit the protective capacity of the vaccine. We provide two lines of evidence to suggest this may not be the case. First, we demonstrate that BCG can induce activation of Ag-specific CD8 T cells, with extensive proliferation of transferred OT-I CD8 T cells in BCG-vaccinated newborns (23), and the activation of CD8 T cells in animal models of BCG infection (24, 25). Second, we show that BCG-induced CD8 T cells can protect against *M. tuberculosis* infection, and these cells are as protective as T cells derived from *M. tuberculosis* infected animals (Fig. 5). This observation indicates that BCG and *M. tuberculosis* express a similar repertoire of protective CD8 T cell epitopes. Further, these data suggest that the absence in BCG of *M. tuberculosis*-specific proteins recognized by CD8 T cells may not be a major contributing factor to the variable efficacy of BCG in humans, despite that fact that some *M. tuberculosis*-specific Ags can induce protective CD8 T cell responses in animal models (26).

The initial priming of OT-I CD8 T cells after BCG infection was delayed and of a reduced magnitude when compared with infection with *M. tuberculosis* (Fig. 1), and this effect correlated with reduced Ag load in the DLN (Fig. 3). This may explain the previous observation of reduced generation of CD8 T cells recognizing the TB10.4 Ag of *M. tuberculosis* upon infection of mice with mycobacterial strains of differing virulence (25). These data, together with a recent study by Russell et al. (27), demonstrate that the well-documented dependence on Ag load for shaping the rate of CD8 T cell activation during viral and acute bacterial infections (28) also extends to chronic bacterial infection. There are a number of possible mechanisms that may account for the differences in CD8 T cell priming observed in our study. Cross-presentation of mycobacterial Ags via the transfer of apoptotic vesicles from infected macrophages to bystander DCs may be important (10). Macrophage apoptosis has been shown to be more pronounced after infection with *M. tuberculosis* compared with BCG (9), and this model would predict that Ag loads transferred to DCs would be greater after *M. tuberculosis* infection. Additionally, exogenous Ag can be cross-presented to CD8 T cells by at least two distinct intracellular pathways (29, 30). In the lysosomal model, Ag is transferred from the phagosome to the cytoplasm and presentation via MHC class I occurs by a proteosome and TAP-dependant process. In the vacuolar model, peptides generated in the phagosome are loaded on to MHC class I molecules and processing does not require TAP or trafficking through the endoplasmic reticulum. Ags such as OVA can be presented to CD8 T cells by the two pathways (31, 32), and both cytosolic and alternative models of Ag presentation have been shown to contribute to the recognition of mycobacterial Ags (33–35). It is possible that both these pathways contribute to CD8 T cell activation after BCG or *M. tuberculosis* infection, and the greater Ag load available for MHC class I loading after infection with *M. tuberculosis* results in improved T cell priming. However, BCG lacks genes contained in the region of deletion I (RD1), which is associated with cytotoxic entry by *M. tuberculosis* (12), and it is tempting to speculate that the absence of RD1 directs BCG-derived Ags to the vacuolar pathway of Ag presentation. This is particularly relevant as the vacuolar pathway appears less efficient than the phagosome-to-cytosol pathway in the generation of immune responses in vivo (30). It should be noted that the absence of RD1-encoded proteins does not appear to affect the capacity of DCs to present secreted *M. tuberculosis* Ags to human CD8 T cells via the lysosomal pathway (35), however detailed comparison of the precise Ag processing and presentation mechanisms used to activate either BCG- or *M. tuberculosis*-reactive CD8 T cells has yet to be performed.

Our results demonstrate that the presence of *M. tuberculosis* itself did not appear to influence the rate of priming of BCG-OVA-reactive CD8 T cells (Fig. 4), despite the fact that *M. tuberculosis* induces significant inflammatory response after infection. This was confirmed by an increased cellular infiltrate in the DLNs of *M. tuberculosis*/BCG-OVA infected mice at day 7 postinfection as compared with those infected with BCG-OVA (data not shown). Inflammatory cytokines, such type-I IFNs or IL-12, play an important role in determining the extent of CD8 T cell expansion during viral infection (36, 37). The magnitude of CD8 T cell expansion is programmed early after Ag encounter (28), and the level of inflammatory cytokines induced very early after *M. tuberculosis* coinfection with BCG may not be sufficient to influence T cell priming. How inflammatory processes influence other stages of the CD8 T cell response after mycobacterial infection, such as the extent of T cell contraction and the generation of protective memory T cell responses, is currently under investigation in our laboratory.

Protection against *M. tuberculosis* relies on the ability of Ag-specific T cells to respond in the lung upon secondary exposure to Ag (38), and as such analysis of the distribution and differentiation of activated T cells following vaccination may reveal important correlates of the protective response. After infection of mice with either BCG or *M. tuberculosis* activated T cells circulate rapidly to other sites such as the spleen and lung, however a greater number of cells were detected after infection with *M. tuberculosis* (Fig. 2). Indeed, at the timepoint analyzed, the number of OT-I CD8 in the lung after BCG/OVA was not significantly greater than that seen in noninfected mice (Fig. 2C). This may reflect one limitation of BCG vaccination, however it remains to be determined how these low numbers of Ag-experienced cells respond to secondary stimulation with Ag. Ag-specific CD8 effector cells accumulate in the lung more rapidly than naive cells in response to pulmonary influenza challenge (39), and we are currently investigating whether differences in the capacity of candidates tuberculosis vaccines to invoke expansion and migration of effector T cells to the lung correlates with protective efficacy against *M. tuberculosis*. We also observed that unlike infection with *M. tuberculosis*, infection with BCG did not result in dissemination of the vaccine
The authors have no financial conflict of interest.

Disclosures

We thank Prof. W. Heath (Walter and Elisa Hall Institute, Melbourne, Australia) for providing the OT-1 transgenic mice.

References


