Influence of *Mycobacterium bovis* BCG Vaccination on Cellular Immune Response of Guinea Pigs Challenged with *Mycobacterium tuberculosis*\(^\text{V}\)

Diane Ordway,\(^*\) Marcela Henao-Tamayo, Crystal Shanley, Erin E. Smith, Gopinath Palanisamy, Baolin Wang, Randall J. Basaraba, and Ian M. Orme

Mycobacteria Research Laboratories, Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, Colorado 80523

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*Mycobacterium bovis* bacillus Calmette-Guérin (BCG) currently remains the only licensed vaccine for the prevention of tuberculosis. In this study, we used a newly described flow cytometric technique to monitor changes in cell populations accumulating in the lungs and lymph nodes of naïve and vaccinated guinea pigs challenged by low-dose aerosol infection with virulent *Mycobacterium tuberculosis*. As anticipated, vaccinated guinea pigs controlled the growth of the challenge infection more efficiently than controls did. This early phase of bacterial control in immune animals was associated with increased accumulation of CD4 and CD8 T cells, including cells expressing the activation marker CD45, as well as macrophages expressing class II major histocompatibility complex molecules. As the infection continued, the numbers of T cells in the lungs of vaccinated animals waned, whereas the numbers of these cells expressing CD45 increased. Whereas BCG vaccination reduced the influx of heterophils (neutrophils) into the lungs, an early B-cell influx was observed in these vaccinated animals. Overall, vaccine protection was associated with reduced pathology and lung damage in the vaccinated animals. These data provide the first direct evidence that BCG vaccination accelerates the influx of protective T-cell and macrophage populations into the infected lungs, diminishes the accumulation of nonprotective cell populations, and reduces the severity of lung pathology.

Well over 2 million people now die each year from tuberculosis (5–7, 9, 12, 23). At present, the only available vaccine against tuberculosis, *Mycobacterium bovis* bacillus Calmette-Guérin (BCG), has proven unreliable in being able to protect against pulmonary tuberculosis in adults (2, 4, 32). To date, the specific activity of BCG has been thought to lie in its ability to generate a state of immunological memory in the host and thus accelerate the emergence of a TH1 protective response upon infection (8, 18, 32). However, recent studies seem to imply that the ability of BCG to induce stable memory T-cell populations may be lacking (19). Hence, further research into the actual immunological activity of BCG is warranted to guide rational vaccine design.

The two small animal models used most often for preclinical tuberculosis vaccine screening are the low-dose aerosol mouse and guinea pig models (1, 25, 26, 31, 33). The majority of this work has been carried out in various mouse models due to their low cost and the wealth of immunological reagents (32). Low-dose aerosol infection of the guinea pig with *Mycobacterium tuberculosis* produces a well-characterized disease that shares important morphological and clinical features with human tuberculosis (25, 26). The ability to precisely characterize the protective immune response induced by BCG during *M. tuberculosis* infection in the guinea pig would greatly improve the usefulness of this animal model for the testing and evaluation of urgently needed new vaccines.

Effective resistance to *Mycobacterium tuberculosis* infection is mediated by both innate and adaptive mechanisms of immunity (8). After pulmonary infection with *M. tuberculosis*, alveolar macrophages and dendritic cells phagocytose bacilli, and it is thought that dendritic cells carry both intact bacteria and their antigens to draining lymph nodes, where recognition by T cells generates cell-mediated immunity (3, 10, 11, 24, 29). Macrophages present antigen via both class I and class II major histocompatibility complex (MHC) molecules, resulting in effector CD4 and CD8 T cells which secrete cytokines, including gamma interferon (IFN-\(\gamma\)), giving rise to macrophage activation and intracellular killing of the organism (8, 17, 22, 28, 34). The development of a pulmonary granuloma is orchestrated by both chemokines and cytokines, resulting in a continuous recruitment of lymphocytes, granulocytes, macrophages, dendritic cells, and monocytes to the local site of the infection (37, 38). Control of the infection relies on this granulomatous response, which is an organized cellular network acting to restrain mycobacterial growth and to limit dissemination.

The progression of tuberculosis in guinea pigs can be divided into acute, subacute, and chronic stages of infection, based on the pattern of bacterial growth and dissemination as well as patterns of pulmonary and extrapulmonary pathology (37, 38). The acute phase consists of a 2-week period of rapid bacterial growth and dissemination of the infection to draining lymph nodes. The subacute phase runs from approximately week 2 to week 4 and is characterized by the emergence of a stationary phase of bacterial replication. The primary granuloma develops during the acute and subacute stages of infection and has

\(^*\) Corresponding author. Mailing address: Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO 80523-1682. Phone: (970) 491-7469. Fax: (970) 491-5129. E-mail: D.Ordway-Rodriguez@colostate.edu.

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a characteristic central core of necrosis which may become mineralized over time. Finally, while the actual growth of the infection is relatively slow, the chronic stage in guinea pigs is characterized by the development of necrosis and secondary lesion progression, killing the animal in about 100 to 150 days.

In this study, we used a new flow cytometric approach (30), combined with immunohistochemistry, in order to characterize how BCG vaccination alters the immune responses toward \textit{M. tuberculosis} infection. The vaccinated guinea pigs were able to contain and control \textit{M. tuberculosis} growth at the peak of acquired immunity more rapidly than were unvaccinated control guinea pigs. The lung granulomas present in these BCG-vaccinated guinea pigs were smaller than those in control animals, with less parenchymal inflammation and slower progression of lung pathology. There was an earlier influx of CD4$^+$ and CD8$^+$ T cells expressing the activation marker CD45$^+$ in the immune animals. In addition, BCG-vaccinated animals showed an early presence of increased numbers of macrophages upregulating MHC class II molecules. During chronic infection, BCG vaccination reduced the influx of heterophils (neutrophils) into the lungs, which has been associated with tissue damage. These data provide the first direct evidence that BCG vaccination induces both qualitative and quantitative immunological differences which can be related to bacterial growth and lung pathology.

**MATERIALS AND METHODS**

Guinea pigs. Female outbred Hartley guinea pigs (~500 g in weight) were purchased from the Charles River Laboratories (North Wilmington, MA) and held under barrier conditions in a biosafety level III animal laboratory. The specific-pathogen-free nature of the guinea pig colonies was demonstrated by testing sentinel animals. All experimental protocols were approved by the Animal Care and Usage Committee of Colorado State University.

Experimental infections in guinea pigs. Guinea pigs were challenged using a \textit{Mycobacterium tuberculosis} H37Rv at a low-dose aerosol of 20 bacilli. Animals were then assayed for lung, mesenteric lymph node, and spleen bacterial loads, histology, and cell homogenates for flow cytometric analysis on days 5, 20, 30, 60, and 90 of the infection. Bacterial counts in the organs of guinea pigs (\(n = 4\)) at each time point of the study were determined by plating serial dilutions of homogenates of lungs on nutrient \(7 \times \text{H}11\) agar and counting CFU after 3 weeks of incubation at 37°C.

**BCG vaccination.** Groups of four animals were vaccinated with BCG Pasteur injected by the intratracheal route at a dose of 10$^6$ viable bacilli and then rested for 1 month prior to aerosol challenge. No adverse reactions at the injection site were noted after injection of BCG.

**Histological analysis in guinea pigs.** The lung lobes and lymph nodes from each guinea pig were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS). Sections from these tissues were stained using hematoxylin and eosin and the Ziehl-Neelsen stain for acid-fast bacilli. In guinea pigs, the concurrent progression of lung and lymph node lesions was evaluated using a histological grading system (38). The method for grading granulomatous lesions was based on inflammatory cell numbers and their infiltrative distribution pattern in the organs assayed. Briefly, scoring of the pathology of lung sections to provide reproducible day 1 animal necropsy and plating of whole-organ infectivity. Bacterial counts in the organs of guinea pigs (\(n = 4\)), with standard errors of the means. A parametric method, Student’s \(t\) test, was used to assess statistical significance of differences between groups of data.

**Flow cytometric analysis of cell surface markers.** Single-cell suspensions from the lungs and portions of the whole spleens and lymph nodes were prepared as recently described (30). Thereafter, cell suspensions from each individual guinea pig were incubated first with antibodies to CD4 (clone FITC CT7) (36), CD8 (clone FITC CT6) (36), pan-T cells (clone APC CT5) (35), CD45 (clone RPE Cl.13.I) (36, 39) at 4°C for 30 min in the dark, after washing of the cells with PBS and incubated with the secondary detection antibody F(ab')3 rabbit anti-mouse conjugated to horseradish peroxidase (SeroSorb Inc., Raleigh, NC). Finally, the reaction was developed using aminoethylcarbazole (BioGenex, San Ramon, CA) as a substrate. The sections were counterstained with Meyer's hematoxylin and thereafter mounted with Crystal/Mount (BioGenex, San Ramon, CA). Our experiments utilized different lung lobes of the guinea pig for immunohistochemistry and flow cytometry because we have shown that the lesions are evenly distributed throughout the infected guinea pig lung by evaluating the histopathology and magnetic resonance imaging of pulmonary lesions in guinea pigs (21).

**Organ cell digestion.** To prepare single-cell suspensions, the lungs and lymph nodes were perfused with 20.0 ml of a solution containing PBS and heparin (50 U/ml in Sigma-Aldrich, St. Louis, MO) through the pulmonary artery, and the caudal lobe was aseptically removed from the pulmonary cavity, weighed, placed in medium, and dissected. The dissected lung tissue was incubated with complete Dulbecco’s modified Eagle’s medium containing collagenase XI (0.7 mg/ml; Sigma-Aldrich) and type IV bovine pancreatic DNase (30 U/ml in Sigma-Aldrich) and type IV bovine pancreatic DNase (30 U/ml; Sigma-Aldrich) for 30 min at 37°C. The digested lungs were further disrupted by gently pushing the tissue twice through a cell strainer (BD Biosciences, Lincoln Park, NJ). Red blood cells were lysed with ACK buffer, washed, and resuspended in complete Dulbecco’s modified Eagle’s medium. Total cell numbers per lung and lymph node were determined by using a hemocytometer and then calculating the cell number per 1.0 g of tissue.

**RESULTS**

BCG vaccination slows the growth of challenge infection over the early stages of the disease process. Guinea pigs were exposed to approximately 20 bacilli of \textit{M. tuberculosis}, based on reproducible day 1 animal necropsy and plating of whole-organ...
homogenates on agar plates. These guinea pigs were evaluated for bacterial loads in the lungs and lymph nodes at the indicated time points (Fig. 1). Control guinea pigs showed an increase of approximately $5.5 \log_{10}$ in lungs over the first 20 days of infection (Fig. 1A), followed by a chronic phase of disease. A similar rise in numbers to those seen in the lungs was observed in the draining lymph nodes (mediastinal lymph node cluster) (Fig. 1B).

FIG. 1. Bacterial growth in the lungs and draining lymph nodes of control and vaccinated guinea pigs infected with *M. tuberculosis*. Bacterial growth in organs from control ($n = 4$) (circles) and vaccinated ($n = 4$) (squares) guinea pigs receiving a low-dose aerosol of *M. tuberculosis* was assayed in the lungs (A) and lymph nodes (B). Groups of control and immune *M. tuberculosis*-infected guinea pig organs were assayed for bacterial loads on days 5, 20, 30, 60, and 90 postchallenge. Results are expressed as the mean $\log_{10}$ bacilli (CFU) ± standard error of the mean (SEM) ($n = 4$). (C and D) The degree of histopathology was determined using a lesion scoring system (36) that showed the significant extent of lung and lymph node disease in the controls compared to that in the vaccinated animals. *, $P \leq 0.05$ (Student's *t* test).
As expected, BCG vaccination reduced the growth of *M. tuberculosis* in the lungs and lymph nodes 20 days after infection. The mean lung log_{10} CFU of *M. tuberculosis* from immune animals (Fig. 1A) was significantly reduced compared to that for control animals, as was the mean log_{10} CFU recovered from the lymph nodes (Fig. 1B).

Figure 1C and D show mean lesion pathology scores for the lungs and lymph nodes of control and vaccinated animals. In controls, the severity of the pathology in both tissues (Fig. 1C and D) worsened progressively over time, with a considerable surge after day 30, as we previously also showed, due to progression of secondary lesions (30). In contrast, this increased at a much lower rate in vaccinated animals, particularly in the lungs.

**Differences in granulomatous responses between control and vaccinated guinea pigs.** Consistent with earlier observations (30), on day 5 of the infection the lung lesions consisted of small aggregations of resident cells close to major airways and blood vessels (Fig. 2A, panel A). By day 30, lesions in the lungs and lymph nodes consisted of foci of mixed inflammation with areas of central necrosis (arrows) composed of nuclear and cytoplasmic debris, whereas this necrosis was not observed in either organ in vaccinated animals. By day 90, lesions in control animals had progressed to form multifocal to coalescing inflammation that effaced large areas of tissue, in contrast to the minimal tissue damage seen in the vaccinated guinea pigs. In controls, substantial dystrophic mineralization was taking place (arrows), whereas in vaccinated animals there was minimal necrosis and abundant functional tissue remaining (arrow). Bars, 20 μm.

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guinea pigs than in those of controls. This early increased in T-cell numbers observed in the vaccinated animals was coincident with an observed decline in bacterial numbers in the vaccinated group. However, in control animals, by day 30, CD4<sup>+</sup> and CD8<sup>+</sup> T-cell numbers peaked, reflective of the progression of secondary lesions in this group. This increase of CD4<sup>+</sup> and CD8<sup>+</sup> T-cell numbers in the lungs of control animals was not sustained, however, and declined after 30 days. Despite this, numbers of these cells still remained higher in the vaccinated animals than in controls during the chronic stage of the disease.

The upregulation of CD45 (leukocyte common antigen) molecules on T cells indicates signal transduction of antigen receptor signaling during immune responses and hence is an indication of T-cell activation (13, 36). We took advantage of an available monoclonal antibody to evaluate the percentage of cells expressing CD45 on CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the BCG-vaccinated animals. Results are expressed as the mean cells/1.0 g of tissue of each analyzed cell population ± SEM (n = 4). *, P ≤ 0.05 (Student’s t test) compared to H37Rv-infected control guinea pigs.

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As shown in Fig. 3C and D, CD45 was upregulated on both CD4$^+$ and CD8$^+$ cells much more rapidly in the vaccinated animals, and these levels were sustained through day 90 of the experiment. Small rises in CD45 expression were observed on CD4$^+$ and CD8$^+$ T cells in control guinea pigs over the first month in both the lungs (Fig. 3C) and lymph nodes (Fig. 3D), but this was not sustained. As shown in Fig. 3G, the flow cytometric dot plots for BCG-vaccinated guinea pigs showed early expression of CD45 on CD4 and CD8 T cells compared to that of the control animals. It is evident that there is more T-cell CD45 expression observed utilizing this gating technique and that it may include some unidentified fine populations of cells, such as NK or γδ T cells, which coexpress CD4$^+$ and CD45$^+$, or dendritic cells coexpressing CD8$^+$. Flow cytometry in the guinea pig is a newly developed technique, and with the development of more commercially available flow cytometric reagents, identification of these fine populations which coexpress multiple markers will be possible.

**Increased numbers of macrophages in the lungs of vaccinated animals after challenge infection.** We used the monoclonal antibody MR-1 to track macrophages in the lungs and draining lymph nodes (20). This technique utilizes SSC versus FSC gating on the viable granulocyte population demonstrated in Fig. 4C and on the isotype control (Fig. 4D). As shown in Fig. 4A and B, there was a substantial increase in macrophages in the lungs and lymph nodes of vaccinated guinea pigs. As shown in Fig. 4E, the flow cytometric dot plots for BCG-vaccinated guinea pigs showed increased expression of MHC class II on MR-1$^+$ macrophages compared to that for the control animals. Interesting, only small numbers of macrophages in the lungs or lymph nodes of control animals stained positive for MHC class II (39). In vaccinated animals, the...
presence of macrophages in the lungs was much earlier and more prominent, with most cells expressing MHC class II. A similar pattern was seen in the lymph nodes, but in this case, only about 25% were positive for MHC class II.

**Kinetics of influx of B lymphocytes and heterophils.** We previously showed that both B cells and heterophils enter lung lesions, accumulating in number after a decline in CD4 cells seen in naïve infected animals (30). To determine if these cells could still accumulate in vaccinated animals, we analyzed tissues by flow cytometry, using SSC<sub>low</sub> versus MIL4<sub>neg</sub> gating, to clearly delineate the MIL4<sup>+</sup> and B-cell populations. As shown in Fig. 5A and B, we unexpectedly observed a rapid increase in the number of B cells in the lungs over the first 30 days of infection in the vaccinated guinea pigs, which then flattened. In controls, B-cell numbers remained low through day 30 and then increased progressively, consistent with our early studies (30). As shown in Fig. 5C, the flow cytometric dot plots for BCG-vaccinated guinea pigs showed more B cells than did those for control animals during subacute infection. An increase in heterophil (MIL4<sup>+</sup>) numbers was seen in controls; this was diminished by vaccination. A similar kinetics was seen in the draining lymph node cluster (Fig. 5B), although in this case the influx of heterophils in control animals was earlier, consistent with the rapid necrosis seen in these tissues in control animals. As shown in Fig. 5D, the flow cytometric dot plots for BCG-vaccinated guinea pigs showed fewer heterophils than those for the control animals during chronic disease.

**Positioning of T cells revealed by immunohistochemical staining.** Having established the cell numbers in the lungs and lymph nodes, in a final series of studies we used immunohistochemical staining of lung sections to determine the actual organ distribution of these cell types (Fig. 6).

On day 30 of the infection, aggregates of CD4 cells were observed in large rims surrounding the developing central necrotic core of the primary lesions in the lungs (Fig. 6A and B). These aggregates were less prominent in primary lesions in vaccinated animals (Fig. 6C and D) (secondary lesions [data not shown] were far less prominent in these animals, indicating that they were prevented by the vaccination process, as suggested by our previous pathological evaluation of histology and magnetic resonance imaging data [21]). In control animals, CD8 cells were also detected, but they were more scattered and more toward the periphery of the lesions (Fig. 6E and F). A similar distribution was seen in vaccinated animals, with fewer CD8<sup>+</sup> cells again forming a diffuse rim on the edges of the lesions (Fig. 6G and H). Macrophages were widely distributed in clusters (consistent with “sheets” of epithelioid macrophages found in the granuloma) but were far more prominent in the vaccinated animals (Fig. 6K and L), consistent with the more rapid influx indicated by the flow cytometric data.
Again consistent with that analysis, B cells were scattered across lesions in control animals, mostly on the peripheries of lesions (Fig. 6M and N), but were more obvious in vaccinated animals as loose clusters of cells (Fig. 6O and P). As we reported before (24), heterophils were associated almost exclusively with the areas of central necrosis (Fig. 6Q to T) and were increased in control animals.

**DISCUSSION**

The results of this study show that the protective properties of BCG vaccination at the peak of acquired immunity result in the generation of immunity in the lung, leading to a reduction in bacterial growth of about 1 log (15). In addition, the protective properties of BCG are associated with reduced lesion scores, indicative of reduced number and size of granulomas, combined with less T-cell infiltration and parenchymal inflammation, a finding which is directly associated with bacterial containment. The immune guinea pigs also showed a delay in secondary granuloma progression in the lungs. These results are supported by other murine and human studies showing that reduced granuloma size is associated with immune responses able to control bacterial growth (29).

One central finding in this study is that immune animals
show a substantial increase in CD4 T-cell numbers during the acute and subacute phases of infection, which thereafter level off as bacterial control is established (many of the cells expressed the activation marker CD45), compared to those in the control animals. We observed a twofold increase in CD4 cells in the lungs by day 5 of the challenge infection in the vaccinated guinea pigs, an observation reminiscent of our earlier studies with the mouse model (19). In addition, an early increased CD8 T-cell response was also seen in the vaccinated guinea pigs, although this occurred more slowly than the CD4 response. These flow cytometric observations were confirmed by immunohistochemical staining of lung tissues showing that as BCG-vaccinated animals establish bacterial control, fewer CD4+ and CD8+ T cells are present in immune animals than in controls. Murine studies (18, 19) have shown that reduced numbers of bacteria in BCG-vaccinated mice infected with M. tuberculosis are associated with a preexisting pulmonary Th1 response characterized by fewer numbers of CD4+ T cells producing IFN-γ. Immune guinea pigs have been shown to produce protective cytokines, such as IFN-γ and tumor necrosis factor alpha, during the subacute phase of infection (25, 26, 27). However, in contrast to murine models, in the guinea pig this rapid increase in CD4 T-cell numbers is followed by CD4+ T-cell decline and resurgence of disease.

Staining with an antibody to CD45 provided insight into activated T-cell numbers, potentially indicating antigen specificity (8). Increased numbers of these cells remained sustained in vaccinated animals, both within the lungs and, interestingly, in the lymph nodes, despite the increasing pathological damage in the latter tissues. Thus, one of the protective properties of BCG vaccination may be the ability of T cells to upregulate and express CD45. Although we lack the reagents as yet to be more definitive, these observations do allow us to hypothesize that BCG causes a more rapid focusing of effector T cells into the infected tissues and that while the total numbers of CD4 and CD8 cells clearly drop, the numbers of CD4+ activated cells appear to be sustained. A similar situation is suggested by observations in the mouse model (19).

We still do not fully understand why the CD4+ and CD8+ T-cell numbers decline during chronic disease. However, we know that the drop in CD4+ and CD8+ T-cell numbers appears to represent a true effect and is not caused by the tissue processing technique or by dilution by other cell types entering the granulomas. Our previous studies (38) have confirmed this, as the lymphocyte “mantle,” an early characteristic of the guinea pig granuloma, showed a reduction in the intensity of immunohistochemical staining after day 30. This was further confirmed in subsequent flow cytometric and immunohistochemical studies (30) showing that CD4 cells took up a position surrounding the developing core of necrosis, but by day 60 this layer was smaller and staining for CD4 was more diffuse. We know that in the draining lymph nodes the early T-cell response was much more rapid but then dropped considerably during chronic infection as necrosis developed in this organ. Also, the draining lymph nodes are the first extrapulmonary sites to encounter the bacilli and the first site of rapidly progressive destructive pathology (37, 38). The dendritic cells in the draining lymph nodes are responsible for antigen presentation to naïve T cells and for T-cell priming (8, 10, 24). We hypothesize that in chronic infection the rapid involvement and destruction of the draining lymph nodes may cause dendritic cells to become unable to function as antigen-presenting cells, eliminating T-cell priming and causing these cells to be depleted in the lungs during the chronic infection. Presently, a commercially available antibody does not exist for dendritic cells in the guinea pig model, and thus, we are currently using cross-reactive dendritic cell markers to further investigate this hypothesis.

Another important finding is the early increase in numbers of macrophages present in the lungs of the vaccinated guinea pigs. We hypothesize that macrophage influx into the lungs is due to the production of recruiting chemokines and to appropriate integrin expression on local blood vessel walls, which may be very early events in these animals. Moreover, most of these cells stained positive for expression of class II MHC molecules. In control animals, in contrast, the response was much smaller and slower. Moreover, very few of these cells expressed class II molecules, even by day 90. A faster influx of macrophages in vaccinated guinea pigs was also observed in the lymph nodes, but in stark contrast, in this organ macrophages in both vaccinated and control groups poorly expressed class II molecules. Thus, these results together support the hypothesis that a protective property of BCG vaccination in guinea pigs is that macrophages have a better ability to activate and to function as antigen-presenting cells by upregulation of MHC class II molecules. It is known that M. tuberculosis infects macrophages and that these cells are capable of eliminating bacteria very efficiently after activation by IFN-γ (24, 27, 29). Until we can directly measure IFN-γ secretion by T cells, we cannot explain this, but it suggests that the T cells capable of entering the lymph nodes are either not making IFN-γ (we can only measure expression as yet) or are not getting close enough to these macrophages to activate them. The second option seems more likely, especially given the difficulty that T cells probably encounter in moving through the lymph node tissues due to the rapid development of necrosis.

BCG-vaccinated guinea pigs showed reduced numbers of heterophil influx during chronic infection compared to those in controls. We further confirmed this by immunohistochemical staining of heterophils, which were localized to foci of central necrosis in the primary lesions and were reduced in BCG-vaccinated animals compared to those in controls. Previous studies (30) have shown that large numbers of heterophils are present within airway lumens associated with secondary lesions during the chronic stage of the disease. One of the protective properties of BCG vaccination is that secondary granuloma formation is delayed, and this could be the reason for a reduction in heterophils. We previously suggested (30) that the presence of heterophils in the foci of the primary lesion may be related directly to lesion necrosis and may reflect the continual degranulation of heterophils in these lesions. In addition, heterophils are directly related to Th2 immunity and are the cell phenotype responsible for the immunopathogenesis of asthma and allergic rhinitis (30, 39). Therefore, one protective property of BCG vaccination in the guinea pig model may be to limit heterophil accumulation in primary lesions and airway lumens associated with secondary lesions, resulting in reduced tissue necrosis.
Unexpectedly, we observed a rapid influx of B cells early into the lungs of the vaccinated guinea pigs, which then ceased. We speculate that the observed B-cell influx may simply be due to integrin expression on blood vessels, as B cells produce proinflammatory cytokines and express chemokine receptors, such as CXC5 and CCR7, that may promote their migration to inflammatory sites (30). However, in the mouse model at least, there is no evidence that these cells aid or interfere with protective immunity (8), although they are found in large sheets in the granulomas in murine tuberculosis (8).

We propose that this new information on the protective properties of BCG vaccination in the guinea pig model could act as a novel template with which to compare the efficacies of new vaccine candidates in this important animal model. Potential surrogate markers for protection in the guinea pig model could be early increased CD45 expression on CD4 macrophages as a measure of vaccine efficacy. One advantage of this would be to shorten experiments by focusing on the subacute phase of infection rather than waiting for the chronic disease on days 60 and 90. Our study showed that another protective property of BCG vaccination in this model is reduced heterophil influx during chronic infection. The presence of reduced heterophils could be utilized as a potential surrogate marker for protection in post-M. tuberculosis exposure vaccine testing.

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