Immune Response to Postprimary Tuberculosis in Mice: *Mycobacterium tuberculosis* and *Mycobacterium bovis* bacille Calmette-Guérin Induce Equal Protection

Hans-Joachim Mollenkopf,* Mischo Kursar, and Stefan H. E. Kaufmann
Department of Immunology, Max-Planck-Institute for Infection Biology, Berlin, Germany

We addressed the question of whether protective immunity induced by natural infection with *Mycobacterium tuberculosis* and that induced by vaccination with *Mycobacterium bovis* bacille Calmette-Guérin (BCG) differ in the murine model. We infected mice with *M. tuberculosis* Erdman, cured them by chemotherapy, and subsequently reinfected them with a low dose of *M. tuberculosis* H37Rv. The course of tuberculosis was compared with that in mice previously vaccinated with BCG Danish 1331. Protection against postprimary *M. tuberculosis* infection did not differ significantly between the 2 groups. After challenge infection, numbers of interferon-γ–positive splenocytes did not differ between mice with primary infection and vaccinated mice. Splenocytes from primary *M. tuberculosis*–infected mice conferred marginally higher protection than did those from BCG-vaccinated mice. Serum transfer did not protect against reinfection in either group. Our data emphasize that natural infection with *M. tuberculosis* and vaccination with BCG do not differ in their capacity to induce protective immunity against tuberculosis and support the notions that reinfection contributes to the development of active disease and that any novel vaccine against tuberculosis has to perform better than both vaccination with BCG and immunity evoked by natural infection.

Tuberculosis caused by the intracellular pathogen *Mycobacterium tuberculosis* is one of the leading causes of morbidity and mortality worldwide [1]. Since the first isolation of the tubercle bacillus by Robert Koch in 1882, different animal models have been applied [2, 3]. Although the guinea pig model has the advantage of a pathological process similar to that in human pulmonary tuberculosis, the mouse provides the best immunologically characterized model [4, 5].

A vaccine against tuberculosis was developed by Calmette and Guérin at the beginning of the last century [6]. The bacille Calmette-Guérin (BCG) vaccine is an attenuated strain of *Mycobacterium bovis* and represents the world’s most widely used live vaccine, with >2 billion vaccinations performed to date [7]. BCG protects against miliary tuberculosis in children [8] but fails to consistently protect against pulmonary tuberculosis in adults, the most prevalent form of the disease [9–11], with a variability of 0%–80% [8, 12–14]. This variability could be due to genetic variation of vaccinated individuals, unique features of environmental mycobacteria in different parts of the world, differences in vaccination schedules, or the use of different BCG strains and dosages [10, 11, 15–21].

It is generally assumed that postprimary active tuberculosis is primarily caused by reactivation of endogenous infection with dormant *M. tuberculosis*, rather than by exogenous infection [22–24]. *M. tuberculosis* is a successful pathogen because of its ability to persist in the
host for long periods of time in face of an ongoing immune response [25–30]. Indeed, one-third of the world’s population is estimated to be infected with *M. tuberculosis*, but only 10% of infected individuals ever develop the disease. This phenomenon could be taken as evidence that natural infection provides sufficient protection against reinfection in a significant proportion of infected individuals. More-recent evidence emphasizes that a considerable number of cases of active tuberculosis are caused by exogenous reinfection, suggesting that immunity to natural primary infection fails to provide satisfactory protection. We addressed this issue in a murine model by comparing postprimary tuberculosis in mice previously infected with *M. tuberculosis* or vaccinated with BCG.

**MATERIALS AND METHODS**

**Bacterial strains and cultures.** *M. tuberculosis* H37Rv, *M. tuberculosis* Erdman, and *M. bovis* BCG Danish 1331 (Statens Serum Institute) were grown in Middlebrook 7H9 broth (Difco) supplemented with Middlebrook albumin, dextrose, and catalase (ADC) enrichment (Difco). After passage through mice, strains were grown on Middlebrook 7H11 agar supplemented with oleic acid and ADC enrichment 1339 (Difco) and, subsequently, on Middlebrook 7H9 liquid medium containing ADC enrichment (Difco), under shaking for ∼14 days, until bacterial growth reached an OD$_{600}$ of ∼0.7, equivalent to a cell density of ∼10$^8$ cells/mL. Bacteria were harvested by centrifugation, washed with PBS without Ca$^2+$, and maintained in 10% glycerol at −70°C until use.

**Animals.** BALB/c mice were bred at the central animal facilities of the Max-Planck-Institute for Infection Biology at the Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (Berlin, Germany). Mice were kept under specific pathogen–free conditions and fed autoclaved food and water ad libitum. Female mice were used at age 8 weeks. Groups of at least 5 mice were used in all experiments. Animal experiments were approved by the Landesamt für Arbeitsschutz, Gesundheitsschutz, und technische Sicherheit (LAGetSi Fachgruppe 5.2, Veterinärwesen, Berlin, under the registration number G0252/00).

**M. bovis BCG vaccination and M. tuberculosis infection.** Mice were vaccinated with 5 × 10$^5$ live *M. bovis* BCG by intravenous (iv) injection into the lateral tail vein. Primary *M. tuberculosis* infection (iv) was performed in a parallel group of mice with 5 × 10$^5$ live *M. tuberculosis* Erdman organisms. For up to 21 weeks, mice were treated with rifampicin (100 mg/L) and isoniazide (200 mg/L) in the drinking water, with the treatment starting at 2 weeks after vaccination or infection. Cure was verified by enumeration of bacterial titers at different time points after vaccination or infection. At week 24 (day 0), all groups (cured mice, mice that received serum or splenocytes, and naive control mice) were challenged by use of an aerosol exposure system (Glas-Col) using an infection dose of 100–200 bacilli/lung. Mice were killed at different time points after challenge, and spleens and lungs were removed aseptically. Organ homogenates were prepared, and colony-forming unit counts were determined. For control purposes, primary-infected or vaccinated mice were treated as described above and then were treated subcutaneously with dexamethasone (Sigma) at week 32. Reactivation of *M. tuberculosis* from this group was determined at days 90 and 120 by analysis of colony-forming unit counts. Colony-forming units were enumerated by plating serial dilutions of organ homogenates on Middlebrook 7H11 agar.

**Preparation of serum and splenocytes.** Naive mice, *M. bovis* BCG–vaccinated mice, and *M. tuberculosis* Erdman–infected mice were bled by heart puncture under anesthesia. The blood was coagulated for 30 min at room temperature, and the blood clot was separated from the serum by centrifugation. One hour before challenge with *M. tuberculosis* H37Rv, 50 µL of serum was transferred passively (iv).

Spleens of naive, *M. bovis* BCG–vaccinated, and *M. tuberculosis*–infected mice were removed aseptically. Single-cell suspensions were prepared by use of an iron mesh sieve, and erythrocytes were lysed as described elsewhere [31]. Splenocytes were washed 3 times at 37°C and were passed through 70-µm nylon-mesh cell strainers (Becton Dickinson). One hour before challenge with *M. tuberculosis* H37Rv, 5 × 10$^7$ cells in 200 µL of PBS were transferred adoptively (iv).

Splenocytes for ELISPOT and fluorescence-activated cell sorter (FACS) analyses were cultured with an initial density of ∼10$^7$ cells/5 mL in 6-well flat-bottom plates for 3 days in RPMI 1640 medium supplemented with glutamine, 10% fetal calf serum, 50 µg/mL streptomycin, 200 µg/mL amikacin, and 1 U of recombinant interleukin-2 at 37°C under 7% CO$_2$ in the presence of 0.1 µg/mL purified protein derivative (PPD) from *M. tuberculosis* (Statens Serum Institute). Cultures without PPD were used as negative controls. After 3 days, cells were washed with PBS, and viability was analyzed by use of Trypan blue staining.

**Interferon (IFN)–γ ELISPOT assays.** Frequencies of IFN-γ–secreting T lymphocytes were determined after stimulation of splenocytes for 3 days by use of ELISPOT assay. In brief, Millititer HA Multiscreen 96-well nitrocellulose filtration plates (Millipore) were coated overnight at 4°C with 4 µg/mL rat anti-murine IFN-γ monoclonal antibody (MAb) RA42 diluted in PBS (without Mg and Ca). The plates were blocked for 90 min at 37°C with PBS plus 1% bovine serum albumin (BSA). Washed splenocytes were restimulated with 20 µg/mL PPD for 3 days in 6-well plates and seeded into coated ELISPOT plates. Cells cultured without PPD for 3 days were also restimulated with 20 µg/mL PPD in the ELISPOT assay and served as nonspecific controls.

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Concanavalin A–stimulated splenocytes served as positive controls. Cells were plated in triplicates at 10-fold dilutions, starting with $5 \times 10^5$ cells/well, and were incubated for 50 h in 5% CO$_2$ water-saturated atmosphere at 37°C. After thorough washing with PBS plus 0.5% Tween 20, biotin-conjugated rabbit antimurine MAb AN-18 (1 μg/mL) in PBS plus 0.1% BSA was incubated for 90 min at 37°C. After washing with PBS plus 0.5% Tween 20, streptavidin alkaline phosphatase conjugate (Dianova) diluted 1:20,000 in PBS plus 0.1% BSA were added, and the plates were incubated for 1 h at 37°C. After washing, a solution of Sigma Fast 5-bromo-4-chloro-3-indolyl phosphate nitroblue tetrazolium (Sigma) was used according to the supplier’s instructions, and the plates were incubated at 37°C. After 15 min, when spots became visible, the color reaction was stopped by rinsing the plates with distilled water. Wells with mismatched detection antibodies, without capture antibodies, or without cells were used as negative controls. Stained spots were automatically counted with a Bioreader 2000 (Biosys). Numbers of spot-forming cells are expressed as averages of triplicates per 10$^5$ spleen cells.

**FACS analysis.** The frequency of IFN-γ–secreting T lymphocytes was determined by use of flow cytometry. PPD-stimulated cells and unstimulated control cells were used. Splenocytes ($4 \times 10^5$ cells) were cultured in a volume of 1 mL of RPMI 1640 medium and were stimulated for 5 h with 0.2 μg of PPD/mL of complete RPMI 1640 medium. During the final 4 h of culture, 10 μg/mL Brefeldin A (Sigma) was added. Cultured cells were washed and incubated for 10 min with rat IgG antibodies and anti-CD16/CD32 MAb to block nonspecific antibody binding. Subsequently, cells were stained with Cy5-conjugated anti-CD4 MAb or anti-CD8 MAb, and, after 30 min on ice, cells were washed with PBS and fixed for 20 min at room temperature with PBS plus 4% paraformaldehyde (Sigma). Cells were washed with PBS and 0.1% BSA, permeabilized with PBS plus 0.1% BSA and 0.5% saponin (Sigma), and incubated in this buffer with rat IgG antibodies and anti-CD16/CD32 MAbs. After 5 min, fluorescein isothiocyanate (FITC)–conjugated anti–IFN-γ or isotype control MAb was added. After a further 20 min at room temperature, cells were washed with PBS and fixed with PBS plus 2% paraformaldehyde overnight. Cells were analyzed by use of a FACSCalibur and CellQuest software (both from Becton Dickinson). We routinely acquired 20,000–50,000 lymphocyte-gated CD4$^+$ T cells or CD8$^+$ T cells from each sample.

**Statistical analysis.** Significance of differences was calculated according to the nonparametric Mann-Whitney U test, and $P < .05$ was considered to be significant. The individual groups in each experiment contained at least 6 and, occasionally, 10 mice. Mean values of in vitro studies are based on 3 replicates.

**RESULTS**

**Persistence of M. bovis BCG and M. tuberculosis during chemotherapy.** Groups of mice were vaccinated with $5 \times 10^4$ BCG or infected with the same inoculum of M. tuberculosis Erdman. Chemotherapy was initiated 2 weeks after vaccination or infection. Bacterial titers in spleens and lungs of vaccinated or infected mice were determined at different time points during chemotherapy, starting at week 6 after vaccination or infection. The experimental schedule is depicted in figure 1. During chemotherapy, numbers of M. tuberculosis bacilli in spleens and lungs were higher than those of BCG (figure 2). Both
groups showed higher titers in spleens than in lungs at same time points. Highest counts were determined in the spleens of *M. tuberculosis* infected mice at week 6 after infection. At this time point, the titers of *M. tuberculosis* were ~2 log lower in lungs than in spleens. Moreover, at this time point, numbers of BCG in spleens were ~3 log lower than numbers of *M. tuberculosis* bacilli. In lungs of BCG-vaccinated mice, we observed reduction of >2 logs, compared with *M. tuberculosis*-infected mice. A constant decrease of both mycobacterial strains was observed over the course of the following 11 weeks of chemotherapy, leading to sterile eradication of BCG in lungs by ~10 weeks and in spleens by 17 weeks. At week 20, sterile eradication was achieved in all mice.

Cured but not reinfected mice were treated with dexamethasone, to determine whether latent bacilli persisted and thus had allowed reactivation of latent infection after induction of immune suppression. Reactivation of mycobacteria was not observed in either spleens or in lungs in whole-organ homogenate cultures (complete organs homogenized in 500 μL of sterile PBS on Middlebrook 7H11 agar plates; data not shown). We assume that latent primary tuberculosis did not account for the higher bacillary load after postprimary *M. tuberculosis* infection.

**Protection against postprimary tuberculosis in *M. bovis BCG*-vaccinated and *M. tuberculosis*-infected mice.** At 22 weeks after vaccination or postprimary infection (day 0), mice were challenged by the aerosol route with *M. tuberculosis* H37Rv. At different time points thereafter, mycobacterial growth in lungs was analyzed (figure 3). Numbers of tubercle bacilli in mice steadily increased over time, with the highest colony-forming unit counts in naive mice. In this group, a maximum was reached between days 60 and 90, whereas the BCG-vaccinated mice and the *M. tuberculosis* postprimary–infected mice still showed some increase in bacterial load, although at a lower level. The colony-forming unit counts were equally reduced in BCG-vaccinated mice and in primary *M. tuberculosis*-infected mice, compared with naive control mice. Even in postprimary *M. tuberculosis*-infected mice, colony-forming unit counts increased steadily over time after challenge infection, reaching a plateau at ~90 days after challenge. Thus, protection of BCG-vaccinated and postprimary *M. tuberculosis*-infected mice was comparable and did not differ significantly.
Characterization of the immune response induced by vaccination or primary infection. To characterize the immune response of *M. bovis* BCG–vaccinated and primary *M. tuberculosis* Erdman–infected mice, we obtained serum samples and isolated splenocytes. Serum or splenocytes were passively or adoptively transferred into naive mice 1 h before challenge infection. Bacterial titers of recipient, postprimary-infected, vaccinated, and naive mice were determined in lungs at various time points after challenge (figure 4). At days 7 and 14, no differences were observed among the 5 groups (figure 4). At days 30 and 60, adoptive transfer of splenocytes reduced colony-forming unit counts slightly, and recipients of cells from primary *M. tuberculosis* Erdman–infected mice had a greater reduction in colony-forming unit counts than did recipients of cells from *M. bovis* BCG–vaccinated mice. In both cases, however, the reduction in colony-forming unit count was smaller than that achieved by active vaccination with *M. bovis* BCG or by primary infection with *M. tuberculosis*. The greatest reduction in colony-forming unit count was observed after transfer of splenocytes from primary *M. tuberculosis*–infected mice at day 30 after challenge (figure 4A), with a significant difference from that in naive mice. The transfer of serum had virtually no effect over the course of the whole observation period.

Frequencies of IFN-γ–secreting splenocytes. Numbers of IFN-γ–secreting cells were enumerated by use of ELISPOT analysis of T lymphocytes from spleens at various time points after challenge infection (figure 5). Splenocytes from naive mice did not respond to stimulation with PPD and were at background levels, whereas splenocytes from BCG-vaccinated mice were ≈2-fold induced by PPD, compared with those in control mice. T lymphocytes from primary *M. tuberculosis* Erdman–infected mice were stimulated 10-fold by PPD, compared with the respective unstimulated controls (day 0).

At day 7 after aerosol challenge, the BCG-vaccinated/infected mice and the primary/postprimary *M. tuberculosis*–infected mice displayed an ≈2-fold induction of IFN-γ–secreting cells after stimulation with PPD, compared with control groups. T lymphocytes isolated at day 14 after challenge of the BCG-vaccinated/infected mice were only slightly stimulated by PPD, whereas T cells from primary/postprimary *M. tuberculosis*–infected mice responded with ≈3-fold induction, compared with the controls. The highest numbers of IFN-γ–producing spleocytes were induced by PPD in primary/postprimary *M. tuberculosis*–infected mice at day 14 after challenge. At day 30 after challenge, in the BCG-vaccinated, postprimary-infected mice and the primary/postprimary *M. tuberculosis*–infected mice, a nearly 5-fold induction was observed, compared with the respective control mice. At day 60 after challenge infection, all groups showed a decline in numbers of IFN-γ–secreting spleocytes, compared with those at day 30. Surprisingly, the numbers of PPD-induced T lymphocytes in the BCG-vaccinated
Figure 5. ELISPOT analyses of interferon-γ–producing cells at different time points after aerosol challenge infection with Mycobacterium tuberculosis H37Rv in naive, bacille Calmette-Guérin (BCG)–vaccinated, and primary M. tuberculosis Erdman–infected mice in the presence or absence of stimulation with purified protein derivative (PPD). The experiment was performed twice with similar results. Bars represent means of spot-forming unit (sfu) counts, and error bars indicate SEM of each group. P values were determined by use of the 1-tailed Mann-Whitney U test with a 95% confidence interval (P<.05). Asterisks, significance for all data points for a given time point; black circles, significance for all data points except BCG PPD; and black squares, significance for all data points except cells from primary M. tuberculosis Erdman–infected mice in the absence of stimulation.

To verify the results from the ELISPOT analyses and to determine the phenotype of the IFN-γ–producing cells, IFN-γ–producing splenocytes were characterized by use of intracellular cytokine staining and flow cytometry. After restimulation with PPD, splenocytes were surface stained with Cy5-conjugated anti-CD4 or anti-CD8 MAb and with FITC-conjugated anti–IFN-γ MAb (figure 6). Splenocytes of naive mice showed background numbers of CD4+ IFN-γ–positive and CD8+ IFN-γ–positive cells after restimulation with PPD (data not shown). Splenocytes from vaccinated/infected and primary/postprimary–infected mice produced IFN-γ after stimulation with PPD. Although modest production of IFN-γ was detectable within the CD4+ T cell compartment of either group, only background levels of CD8+ IFN-γ–positive T cells were measured in either group. At day 60 after aerosol challenge infection, similar frequencies of CD4+ IFN-γ–positive cells were identified in BCG-vaccinated/infected and M. tuberculosis primary/postprimary–infected mice. Thus, PPD antigen–specific CD4+ T cells from immune mice produced the central cytokine of protection against tuberculosis.

DISCUSSION

Experimental infection of mice with M. tuberculosis is widely used for deciphering cell-mediated protection, notably because the murine immune system is understood extremely well. Studies of latent tuberculosis in mice have been published recently [32–34], but experiments on postprimary murine M. tuberculosis infection have not been performed recently. The issue of whether progressive tuberculosis is caused primarily through reactivation of dormant bacilli or through reinfection with a second M. tuberculosis strain is controversial and has been addressed mainly in human tuberculosis [27, 30, 35–40]. In general, reactivation of latent M. tuberculosis is considered to be a major cause of disease, with reinfection thought to be the exception [39, 41]. However, a recent study has unequivocally demonstrated reinfection with M. tuberculosis in patients in whom primary tuberculosis had been successfully eradicated by chemotherapy [24, 42]. Thus, reinfection is a matter of fact in tuberculosis that deserves further consideration [22, 43].

We used 2 different M. tuberculosis strains, Erdman and H37Rv, to allow their follow-up identification by strain typing. However, this was not necessary, because no reactivation of
latent *M. tuberculosis* Erdman was detectable, even after immune suppression of cured mice with dexamethasone. We have demonstrated that protection against aerosol challenge with *M. tuberculosis* H37Rv in mice does not differ significantly between vaccination with BCG and primary infection with *M. tuberculosis* Erdman. We had expected a much greater difference, even though we did not assume sterile protection. *M. tuberculosis* Erdman, the strain used for primary infection, is a laboratory strain that infects and replicates in experimental mice; yet, its virulence seems reduced, compared with that of clinical isolates or *M. tuberculosis* H37Rv [44, 45]. In addition, it can be argued that immunity could be impaired by chemotherapy. We have observed that the persistence of *M. tuberculosis* during chemotherapy was more marked, compared with that of BCG. However, this difference did not translate into superior protection against challenge infection. The persistence of *M. tuberculosis* during treatment was comparable to that described elsewhere [34], with a complete elimination of the bacilli without reactivation of dormant bacilli. In drug-induced models of latent tuberculosis, dormant *M. tuberculosis* could be reactivated by treatment with immunosuppressive agents, notably dexamethasone. The difference of induced reactivation of dormant tuberculosis, as observed in the Cornell model and in the present study, is probably due to methodological differences. Cornell model–based studies depend highly on the parameters used [46]. The Cornell model uses an inoculation dose range of $10^3$–$10^6$ cfu count, an infection period of 30 min to 4 weeks, distinct types and concentrations of chemotherapeutic drugs, a varying length of treatment, and a posttherapy rest period. We used prolonged drug treatment for up to 21 weeks to achieve culture-negative sterile clearance in all organs examined. No mycobacteria were recovered, nor did we detect reactivation of latent bacilli by immune suppression with dexamethasone. Furthermore, our experiments differ from Cornell model–based studies with regard to the inoculum dose, infection period, combination with drug regimens, and duration of treatment.

Our result that primary infection with *M. tuberculosis* and vaccination with BCG induced comparable levels of protection could be interpreted to mean that it is impossible to achieve efficient protection against tuberculosis. We consider this conclusion to be false. It is well established that *M. tuberculosis* infection progresses to disease in only ~10% of all infected...
individuals. In the vast majority of infected individuals, an effective immune response that is capable of containing the pathogen must develop so that the pathogen does not cause disease. Only susceptible individuals will develop disease, whether due to reinfection or to reactivation. It remains to be clarified whether the failure of BCG vaccination and primary *M. tuberculosis* infection to protect against challenge with *M. tuberculosis* is due to identical or different mechanisms. It is conceivable that BCG vaccination induces an insufficient immune response, whereas the immune response induced by primary *M. tuberculosis* infection is concomitantly suppressed [47, 48]. Since the mechanisms induced by BCG vaccination or by primary *M. tuberculosis* infection are disparate, elucidation of the underlying mechanisms could provide guidelines for developing an improved vaccine. Future studies aimed at careful dissection of the immune responses caused by natural infection or by vaccination will be needed to clarify this issue.

Adoptive cell transfer of splenocytes from *M. tuberculosis*-infected donors into naive recipients resulted in marginally lower colony-forming unit counts, compared with transfer of cells from BCG-vaccinated donors. This result is in accordance with that of a report by Orme and Collins; adoptive transfer of protection against tuberculosis can be achieved, provided that the recipient mice are rendered T cell deficient before infusion of donor T cells [49]. Accordingly, protective immunity was expressed in the lungs by a sufficiently large inoculum of immune T cells obtained from donors at the height of the primary immune response. Although protection in T cell–deficient mice increased over time after adoptive transfer and challenge infection [49], we detected an increasing loss of protection, as revealed by higher colony-forming unit counts in lungs over time. More recently, it was reported that adoptive transfer of Th1 cells into intact naive recipient mice resulted in protective immunity against subsequent challenge with *M. tuberculosis* [50]. Similar to our experiments, recipients were not irradiated in that study. As expected, the transfer of serum did not reduce colony-forming unit counts after BCG vaccination or *M. tuberculosis* primary infection. This result confirms that primed T cells, rather than antibodies, are critical to protection [51]. Primary *M. tuberculosis* infection induced higher numbers of IFN-γ–secreting T cells than did BCG vaccination. This difference could be due to the lack of protective antigens in BCG, compared with *M. tuberculosis*, a lower immunogenicity of BCG, and/or to the prolonged persistence of *M. tuberculosis*. After postprimary infection, a similar picture existed until day 60 after challenge, with the highest numbers of IFN-γ–positive cells between days 7 and 30. This observation is in agreement with the time course of induction of a cellular immune response in tuberculosis. At day 60 after challenge, numbers of IFN-γ–secreting T lymphocytes decreased, most likely due to the partial disappearance or suppression of PPD-reactive T cells. Despite the higher frequency of IFN-γ–secreting splenocytes in postprimary-infected mice, these cells did not confer substantially superior protection, compared with BCG-vaccinated mice. It is challenging to ask whether the IFN-γ–positive splenocyte population is homogeneous and whether all cells contribute to protective immunity equally regardless of their biological nature. To determine the phenotype of the IFN-γ–secreting splenocytes, surface CD4 and CD8 staining, together with intracellular IFN-γ staining, were performed. FACS analyses revealed that the source of IFN-γ–secreting cells could be assigned mainly to the CD4+ cell population, whereas the population of CD8+ IFN-γ–positive cells was smaller. However, we are aware that the system used is biased toward preferential restimulation of PPD-specific CD4+ T cells.

Our findings are compatible with the concept that a future vaccine against tuberculosis needs to perform better than either natural infection with *M. tuberculosis* or BCG vaccination. Although such a vaccine may be considered to be an ambitious goal, precedent exists that vaccination can induce a superior immune response, compared with natural infection. For example, conjugate vaccines induce an immune response that can protect newborns against encapsulated bacteria, including pneumococci and meningococci, although natural infection fails to produce effective immunity in toddlers [52–54]. To address the risk of reinfection in previously infected individuals, the outcome of reinfection in mice with a low-level persistent and latent infection should be addressed in subsequent studies. Latency occurs in the majority of *M. tuberculosis*–exposed individuals in areas where the bacteria are endemic. The large number of individuals vaccinated with BCG should also be taken into consideration in future clinical studies of postprimary tuberculosis.

In summary, our experiments provide evidence that reinfection, in addition to reactivation, contributes to pathogenesis and disease progression in tuberculosis. We, therefore, assume that exogenous reinfection is a significant cause of postprimary tuberculosis, even after successful therapy of primary *M. tuberculosis* infection. Our findings not only emphasize the risk of PPD-positive contacts to acquire disease from patients with active tuberculosis but also underscore the difficulties in the development of an effective new tuberculosis vaccine [55].

References

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