Induction of an AIDS Virus-Related Tuberculosis-Like Disease in Macaques: a Model of Simian Immunodeficiency Virus-Mycobacterium Coinfection

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The mechanism by which human immunodeficiency virus (HIV)-Mycobacterium tuberculosis coinfection facilitates development of HIV-related tuberculosis is poorly characterized. Macaque models of simian immunodeficiency virus (SIV)-Mycobacterium bovis BCG coinfection were employed to explore the pathogenesis of AIDS virus-related tuberculosis. Following BCG coinfection, SIV (SIV)-infected macaques with high viral loads developed an SIV-related tuberculosis-like disease. This disease was characterized clinically by a syndrome of diarrhea, anorexia, weight loss, and altered levels of consciousness and pathologically by the presence of disseminated granulomas. In contrast, SIVmac-infected macaques with low viral loads either showed no evidence of BCG-induced disease or developed focal granulomatous lesions. Pathogenic SIV-BCG interactions appeared to play a critical role in triggering the development of this SIV-related tuberculosis-like disease. BCG coinfection enhanced the destruction of CD4+ T cells in SIVmac-infected macaques whose viral loads were high. Reciprocally, exacerbations of SIV disease led to marked suppression of BCG-specific T-cell responses, persistence of the BCG infection, and development of an SIV-related tuberculosis-like disease. Furthermore, development of this SIV-related tuberculosis-like disease was also seen in naïve macaques simultaneously inoculated with SIVmac and BCG. These results provide in vivo evidence that coinfection of AIDS virus-infected individuals with an avirulent mycobacterium can lead to development of a tuberculosis-like disease.

Mycobacterium tuberculosis-induced tuberculosis remains the leading cause of morbidity and mortality among infectious diseases (13, 35). It is estimated that one-third of the world’s population is infected with M. tuberculosis (15). However, only 5 to 10% of infected individuals go on to develop primary or reactivation tuberculosis, suggesting that host factors contribute to the pathogenicity of M. tuberculosis (15). Individuals with human immunodeficiency virus (HIV)-induced immune suppression appear to be particularly susceptible to M. tuberculosis infection even at moderate stages of the viral infection. It has been shown that HIV-infected persons are at marked risk for developing both primary and reactivation tuberculosis (10, 16, 31, 33). In addition, the manifestations of tuberculosis appear to be more systemic and life-threatening in HIV-infected persons (1). Elucidating precisely how HIV-induced damage to the immune system predisposes an individual to tuberculosis would facilitate exploration of disease pathogenesis and control of HIV-related tuberculosis.

An animal model of coinfection with HIV and a mycobacterium would facilitate exploration of disease pathogenesis and treatment of HIV-related tuberculosis. Simian immunodeficiency virus (SIV)-infected nonhuman primates have proven to be powerful models for the study of AIDS (11, 22). However, little has been done to develop a macaque model for AIDS virus-related tuberculosis. The difficulty in developing a macaque model for AIDS virus-mycobacterium coinfection can be attributed, at least in part, to the unusual susceptibility of macaques to tuberculosis. It has been shown that following inhalation of M. tuberculosis or Mycobacterium bovis, rhesus monkeys develop an acute, fulminant, and fatal form of tuberculosis (38, 40). The rapid progression of tuberculosis in macaques makes it difficult to use nonhuman primates as models for exploring the pathogenesis of HIV-M. tuberculosis coinfection.

We reasoned, however, that utilizing an attenuated mycobacterium for coinfection of SIV-infected macaques may provide an alternative strategy to study this process. The potential utility of this strategy is supported by clinical case reports that have documented M. bovis disease in HIV type 1 (HIV-1)-infected humans who have been vaccinated with M. bovis BCG (2, 5, 27, 28, 34). Moreover, M. bovis infection in humans can induce a zoonotic tuberculosis that is indistinguishable from that caused by M. tuberculosis in terms of pathogenesis, lesions, and clinical findings (3, 8, 24, 30).

We have recently adapted SIVmac-infected macaques to an SIV-BCG coinfection model for the study of AIDS virus-related tuberculosis. In a previous study, we showed that BCG coinfection can have an impact on SIVmac-induced disease, accelerating the progression to AIDS in coinfected macaques (39). This accelerated disease progression appeared to be linked to the prolonged T-cell activation associated with coinfection (39). In the present study, we examined the impact of macaque model of SIV (SIVmac)-BCG coinfection on mycobacterial disease in coinfected macaques. We found that exacerbation of SIV-induced disease in these monkeys led to development of tuberculosis.

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a Focal lesions were identified at necropsy, 4 to 8 months after coinfection.
b The fatal syndrome occurred 1.5 to 2 months after coinfection; 90% of the macaques that developed the fatal syndrome were coinfected with BCG 2 to 3 months after SIVmac infection.
c The fatal syndrome occurred 3 to 4 months after coinfection.

MATERIALS AND METHODS

Animals and virus. Rhesus (Macaca mulatta) and pigtailed (Macaca nemestrina) macaques that were 2 to 5 years old were used in these studies. Six of the animals studied were pigtailed macaques; the rest were rhesus macaques. Three of the six pigtailed macaques were subjected to simultaneous SIVmac-BCG coinfection, and three were in the group of monkeys that were infected with SIV and then with BCG. Rhesus and pigtailed macaques were used in the SIVmac-BCG coinfection study since these two species displayed comparable immune and inflammatory responses after Staphylococcus enterotoxin B superantigen and BCG challenges. There have been no strong data indicating that there are significant differences in the disease patterns resulting from BCG infection in these two species, although the pigtailed macaques appear to be more sensitive to simultaneous coinfection with SIVmac and BCG. The animals were maintained in accordance with the guidelines of the Committee on Animals for Harvard Medical School and the Guide for the Care and Use of Laboratory Animals (25a).

For SIVmac infection, macaques were inoculated intravenously with 10^9 50% tissue culture infectious doses of SIVmac strain 251, as described previously (6). The animals that were infected with SIVmac for 4 months or less before BCG coinfection were referred to as early SIVmac-infected macaques in this study. As controls, three macaques were infected with nonpathogenic chimeric simian-human immunodeficiency virus (SHIV)-89.6 as described previously (6) prior to BCG inoculation.

M. bovis BCG coinfection. M. bovis BCG (Pasteur strain) was stored in liquid nitrogen and thawed immediately before inoculation. Three BCG inocula, 10^9, 10^8, and 10^7 CFU, were used for intravenous inoculation (Table 1). Since numerous animals were used, it was impossible to infect all of the animals with SIVmac or to coinfect them with BCG at a single time. However, the macaques with high and low viral loads in small cohorts were coinfected at the same time with a defined BCG inoculum. To examine the impact of SIVmac infection on BCG-specific memory immune responses, eight macaques were infected sequentially with BCG, with SIVmac, and finally with BCG again at 2-month intervals. Seven naive macaques were inoculated simultaneously with SIVmac and BCG. All other monkeys were infected with SIVmac strain 251 for 3 to 16 months and then inoculated with BCG. After BCG inoculation the monkeys were observed for signs of clinical illness. The SIVmac-BCG-coinfected macaques that died from the coinfection were subjected to necropsy studies. Specimens were collected for pathological analyses when the animals were moribund or dead due to the terminal BCG disease.

Isolation and fractionation of lymphocyte populations from blood and lymph nodes. Peripheral blood mononuclear cells were isolated from EDTA-anticoagulated blood of the monkeys by Ficoll-diatrizoate gradient centrifugation. Peripheral lymph nodes were obtained by standard biopsy procedures before and after BCG inoculation and were carefully teased to generate single-cell suspensions. CD4^+ T cells were enriched by using anti-CD4 or anti-CD8 antibody-conjugated Dynabeads (Dynal, Inc., Great Neck, N.Y.), as described previously (6, 39). Peripheral blood mononuclear cells or lymph node cells were incubated with these immunomagnetic beads for 30 min at room temperature and then selected in two cycles with a magnetic particle concentrator. CD4^+ T cells enriched by these methods (CD5-depleted peripheral blood lymphocytes [PBL]) contained less than 5% CD8^+ T lymphocytes.

Immune flow cytometry analyses of CD4^+ T cells. CD4^+ PBL counts were calculated based on the results of complete blood count data and immune flow cytometry data showing the percentage of CD4^+ PBL. Three-color cytometric analyses of whole blood were performed with an XL flow cytometer (Coulter, Hialeah, Fla.). The following anti-human monoclonal antibodies that cross-react with the corresponding macaque antigens were used: phosphatidylethanolamine (PE)-conjugated anti-rhesus monkey CD3 (FN18; Biosource, Camarillo, Calif.), PE-conjugated anti-human CD4 (Ortho Diagnostic Systems, Raritan, N.J.), and PE-conjugated anti-human CD8 (Dako Corporation, Carpinteria, Calif.).

Proliferation assay. A conventional proliferation assay was carried out as described previously. Briefly, macaque PBL (1 × 10^8 cells per well) or enriched CD4^+ T cells were cultured in triplicate in 96-well plates in the presence of BCG purified protein derivative (PPD) (1, 5, or 25 μg/ml), concanavalin A (5 μg/ml), bovine serum albumin (3 μg/ml), or medium alone. Five days later, the cells were pulsed with [3H]thymidine (1 μCi per well), and uptake was measured 8 h later with a 1450 Microbeta scintillation counter (Wallac, Gaithersburg, Md.). The stimulation index was defined as the ratio of the mean counts per minute for PPD- or concanavalin A-stimulated wells to the mean counts per minute for the control wells (medium alone).

Quantitative measurement of plasma SIV RNA. The amount of plasmid SIV RNA was determined by quantitative-competitive (QC)-PCR as described previously (39). Briefly, viral RNA was extracted by following the instructions of an RNA extraction kit obtained from Qiagen (Valencia, Calif.). The extracted RNA was placed in six tubes which contained defined copies of SIVmac gag competitor RNA. The RNA mixtures were reverse transcribed to cDNA and competitively amplified by using a 35-cycle PCR performed with a pair of SIVmac-gag-specific primers (39). The amplified PCR products containing the wild type and competitor were separated on 2% agarose gels, and their densities were measured with a GS 700 imaging densitometer (Bio-Rad). Amounts were determined by analyzing the data with the Molecular Analyst system software (Bio-Rad). The coefficients of variation within and between assays when this protocol was used were less than 20%. The sensitivity of the QC-PCR was 4 × 10^3 RNA copies per ml of plasma. As a complementary study, the amount of plasma SIV RNA was also determined by a branched DNA assay (Chiron, Emeryville, Calif.). This assay can detect a minimum of 1,500 RNA copies/ml.

BCG colony counts for quantitation of mycobacteria. The viable BCG counts for lymph nodes were calculated by counting the BCG CFU in cell lysates from lymph node cells (32). The red blood cell-depleted cell pellets obtained from 10^8 lymph node cells were lysed with 10% saponin to release the intracellular BCG. Fivefold dilutions of each lysate were plated in duplicate on Middlebrook 7H10 agar plates (Difco). The CFU were counted after 3 weeks of incubation at 37°C.

Statistical analysis. The Student t test and nonparametric methods, as described previously (14), were employed to examine whether there were any statistically significant differences in viral loads, CD4^+ PBL counts, or BCG loads between SIVmac-BCG-coinfected macaques and control macaques. In addition, the correlation coefficient was calculated with Prism to determine the correlation between changes in BCG loads and numbers or function of CD4^+ T cells.

TABLE 1. BCG coinfection of SIVmac-infected macaques induced the SIV-related tuberculosis-like disease

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a Focal lesions were identified at necropsy, 4 to 8 months after coinfection.
b The fatal syndrome occurred 1.5 to 2 months after coinfection; 90% of the macaques that developed the fatal syndrome were coinfected with BCG 2 to 3 months after SIVmac infection.
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RESULTS

Highly active SIVmac infection predisposed macaques to develop a tuberculosis-like disease after BCG coinfection. To examine the impact of an AIDS virus infection on a mycobacterial infection, SIVmac-infected monkeys were inoculated intravenously with 10^3, 10^6, or 10^8 CFU of BCG and then assessed to determine their ability to contain the mycobacterium. As controls, macaques that were not infected with SIVmac or were infected with nonpathogenic SHIV were similarly inoculated with BCG. BCG coinfection of SIVmac-infected macaques could induce a tuberculosis-like disease (Table 1). The susceptibility of the monkeys to a fatal form of tuberculosis was associated with the status of SIVmac infection. The early SIVmac-infected macaques with plasma SIV RNA levels greater than 10^6 copies/ml showed rapid disease progression and died from the tuberculosis-like disease 1.5 to 4 months after BCG coinfection (Fig. 1 and Table 1). These macaques developed a fatal clinical syndrome characterized by a progression of diarrhea, anorexia, weight loss, and altered levels of consciousness. Necropsy studies of the coinfected macaques revealed dissemination of nonnecrotic granulomas in multiple organs (Fig. 1). In fact, some of the dying macaques had clinical evidence of organ failure that coincided with the development of multiple granulomas in the corresponding organs, suggesting that they died from SIV-related tuberculosis-like disease. In contrast, although they all died eventually from SIV-induced disease, the SIVmac-infected macaques with plasma SIV RNA levels less than 10^5 copies/ml did not exhibit clinical evidence of mycobacterial disease. Nevertheless, complete necropsy studies identified focal lesions of granulomatous inflammation in one-third of the macaques with relatively low levels of plasma SIV RNA (Table 1). The nonpathogenic SHIV-infected and uninfected macaques survived the systemic BCG infection, and no granulomatous lesions were found in biopsy specimens of lymph nodes and rectal mucosae (data not shown). These results suggest that SIV infections with high levels of persistent viral replication predispose macaques to develop a fatal tuberculosis-like disease following BCG coinfection.

Enhanced destruction of CD4^+ T cells following SIVmac-BCG coinfection coincided with development of the tuberculosis-like disease. We then sought to determine whether the dramatic loss of CD4^+ T cells that resulted from SIVmac-BCG coinfection was associated with development of the fatal SIV-related tuberculosis-like disease in the macaques. SHIV- and SIVmac-infected macaques with low viral loads did not show a significant decline in CD4^+ PBL counts after BCG coinfection, although their plasma SIV RNA levels did transiently increase to a mean of 3.2 × 10^5 copies/ml (Fig. 2A). In contrast, SIVmac and BCG infections had significant impacts on the immune systems of the early SIVmac-infected macaques whose plasma SIV RNA levels were more than 1 × 10^6 copies/ml. In these monkeys BCG coinfection was associated with increases in the mean plasma SIV RNA levels to 8.8 × 10^6 copies/ml (Fig. 2A). In addition, in these monkeys there were marked declines in the CD4^+ PBL counts (Fig. 2B).

These changes were associated with inefficient control of the BCG coinfection. Following BCG inoculation, viable BCG was persistently isolated from the lymphoid tissue of the macaques with high SIV loads (Fig. 3A). The persistence of active BCG infection coincided with the decline in the number of CD4^+ PBL in these monkeys (Fig. 3B). More importantly, the marked decrease in CD4^+ PBL counts was inevitably associ-
ated with the occurrence of the fatal tuberculosis-like disease. Consistently, the necropsy studies revealed marked depletion of T cells in lymph nodes and spleens in the macaques that died from the SIV-associated tuberculosis-like disease (data not shown). These results suggest that the enhanced destruction of CD4+ T cells that occurs as a consequence of SIVmac-BCG coinfection is one of the major pathogenic events linked to development of the fatal SIV-associated tuberculosis-like disease.

Loss of BCG-specific T-cell responses in the SIV-BCG-coinfected monkeys was associated with development of the fatal tuberculosis-like disease. Since BCG coinfection accelerated destruction of CD4+ T cells in the SIVmac-infected monkeys, we reasoned that antimycobacterial immunity in the monkeys may have been damaged. To address this issue, PBL of the SIVmac-infected macaques were assessed for PPD-driven proliferation after BCG inoculation. SIVmac-BCG-coinfected macaques with low CD4+ PBL counts exhibited very low PPD-specific T-cell responses compared to the responses seen in PBL of the coinfected animals with less profound decreases in their CD4+ T cells and in PBL of control monkeys infected with BCG alone (Fig. 4A, B, and D).

In addition, while the high level of viral replication in early SIVmac infection was associated with low memory PPD-specific T-cell responses, reduced boosting of the response after the second BCG inoculation was seen in the macaques sequentially infected with BCG, with SIV-, and finally with BCG again (Fig. 4C). This finding contrasted with the findings obtained for the macaques that were not infected with SIVmac, in which memory PPD-specific responses were prolonged after the first BCG inoculation and were boosted following reinfection with BCG.

FIG. 2. Accelerated SIV-induced disease following BCG coinfection contributed to development of the SIV-related tuberculosis-like disease in SIVmac-infected macaques. Following BCG inoculation, the SIVmac-infected macaques with high viral loads showed a marked increase in the level of plasma SIV RNA (A) and an accelerated decline in the number of CD4+ PBL (B), and they developed a fatal SIV-related tuberculosis-like disease. The plasma SIV RNA data in panel A were derived from QC-PCR and were verified by a branched DNA assay. The P values were <0.05 for the differences in plasma SIV RNA levels for the groups with and without the fatal BCG-associated disease; the P values were <0.01 for the different levels of CD4+ PBL in the groups with and without the fatal BCG-associated disease. For the macaques with high viral loads, the data obtained until the week when the animals developed the fatal SIV-related tuberculosis-like disease are shown. The symbols indicate means, and the error bars indicate standard errors of the means. The numbers of animals analyzed (n) are also indicated. TB, tuberculosis.

FIG. 3. Persistent high levels of BCG detected in lymphoid cells of coinfectected monkeys in association with the enhanced decline in CD4+ PBL counts. (A) BCG CFU quantitated by using lysates of 10^6 cells from lymph nodes obtained from macaques after BCG coinfection. (B) Correlation between the enhanced decline in CD4+ PBL in the groups with and without the fatal BCG-associated disease and persistence of viable BCG. The symbols indicate means, and the error bars indicate standard errors of the means. The numbers of animals analyzed (n) are also indicated. The P values were <0.01 for the differences in CFU in week 4 and thereafter for the groups with and without a fatal outcome. SIV^H1, high SIV load; SIV^L2, low SIV load; cont, control.
BCG (Fig. 4D). Furthermore, diminished PPD-specific T-cell responses were associated with persistence of active BCG replication and development of the fatal tuberculosis-like disease (Fig. 3 and 4C). These results suggest that diminished antimycobacterial T-cell immunity contributes to development of a fatal tuberculosis-like disease in SIVmac-BCG-coinfected macaques.

Simultaneous coinfection with SIV mac and BCG caused immune compromise and a rapidly fatal tuberculosis-like disease. Finally, we sought to determine whether the same acceleration of disease was apparent in macaques that were simultaneously coinjected with SIVmac and BCG. Naive macaques were inoculated simultaneously with SIVmac and BCG and then assessed to determine the consequences of this coinfection. Like chronically SIVmac-infected macaques that were subsequently infected with BCG, these monkeys developed a fatal SIV-related tuberculosis. All five macaques that were inoculated with SIVmac and $10^8$ CFU of BCG developed a fatal clinical syndrome or disseminated granulomas; the fatal syndrome occurred 1 to 1.5 months after coinfection, and three of the five animals tested were pigtailed macaques. In contrast, none of the three macaques inoculated with only $10^8$ CFU of BCG developed a fatal clinical syndrome or disseminated granulomas. The CD4+ PBL counts of the monkeys declined rapidly (Fig. 5A), and their PPD-specific T-cell proliferative responses were suppressed (Fig. 5B). The BCG infections also persisted in these animals (Fig. 6A), and the BCG colony counts increased as the CD4+ T-cell function declined (Fig. 6B and C). Disseminated granulomas were found in the macaques when the CD4+ PBL counts dropped to 50 to 300 cells per $\mu L$.

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**FIG. 4.** Inhibition of BCG-specific T-cell responses resulting from SIV-induced disease was associated with development of the SIV-related tuberculosis-like disease. The data are from proliferation assays in which we used PBL from coinjected macaques that exhibited an accelerated decline in CD4+ PBL counts and a tuberculosis-like disease (A and C), from macaques that did not exhibit accelerated depletion of CD4+ T cells or tuberculosis-like disease (B), and from controls that were not infected with SIVmac (D). The SIVmac-infected macaques whose data are shown in panel C and controls whose data are shown in panel D were inoculated twice with $10^8$ CFU of BCG. The macaques whose data are shown in panel B exhibited no evidence of a fatal tuberculosis-like disease and died from SIV-induced disease characterized by lymphoid depletion and other opportunistic infections. The symbols indicate means, and the error bars indicate standard errors of the means. The $P$ values were <0.05 for the differences in proliferation indexes in week 8 and thereafter for the groups with and without a fatal outcome; the $P$ values were <0.01 for the data obtained at any time for normal controls and the coinjected macaques with a fatal outcome. ConA, concanavalin A; BSA, bovine serum albumin; TB, tuberculosis.
Fig. 5A; see above). These results provide further evidence that the effects of SIVmac and BCG on the immune system potentiate the evolution of a tuberculosis-like disease in macaques.

**DISCUSSION**

SIVmac-infected macaques have proven to be a valuable animal model for studying the pathogenesis and treatment of HIV infection in humans (11, 22). In the present study, we used SIVmac-BCG coinfection to develop a model of an SIV-related tuberculosis-like disease. The induction of this SIV-related tuberculosis-like disease observed in macaques suggests that coinfection with HIV-1 and avirulent M. tuberculosis or M. bovis may also result in development of a tuberculosis-like disease in humans. In fact, humans coinfected with HIV-1 and M. tuberculosis can develop tuberculous lesions characterized by small granulomas without caseation or cavitation (36). Such lesions appear to be similar to the nonnecrotizing granulomas found in SIVmac-BCG-infected macaques and small laboratory animals infected with BCG (12, 21, 23). On the other hand, the frequency of granulomatous inflammation appears to be lower in humans coinfected with HIV and M. tuberculosis than in patients infected with M. tuberculosis alone (12). The data for HIV-infected humans and SIVmac-infected macaques suggest that host factors may determine the phenotypes of mycobacterial diseases that occur as a result of AIDS virus-mycobacterium coinfection.

A number of factors appear to contribute to induction of the tuberculosis-like disease in monkeys. The global immune status of the monkeys is certainly important. SIVmac-infected macaques with high viral loads rapidly progressed to the fatal SIV-related tuberculosis-like disease after systemic BCG coinfection. A clinically latent BCG disease is induced in BCG-SIVmac-infected macaques with lower viral loads. Presumably, the monkeys with high viral loads have poor immune function and the monkeys with low viral loads have more intact immune systems. The size of the BCG inoculum also appeared to be an important determinant in development of a tuberculosis-like disease in the coinfected macaques. Three BCG inocula were used to assess the pathogenic potential in the macaques (Table 1). Macaques inoculated with $10^5$ CFU of BCG did not show any clinical evidence of mycobacterial disease during a 1-year
follow-up study. Although inoculation with either $10^6$ or $10^8$ CFU of BCG induced a fatal SIV-related tuberculosis-like disease in SIV$_{mac}$-infected macaques with high viral loads, the disease course was shorter in the macaques inoculated with $10^8$ CFU of BCG (Table 1). A large BCG inoculum may make a BCG coinfection more productive and persistent in SIV$_{mac}$-infected macaques.

The results of our studies provide evidence which suggests that enhanced depletion of CD4$^+$ T cells triggers development of the SIV-related tuberculosis-like disease in macaques. In the SIV$_{mac}$-infected macaques with high viral loads, a fatal granulomatous disease was seen when BCG-induced increases in SIV loads resulted in an accelerated decline in CD4$^+$ PBL counts. In the SIV$_{mac}$-infected macaques with low viral loads, however, BCG coinfection resulted in a transient increase in plasma SIV RNA but not accelerated depletion of CD4$^+$ T cells. A limited increase in viral replication and the absence of accelerated depletion of CD4$^+$ PBL did not lead to development of a fatal tuberculosis-like disease. A certain amount of SIV replication and certain numbers of CD4$^+$ PBL may be required to trigger BCG-enhanced SIV disease and reciprocal development of massive granulomatous BCG disease. Although several studies with humans have suggested that low CD4$^+$ PBL counts are associated with increased incidence of tuberculosis in HIV-1-infected persons (19, 25, 37), the pathogenic consequences of accelerated depletion of CD4$^+$ T cells during mycobacterium-virus interaction are more readily seen in the experimental macaque SIV-BCG coinfection model. In humans coinfected with HIV-1 and M. tuberculosis, the magnitude of the virus-mycobacterium coinfection may not be as great as that seen in the macaques coinfected with SIV$_{mac}$ and BCG. The levels of plasma viral RNA in chronically HIV-1-infected humans are usually not as high as those in the SIV$_{mac}$-infected macaques evaluated in this study (26). Moreover, mycobacterial loads may also be low in humans during a newly transmitted M. tuberculosis coinfection or during reactivation of a latent M. tuberculosis infection, given the clinical difficulty in identifying mycobacteria from specimens collected from patients (4, 20). In the present study, however, the macaques were coinfected with large SIV$_{mac}$ and BCG inocula. The highly active SIV$_{mac}$-BCG coinfection allowed us to determine how an AIDS virus and mycobacterium reciprocally affect development of an AIDS virus-related tuberculosis-like disease.

The present study demonstrated that there is an association between marked suppression of a BCG-specific T-cell immune response and development of an SIV-related tuberculosis-like disease in naïve and SIV$_{mac}$-infected macaques. The results suggest that damaging antimycobacterial T-cell immunity can facilitate development of this mycobacterial disease. Depletion of CD4$^+$ T cells can certainly suppress the immune response to mycobacteria in SIV$_{mac}$-BCG-coinfected macaques. This suggestion is supported by the finding that inhibition of the PPD-specific T-cell responses was most evident when the animals exhibited the greatest decline in their CD4$^+$ PBL counts and developed the SIV-BCG-related clinical syndrome (Fig. 2B and 4). In addition, profound suppression of antimycobacterial immunity may also result from disturbance of cytokine profiles due to the massive stimulation of the immune system by SIV$_{mac}$ and BCG (6).

We realize that the SIV$_{mac}$-BCG coinfection models have some limitations in terms of mimicking the natural coinfection of HIV and M. tuberculosis in humans. M. tuberculosis infection in humans is usually transmitted through inhalation of limited numbers of M. tuberculosis organisms. In addition, susceptibil-
ity of HIV-infected humans to *M. tuberculosis* infection or reactivation may depend on the stage of the viral infection or on the degree of immune suppression. The SIV-related tuberculosis-like disease is induced by intravenous inoculation of numerous BCG cells into SIVmac-infected macaques. Moreover, a high degree of viral replication in early SIVmac-infected macaques appears to be required for successful induction of the fatal BCG disease. This requirement may indeed provide an explanation for why SIV-BCG coinfection cannot uniformly induce the tuberculosis-like disease in nonhuman primes (7, 9). Modeling virus-mycobacterium coinfection for HIV-related tuberculosis in macaques certainly remains challenging. Nevertheless, the SIVmac-BCG coinfection models may be relevant to the tuberculosis-like diseases in AIDS from an immunological standpoint, although coinfection with virulent *M. tuberculosis* strains is more likely to induce a disease process that resembles HIV-related tuberculosis. In fact, development of *M. bovis* BCG-induced tuberculosis-like disease or BCG reactivation has been reported in HIV-infected humans (2, 5, 27, 34). Zoonotic tuberculosis caused by *M. bovis* can also be identified in HIV-infected persons (17, 18, 29). Thus, the presence of a BCG-induced tuberculosis-like disease in SIVmacinfected macaques suggests that the SIVmac-BCG coinfection models might be useful for studying immunopathogenesis and disease consequences of AIDS virus-related tuberculosis.

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REFERENCES


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