Recombinant *Mycobacterium bovis* BCG Prime-Recombinant Adenovirus Boost Vaccination in Rhesus Monkeys Elicits Robust Polyfunctional Simian Immunodeficiency Virus-Specific T-Cell Responses

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While mycobacteria have been proposed as vaccine vectors because of their persistence and safety, little has been done systematically to optimize their immunogenicity in nonhuman primates. We successfully generated recombinant *Mycobacterium bovis* BCG (rBCG) expressing simian immunodeficiency virus (SIV) Gag and Pol as multigenic, nonintegrating vectors, but rBCG-expressing SIV Env was unstable. A dose and route determination study in rhesus monkeys revealed that intramuscular administration of rBCG was associated with local reactogenicity, whereas intravenous and intradermal administration of 10⁶ to 10⁸ CFU of rBCG was well tolerated. After single or repeat rBCG inoculations, monkeys developed high-frequency gamma interferon enzyme-linked immunospot responses against BCG purified protein derivative. However, the same animals developed only modest SIV-specific CD8⁺ T-cell responses. Nevertheless, high-frequency SIV-specific cellular responses were observed in the rBCG-primed monkeys after boosting with recombinant adenovirus 5 (rAd5) expressing the SIV antigens. These cellular responses were of greater magnitude and more persistent than those generated after vaccination with rAd5 alone. The vaccine-elicited cellular responses were predominantly polyfunctional CD8⁺ T cells. These findings support the further exploration of mycobacteria as priming vaccine vectors.

Because of its proven safety and immunogenicity, *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) is currently being explored as a vaccine vector to confer protection against infectious pathogens and cancer. Recombinant BCG (rBCG) has been constructed to express a variety of antigens, including proteins of human immunodeficiency virus type 1 (HIV-1), tuberculosis, and parasites (7, 12, 18, 24, 29, 38). However, rBCG has not demonstrated consistent protection and has failed in human clinical trials as a Lyme disease vaccine (11). The disappointing protection seen in these studies may be a consequence of transgene expression instability or inefficient antigen processing and presentation (9), rBCG constructs may simply not be sufficiently immunogenic to elicit protective immunity when administered intradermally as stand-alone vaccines.

Heterologous prime-boost vaccination regimens using recombinant bacteria, viruses, and proteins have been shown to generate more robust cellular immune responses than vaccine strategies using a simple vaccine modality. In efforts to improve BCG as a vaccine against tuberculosis, prime-boost strategies using BCG as prime and heterologous constructs such as recombinant adenovirus 5 (rAd5), DNA, and recombinant poxviruses as boosting immunogens were shown to enhance CD4⁺ and CD8⁺ T-cell responses to BCG antigens (14, 28, 33, 36, 43–45). A heterologous prime-boost immunization approach has also been shown to increase the immunogenicity of HIV-1 and simian immunodeficiency virus (SIV) antigens expressed by rBCG and other mycobacterial vectors. Ami et al. have shown that priming with rBCG expressing SIV Gag and boosting with a recombinant vaccinia virus generated higher virus-specific cellular responses in vaccinated monkeys than either rBCG or recombinant vaccinia virus alone (2). We recently showed that a recombinant *Mycobacterium smegmatis* vector expressing an HIV-1 envelope protein was able to generate virus-specific CD8⁺ T-cell memory responses in mice, and boosting with rAd5 substantially increased CD8⁺ T-cell responses specific for that HIV-1 antigen (6, 15).

In the present study, we assessed the immunogenicity of rBCG expressing SIV Gag, Pol, and Env antigens in rhesus monkeys using escalating doses of rBCG and various routes of administration. We also explored whether we can augment the immune responses elicited by rBCG by boosting with a replication-defective rAd5 vector. We demonstrate
that intradermal and intravenous administration of rBCG are better tolerated than other routes of rBCG administration and that rBCG prime/rAd5 vector boost vaccination induced robust, persistent, and polyclonal CD8+ T-cell responses.

**MATERIALS AND METHODS**

**Generation of rBCG.** *M. bovis* BCG (Pasteur) was grown in 7H9 medium supplemented with 10% oleic acid-albumin-dextrose-catalase (Difco) and 0.05% Tween 80 (Fisher Scientific). Full-length SIVmac239 gag and pol and a modified env were cloned from codon-optimized DNA plasmids constructed previously (20, 23). The SIV env was modified by removing the signal sequence and the gp41 transmembrane domain, introducing a signal peptide, and the M. tuberculosis M. tuberculosis antigen promoter and the C-terminal signal sequence. For detection of the SIV proteins, a hemagglutinin (HA) tag was fused to the C-terminal end of each viral protein. Within the operon, a kanamycin resistance gene was cloned downstream of the viral gene. Each individual multicopy plasmid containing the viral genes, regulated by the Escherichia coli region. The SIV genes were then individually inserted into the multicopy pJH222 plasmid. Generation of rBCG.

**Guide for the Care and Use of Laboratory Animals** (30) and with the approval of the Institutional Animal Care and Use Committee of Harvard Medical School and the National Institutes of Health.

The immunization schedule for each experimental group of monkeys is summarized in Fig. 1. Monkeys were inoculated intradermally, intramuscularly, or intravenously with 10^6, 10^7, 10^8, or 10^9 CFU of rBCG SIV Gag, Pol, and Env at weeks 0 and 23 with rBCG expressing SIV Gag, Pol, and Env at the indicated doses (10^6, 10^7, 10^8, or 10^9 CFU) either intradermally (ID) or intravenously (IV). All monkeys were subsequently boosted intramuscularly (IM) with 10^10 vp rAd5 expressing SIV Gag/Pol and Env at week 43. Three control MamuA*01+ monkeys were included that were immunized intramuscularly with 10^10 vp rAd5 expressing Gag/Pol and Env alone.

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FIG. 1. Immunization schedule. Ten *MamuA*+ rhesus monkeys were immunized at weeks 0 and 23 with rBCG expressing SIV Gag, Pol, and Env at the indicated doses (10^6, 10^7, 10^8, or 10^9 CFU) either intradermally (ID) or intravenously (IV). All monkeys were subsequently boosted intramuscularly (IM) with 10^10 vp rAd5 expressing SIV Gag/Pol and Env at week 43. Three control MamuA*01+ monkeys were included that were immunized intramuscularly with 10^10 vp rAd5 expressing Gag/Pol and Env alone.

Intracellular cytokine staining analysis. PBMC were isolated from EDTA-anticoagulated blood and frozen in the vapor phase of liquid nitrogen. Cells were then thawed and allowed to rest for 6 h at 37°C in a 5% CO2 environment. The viability of these cells was >90%. PBMC were then incubated at 37°C in a 5% CO2 environment for 6 h in the presence of RPMI–10% fetal calf serum alone (unstimulated), a pool of 15-mer Gag peptides (2 *×* 10^5), a pool of 15-mer Pol peptides (2 *×* 10^5), or a pool of 15-mer Env peptides (2 *×* 10^5) as a positive control. SIV Gag, Pol, and SIV Env pooled peptides were administered by intramuscular injection.
analyzed by using FlowJo software (TreeStar, Ashland, OR). Approximately 500,000 to 1,000,000 events were collected per sample. Doubled cells were excluded by using forward scatter-area versus forward scatter-height. Dead cells were excluded by their staining with amine reactive dye. CD4⁺ and CD8⁺ T cells were determined by their expression of CD3, CD4, or CD8. Functional analysis was done by plotting the expression of each cytokine molecule against another, and a Boolean combination of single functional gates was generated by using FlowJo software. The frequency of cells producing IFN-γ, tumor necrosis factor alpha (TNF-α), and IL-2, either individually or in any combination, was determined from the FlowJo analysis, formatted in PESTLE, and analyzed by using SPICE software (both PESTLE and SPICE software were provided by M. Roederer, NIH). All values used for analysis are background subtracted. Responses were considered positive when the percentage of total cytokine-producing cells was at least twice that of the background, and the cutoff for a positive response was 0.05%.

Statistical analysis. Statistical analyses and graphical presentations were computed with GraphPad Prism (GraphPad Software, San Diego, CA). The two-sided nonparametric exact Mann-Whitney test was used to compare the cellular immune responses between groups of experimental animals. Multiple groups of animals were compared by using the nonparametric one-way analysis of variance Kruskall-Wallis test with a Dunn’s post test. A P value of <0.05 was considered significant. Comparison of cytokine distributions between groups was performed by using a one-sided permutation test in SPICE.

RESULTS

Generation of rBCG expressing SIV Gag, Pol, and Env. The high-expressor multicopy pJH222 plasmid (6) was used to generate rBCG expressing the SIVmac239 Gag, Pol, and Env proteins fused to the 19-kDa signal peptide. Transformation of BCG with the pJH222 plasmid containing either the gag or pol gene produced clones that expressed high levels of the viral proteins. All rBCG clones tested expressed the full-length SIV Gag protein, while both full-length and truncated forms of SIV Pol were expressed in the mycobacteria (Fig. 2). PCR analysis indicated that several transformants contained truncated pol gene inserts (data not shown) consistent with lower-molecular-weight products seen in Western blots. We had difficulty expressing the full-length SIV Env gp160 (unpublished observations). Removal of the leader sequence and gp41 resulted in expression of SIV Env in BCG, albeit at low levels (Fig. 2). However, pJH222 containing this modified SIV env was inefficient in transforming BCG.

Route of administration and dose titration in rhesus monkeys of rBCG expressing SIV Gag, Pol, and Env. Previous studies in small laboratory animals suggested that the dose and route of administration of BCG are both important for the optimal induction of immune responses (16, 34). Rhesus monkeys were therefore inoculated intradermally, intravenously or intramuscularly with increasing doses (10⁶ to 10⁹ CFU) of rBCG expressing SIV Gag, Pol, and Env. We observed severe reactogenicity at the inoculation site in monkeys injected intramuscularly with rBCG. Therefore, this route of BCG administration was not considered in evaluating the immunogenicity of these vaccine constructs. For evaluating the immunogenicity of these constructs, Mamu-A*01+ rhesus monkeys were inoculated with rBCG either intradermally or intravenously at escalating doses (Fig. 1). Two monkeys received 10⁶, 10⁷, 10⁸, and 10⁹ CFU injected intradermally at four different sites on the back. At doses of 10⁶ and 10⁷ CFU bacilli injected intradermally, the rBCG vaccine constructs were also reactogenic in the monkeys. However, the lesions resolved in 3 to 4 weeks. Eight Mamu-A*01+ rhesus monkeys were inoculated intravenously at doses ranging from 10⁶ to 10⁹, using 2 animals per dose. Twenty-three weeks after rBCG priming, the two monkeys that were immunized intradermally received a second injection intradermally with 10⁷ CFU rBCG, and those that were immunized intravenously were injected again with the same vaccine doses. No adverse effects were observed in any of these monkeys after the second rBCG administration. However, transient leukocytosis and lymphocytosis were observed in monkeys that received two inoculations of 10⁷ CFU rBCG intravenously. All 10 rBCG-immunized animals and 3 naive control animals were then vaccinated with 10¹⁰ vp of rAd5 expressing SIV Gag/Pol and 10¹⁰ vp of rAd5 expressing SIV Env 43 weeks after the first rBCG vaccination.
BCG vector-specific immune responses after heterologous priming with rBCG and boosting with rAd5 expressing SIV Gag/Pol and Env proteins. We first assessed BCG vector-specific immune responses in the immunized and control animals. As shown in Fig. 3, BCG PPD cellular responses were seen as early as 1 week after rBCG inoculation. By week 2, strong PPD responses (>3,000 IFN-γ-producing cells) were observed, and this level of immune response was sustained in the weeks after immunization. Interestingly, the second immunization with rBCG resulted in a robust recall response to the BCG vector. Peak responses were seen 1 week after homologous boosting, with a mean greater than the maximum level of detection of 5,000 IFN-γ-producing cells. In the ensuing weeks, this cellular immune response contracted gradually. As expected, there were no increases in the frequency of peripheral blood IFN-γ-producing cells in response to PPD stimulation after rAd5 immunization. Moreover, monkeys that were immunized with rAd5 alone did not develop PPD-specific cellular responses. Taken together, these results suggest that rBCG immunization elicits a strong cellular response to the BCG vector that can be boosted by repeated BCG administration.

SIV-specific T-cell responses after heterologous rBCG prime, rAd5 boost vaccination. After a single or repeated immunization with rBCG expressing SIV Gag, Pol, and Env, rhesus monkeys developed low but detectible SIV-specific T-cell responses. Peripheral IFN-γ enzyme-linked immunospot assay (ELISPOT) responses to Gag and Pol were for the most part less than 100 SFC, and responses to Env were negligible. Furthermore, SIV Gag p11C tetramer-binding CD8+ T cells were detected only after in vitro stimulation of monkey peripheral blood lymphocytes with the Gag p11C peptide and IL-2 (Fig. 4). Heterologous immunization of the rBCG-primed monkeys with rAd5 constructs expressing SIV Gag/Pol and Env generated a high frequency of IFN-γ-producing T cells in response to stimulation with the immunodominant Gag p11C peptide, as well as Gag and Pol peptide pools, but not to the Env peptide pool (Fig. 5). The Gag- and Pol-specific cellular responses were greater than those in rAd5-immunized control animals. T-cell responses in rBCG/rAd5-vaccinated animals peaked 2 to 4 weeks after rAd5 vaccination and subsequently contracted over time. SIV-specific cellular responses remained greater in rBCG-primed animals than in the control animals (Fig. 5).

rBCG-primed monkeys also developed robust expansions of p11C-specific CD8+ T cells compared to the control monkeys after rAd5 administration (Fig. 6). The frequency of tetramer-binding CD8+ T cells peaked 2 to 4 weeks after rAd5 administration and declined in the ensuing weeks. At the peak immune response, there was a trend toward an association between the dose of the rBCG priming immunogen and the magnitude of the vaccine-elicited T-cell response (Fig. 7). This association did not achieve statistical significance in the Kruskal-Wallis test adjusted for multiple comparisons because of the small number of monkeys studied at each dose. The mean frequency of IFN-γ-producing cells increased as the dose of the rBCG priming immunogen increased. Statistical analysis using the Mann-Whitney test showed that Gag ELISPOT and Gag p11C-tetramer responses were higher after rAd5 administration in the rBCG-primed animals than in the control animals (Fig. 8). Although at week 4 after rAd5 immunization a trend toward higher mean cellular immune responses in rBCG/ rAd5-vaccinated animals than in control animals was observed, the difference between the responses in the rBCG/rAd5-vaccinated and the rAd5-vaccinated monkeys achieved statistical significance at weeks 2, 10, and 21 (Fig. 8). The results of these immunogenicity studies suggest that rBCG vaccines can prime effectively for a rAd5 vector boost in rhesus monkeys.

Functional profiles of SIV-specific T-cell responses after rBCG/rAd5 vaccination in rhesus monkeys. Studies suggest that both the magnitude and the quality of the cellular immune response contribute to the control of immunodeficiency virus
infections (3, 40). We therefore assessed the functional profile of the cellular immune responses elicited by the rBCG prime-rAd5 boost vaccination regimen. Peripheral blood lymphocytes isolated from rBCG/rAd5-vaccinated or rAd5-vaccinated monkeys were stimulated with overlapping 15-mer peptides spanning the SIV Gag protein, and the production of IFN-γ, TNF-α, and IL-2 by T cells was measured by intracellular cytokine staining. The total production of these three cytokines by either CD4+ or CD8+ T cells is shown (Fig. 9A, upper panel). Monkeys receiving the rBCG/rAd5 vector vaccine regimen generated higher T-cell responses than those vaccinated with rAd5 alone. Moreover, the cytokine responses were biased toward CD8+ T cells regardless of vaccination strategy.

We next assessed SIV Gag pooled peptide-stimulated production of these three cytokines to characterize the functional profiles of the vaccine-elicited T-cell responses. Total SIV-specific cytokine-producing CD8+ T cells were divided into seven distinct populations based on their production of IFN-γ, TNF-α, and IL-2 by T cells was measured by intracellular cytokine staining. The total production of these three cytokines by either CD4+ or CD8+ T cells is shown (Fig. 9A, upper panel). Monkeys receiving the rBCG/rAd5 vector vaccine regimen generated higher T-cell responses than those vaccinated with rAd5 alone. Moreover, the cytokine responses were biased toward CD8+ T cells regardless of vaccination strategy.

We next assessed SIV Gag pooled peptide-stimulated production of these three cytokines to characterize the functional profiles of the vaccine-elicited T-cell responses. Total SIV-specific cytokine-producing CD8+ T cells were divided into seven distinct populations based on their production of IFN-γ, TNF-α, and IL-2, either individually or in combination. The profiles of the functional capacities of the cells are shown by expressing each type of cytokine response as a proportion of the total response. The mean values for the animals in each vaccine group are shown in a series of pie charts (Fig. 9A, lower panel).

The SIV-specific CD8+ T-cell responses induced by vaccination with either rBCG/rAd5 or rAd5 alone were highly polyfunctional. The predominant population of polyfunctional T cells generated in the vaccinated monkeys produced both
IFN-γ and TNF-α combined. A small fraction of the polyfunctional T cells were also capable of producing IL-2 in conjunction with the other cytokines. Interestingly, the overall functional profile of the CD8<sup>+</sup> T-cell response was similar in both rBCG/rAd5-vaccinated animals and animals vaccinated with only rAd5.

Finally, the CD8<sup>+</sup> T-cell response specific for the immunodominant p11C Gag epitope peptide was assessed. Higher percentages of p11C tetramer-binding CD8<sup>+</sup> T cells were seen in monkeys immunized with rBCG/rAd5 than in those immunized with rAd5 alone (Fig. 9B). However, the proportion of tetramer-binding CD8<sup>+</sup> T cells expressing the memory-associated molecule CD28, as well as producing IFN-γ, TNF-α, and IL-2, was similar in both the rBCG/rAd5 and the rAd5-only groups. Taken together, these findings show that rBCG prime-rAd5 boost vaccination generated large T-cell responses with a polyfunctional phenotype. In addition, rBCG priming did not significantly influence the functional profile of the rAd5 vaccine-elicited T cells.

**DISCUSSION**

A prime-boost vaccination strategy using rBCG as the priming vector and recombinant viruses or protein as the boosting immunogen has been demonstrated to be very effective at eliciting antigen-specific immune responses (4, 27, 31). In the present vaccine immunogenicity study, we found that priming with rBCG and subsequently boosting with recombinant adenovirus expressing SIV antigens linked to the *M. tuberculosis*
ranges of the total cytokine production by CD4 metric analysis. (A) The median percentages and interquartile ranges of the total CD8 cytokine production was measured by MAb staining and flow cytometry. The data shown are the median percentages (with interquartile range) of the total CD8 T-cell response. The antigen-specific CD8 T cells were divided into seven distinct populations based on their production of IFN-γ, TNF-α, and IL-2. The cytokine profiles were determined by expressing each cytokine response as a proportion of the total antigen-specific cytokine-producing CD8 T-cell response. The data were analyzed by using the SPICE software and are presented as the mean values from the 10 animals of the rBCG-primed/rAd5-boosted group (rBCG) and 3 animals of the rAd5-only-immunized group (control) in a pie chart. (B) The cytokine production capacity and CD28 expression of the SIV Gag p11C-specific CD8 T cells were assessed. PBMC isolated from rhesus monkeys 13 weeks after rAd5 SIV Gag/Pol immunization were stained with the p11C tetramer and anti-CD28 MAb. These PBMC were also stimulated with the p11C peptide; cytokine production was measured by staining with MAb and analyzed by flow cytometry. The data shown are the median percentages (with interquartile range) of the total CD8 T cells that bound the p11C tetramer and percentages of p11C tetramer+ CD8 T cells (p11C+CD8+ T cells) that expressed CD28 or produced IFN-γ, TNF-α, or IL-2.

FIG. 9. Heterologous rBCG prime/rAd5 boost immunization elicits polyfunctional SIV Gag-specific CD8+ T cells. PBMC were isolated from rhesus monkeys 13 weeks after boosting rBCG-primed animals with rAd5 SIV Gag/Pol. The PBMC were exposed to pools of overlapping peptides spanning the Gag protein, and cytokine production was measured by MAb staining and flow cytometric analysis. The median percentages and interquartile ranges of the total cytokine production by CD4+ and CD8+ T cells are shown. The antigen-specific CD8+ T cells were divided into seven distinct populations based on their production of IFN-γ, TNF-α, and IL-2. The cytokine profiles were determined by expressing each cytokine response as a proportion of the total antigen-specific cytokine-producing CD8+ T-cell response. The data were analyzed by using the SPICE software and are presented as the mean values from the 10 animals of the rBCG-primed/rAd5-boosted group (rBCG) and 3 animals of the rAd5-only-immunized group (control) in a pie chart. (B) The cytokine production capacity and CD28 expression of the SIV Gag p11C-specific CD8+ T cells were assessed. PBMC isolated from rhesus monkeys 13 weeks after rAd5 SIV Gag/Pol immunization were stained with the p11C tetramer and anti-CD28 MAb. These PBMC were also stimulated with the p11C peptide; cytokine production was measured by staining with MAb and analyzed by flow cytometry. The data shown are the median percentages (with interquartile range) of the total CD8+ T cells that bound the p11C tetramer and percentages of p11C tetramer+ CD8+ T cells (p11C+CD8+ T cells) that expressed CD28 or produced IFN-γ, TNF-α, or IL-2.

19-kDa signal peptide generated robust and polyfunctional SIV-specific T-cell responses in vaccinated rhesus monkeys.

After rBCG vaccination, mycobacterium PPD-stimulated IFN-γ ELISPOT responses were strong; however, cellular responses specific for the SIV antigens were modest at best. A second inoculation with rBCG did not enhance SIV-specific T-cell responses in the animals. These results are consistent with previous studies suggesting that transgenes expressed in rBCG are marginally immunogenic (2, 9). While an explanation for the low ELISPOT responses elicited by rBCG vaccines is not entirely clear, it may be a result of low transgene expression or inefficient presentation of the transgene product to T cells (9).

When rBCG-primed rhesus monkeys were boosted with rAd5 expressing the same SIV antigens, we saw a substantial enhancement of durable SIV Gag- and Pol-specific cellular responses as determined by SIV Gag-p11C tetramer and by IFN-γ ELISPOT responses. We did not evaluate the epitopic breadth of these vaccine elicited T cells. Cellular immune responses to SIV Env, however, were comparable in the rBCG-primed and naive monkeys. The absence of priming for cellular responses to this viral antigen is likely due to the instability of the envelope protein expressed in mycobacteria. We found that the mycobacteria pJH222 plasmid with the SIV env gene insert was inefficient at transforming BCG and the few transformants that grew either did not express SIV gp120 protein or expressed SIV gp120 protein at very low levels. Importantly, however, the absence of envelope priming in monkeys that received this vaccine construct argues against the possibility that the rBCG immunogen primed for enhanced secondary immune responses by nonspecific activation of the immune system.

Although recombinant mycobacterium-induced CD8+ T-cell responses are lower than the responses induced by DNA and other vaccine vectors used as priming immunogens, recombinant mycobacterium-primed T cells have a tremendous capacity for secondary expansion in the setting of heterologous prime-boost vaccination (15). We and others have shown that rBCG and other mycobacteria can induce central memory CD8+ T-cell differentiation in vivo (6, 15, 42). The ability of BCG and other mycobacteria to generate memory CD8+ T cells could be due to the propensity of mycobacteria to stimulate CD4+ T helper cells, which has been shown to be important for driving memory CD8+ T-cell differentiation (37, 39).

It has been suggested that the functional heterogeneity of T-cell responses may be associated with successful containment of microbial infections. The extent of T-cell polyfunctionality has been correlated with protection against leishmaniasis in mice, HIV-1 in humans (1, 3, 8, 10, 32, 41), and SIV in nonhuman primates (13). We previously showed that the magnitude and polyfunctionality of virus-specific CD8+ T-cell responses as determined by SIV Gag-p11C tetramer and by IFN-γ ELISPOT responses were associated with the control of viral replication after SHIV-89.6P challenge in rhesus monkeys (40). In the present study using intracellular cytokine staining and polychromatic flow cytometry, we found a higher frequency of cytokine-producing cells in rBCG-primed monkeys than in control monkeys after an rAd5 boost. There was also a CD8+ T-cell bias in these responses. However, both rBCG/rAd5 and rAd5-alone vaccinations generated T-cell responses with similar functional profiles. In both vaccinated groups of monkeys,
more than half of the virus-specific CD8\(^+\) T cells were poly-functional, capable of producing two or more of the cytokines IL-2, IFN-\(\gamma\), and TNF-\(\alpha\). In addition, the proportion of p11C-specific CD8\(^+\) T cells expressing CD28 and the three cytokines was similar in both groups of animals. Phenotypically, the majority of the cytokine-producing CD8\(^+\) T cells were capable of secreting both TNF-\(\alpha\) and IFN-\(\gamma\), and significant populations of these cells were able to secrete all three or the combination of IFN-\(\gamma\) and IL-2 after Gag peptide stimulation. These IL-2-producing cells are likely long-lived central memory T cells that are capable of rapid expansion after reexposure to cognate antigen (19, 35). The finding of similar functional profiles of vaccine-elicited T cells in the rAd5 alone and rBCG prime/rAd5 boost immunized monkeys was certainly unexpected in view of the reports of a change in the profiles of these cells mediated by a plasmid DNA priming immunization.

Results from the recent STEP clinical vaccine trial showed that rAd5 as a stand-alone vaccine was not effective against HIV-1 (5, 26). Moreover, this trial suggested that rAd5 may enhance HIV-1 acquisition in individuals with preexisting Ad5 immunity. However, rare serotype rAd vectors or recombinant poxvirus vectors combined with other rAd serotypes or other vectors such DNA or BCG in a prime-boost vaccine regimen could potentially confer some protection against an AIDS virus infection since these immunization regimens generate robust anti-HIV-1 immune responses (22). The present study provides further evidence for the potential utility of rBCG immuno-gens for inducing HIV-1-specific immune responses.

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REFERENCES


