Prime-boost vaccination with Bacillus Calmette Guerin and a recombinant adenovirus co-expressing CFP10, ESAT6, Ag85A and Ag85B of *Mycobacterium tuberculosis* induces robust antigen-specific immune responses in mice

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Abstract. Tuberculosis (TB) remains to be a prevalent health issue worldwide. At present, *Mycobacterium bovis* Bacillus Calmette Guerin (BCG) is the singular anti-TB vaccine available for the prevention of disease in humans; however, this vaccine only provides limited protection against *Mycobacterium tuberculosis* (Mtb) infection. Therefore, the development of alternative vaccines and strategies for increasing the efficacy of vaccination against TB are urgently required. The present study aimed to evaluate the ability of a recombinant adenoviral vector (Ad5-CEAB) co-expressing 10-kDa culture filtrate protein, 6-kDa early-secreted antigenic target, antigen 85 (Ag85) A and Ag85B of Mtb to boost immune responses following primary vaccination with BCG in mice. The mice were first subcutaneously primed with BCG and boosted with two doses of Ad5-CEAB via an intranasal route. The immunological effects of Ad5-CEAB boosted mice primed with BCG were then evaluated using a series of immunological indexes. The results demonstrated that the prime-boost strategy induced a potent antigen-specific immune response, which was primarily characterized by an enhanced T cell response and increased production of cytokines, including interferon-γ, tumor necrosis factor-α and interleukin-2, in mice. In addition, this vaccination strategy was demonstrated to have an elevated humoral response with increased concentrations of antigen-specific bronchoalveolar lavage secretory immunoglobulin (Ig)A and serum IgG in mice compared with those primed with BCG alone. These data suggested that the regimen of subcutaneous BCG prime and mucosal Ad5-CEAB boost was a novel strategy for inducing a broad range of antigen-specific immune responses to Mtb antigens *in vivo*, which may provide a promising strategy for further development of adenoviral-based vaccine against Mtb infection.

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Introduction

Tuberculosis (TB) is one of the most prevalent infectious diseases worldwide, accounting for ~1.4 million mortalities and 8.7 million novel cases annually, which occurs as a results of *Mycobacterium tuberculosis* (Mtb) infection (1). Due to Mtb reactivation at a latent state in immunocompromised individuals, slow progress in dealing with drug-resistant Mtb infection and the co-infection of Mtb with human immunodeficiency virus, the global burden of TB remains high, particularly in developing countries (2,3).

Effective vaccines are of key importance in ending the global TB epidemic (1). However, a consistently effective vaccine is not currently available. The only available TB vaccine, attenuated *Mycobacterium bovis* Bacillus Calmette Guerin (BCG) has made a marked contribution to Mtb infection control, especially in juvenile population and newborns (4). However, BCG does not provide effective protection for all age groups, particularly in adults; its protective efficacy is highly varied from different trials, with certain studies observing negative effects associated with BCG revaccination (0-80%) (5,6). Therefore, the development of more effective vaccines or feasible vaccination strategies that provide better protection from Mtb infection are urgently required.

It is widely accepted that homologous boosting with the same vaccine is not sufficient for protecting against Mtb (7); therefore, heterologous prime-boost immunization strategies using BCG and a novel anti-TB vaccine have been investigated. Such prime-boost vaccination strategies have demonstrated the potential to elicit protective immune responses, including

cellular immune responses against Mtb infection in animal models and in humans (8-12).

Mtb is an intracellular pathogen transmitted via a mucosal route; mucosal and cellular immunity have thus been suggested to have pivotal roles in protection against Mtb infection. Therefore, a vector that is able to be delivered via a mucosal route and elicit potent antigen-specific immune responses may be an ideal candidate for anti-TB vaccines. Recombinant adenoviral vectors have gained increasing attention in anti-TB vaccine development due to their properties of type 1 immune adjuvant activity, excellent safety record in humans and high levels of antigen release as well as their suitability for parenteral and intranasal mucosal delivery (13,14). In addition, recombinant adenoviral vectors are highly effective at eliciting robust cellular immunity in experimental animal models (15), implicating them as promising antigen delivery vectors for the development of an anti-TB vaccine. In addition to a delivery vector, proper Mtb antigens used for vaccine development are also key factor for effectiveness of a vaccine candidate (16,17). Previously, a number of microbial antigens of Mtb were tested as TB vaccine candidates, including 10-kDa culture filtrate protein (CFP10), 6-kDa early-secreted antigenic target (ESAT6), the 30-32 kDa family of three proteins [antigen 85 (Ag85)A, Ag85B and Ag85C], the Mtb protein 64 (MPT64) and TB10.4 (a protein of 96 amino acids with a theoretical molecular mass of 10.4 kDa) (18-23). Among them, CFP10 and ESAT6 are immunodominant antigens encoded by region of difference-1 (RD1) that are present in virulent strains of Mtb and *Mycobacterium bovis*; however, these antigens are absent in BCG (24-26). Loss of RD1 was hypothesized to be the contributing factor for the attenuation of BCG (27,28); therefore, RD1-encoded CFP10 and ESAT6 have often been selected as potential antigen candidates in the development of novel anti-TB vaccines (19,29-32). In addition to CFP10 and ESAT6, Ag85A and Ag85B have also been widely employed in anti-TB vaccine development (32-36).

In the present study, BCG and a recombinant adenoviral vector (Ad5-CEAB) co-expressing CFP10, ESAT6, Ag85A and Ag85B of Mtb were used in combination to investigate the effects of this prime-boost strategy in mice.

Materials and methods

Animals. Female ICR mice (n=72, 6-8 weeks old) were purchased from the Animal facility of Ningxia Medical University (Yinchuan, China) and housed in a special pathogen-free environment with free access to food and water and a constant temperature of 18˚C. All animal experiments were performed in accordance with the guidelines of the Chinese Council on Animal Care and were approved by the Committee for Animal Care and Use of Ningxia University.

Bacterial strains and Mtb antigens. The BCG vaccine, which was produced by Chengdu Institute of Biological Products (Chengdu, China), was a gift from the Centers for Disease Control and Prevention in Ningxia Province of China (Ningxia, China) while colony-forming units (CFU) were determined on 7H11 agar plates. For preparation of Mtb antigens of CFP10, ESAT6, Ag85 and Ag85B, the target gene fragments were amplified from Mtb H37Rv genomic DNA, which was extracted using Myco DNAout Kit (Beijing Tiandz Gene Technology Company, Beijing, China), by polymerase chain reaction (PCR), as previously described (18). PCR fragments were codon optimized prior to being subcloned in frame into a prokaryotic expression plasmid pET-28a (Novagen, Madison, WI, USA) for *E. coli* expression of His-tagged proteins (Novagen) (26). The His-tagged CFP10, ESAT6, Ag85A and Ag85B proteins were purified using ÄKTA protein purification system (GE Healthcare, Pittsburgh, PA, USA) according to the manufacturer's instruction. Endotoxins were removed from the purified proteins using ToxinEraser™ Endotoxin Removal kit (GenScript, Piscataway, NJ, USA) prior to use. The antigenic proteins used in the present study had a purity of >85%, which was determined as previously described (18).

Recombinant adenovirus Ad5-CEAB preparation and immunization. The recombinant adenovirus Ad5-CEAB in which the four genes of CFP10, ESAT6, Ag85A and Ag85B were expressed as a mixture of proteins, rather than a fusion protein, was prepared as described previously, and the titer of virus stock was determined by a plaque assay (18). For immunization, mice were randomly divided into three groups (n=8 per group) as follows: Phosphate-buffered saline (PBS; Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) group, mice were treated intranasally with 100 μl PBS three times separated by two-week intervals; BCG group, mice were injected subcutaneously with $1x10^6$ CFU of BCG vaccine three times separated by 2 week intervals; BCG/Ad5 group, mice received a subcutaneous injection with $1x10^6$ CFU of BCG and following a 2 week interval, mice were intranasally boosted with 100 μl $1x10^9$ plaque-forming units (PFU) of Ad5-CEAB twice with 2 week intervals. At 2 weeks following the final immunization, animals were euthanized under ether anesthesia (Tianjin Kemiou Chemical Reagent Co., Ltd., Tianjin, China) by exsanguination for analysis of immune responses.

Flow cytometric analysis of splenocytes. Lymphocytes were isolated from the spleens of mice 2 weeks following the final immunization. Briefly, following sacrifice, spleens were aseptically harvested and the mouse spleen cells were obtained by carefully mashing the spleens with a syringe plunger, passing the product through a cell strainer (BD Biosciences, San Jose, CA, USA) and suspending it in preheated (37˚C) RPMI-1640 medium (Gibco-BRL, Carlsbad, CA, USA). Splenocytes from each mouse were then isolated through density gradient centrifugation (1.092±0.001 g/ml; 500 x g, 20 min) with Mouse Lymphocyte Separation Medium (Solarbio Science & Technology Co., Ltd, Beijing, China). Splenocytes at a concentration of $5x10^6$/ml were cultured in RPMI-1640 medium with 5% fetal calf serum (FCS) (Gibco-BRL, Eggenstein, Germany) supplemented with a mixture of purified Mtb proteins (containing 5 μg/ml of each purified Mtb: CFP10, ESAT6, Ag85A and Ag85B; Mtb CEAB antigen mixture) in an atmosphere of 5% CO_2 at 37˚C for 48 h. The frequencies of $CD4^+$ and $CD8^+$ T cells were characterized through flow cytometric analysis on a FACSCalibur instrument (BD Biosciences). Briefly, splenocytes from each animal were stained with a combination of allophycocyanin (APC) rat anti-mouse CD4 (1 μg/mouse; 553051), phycoerythrin (PE) rat

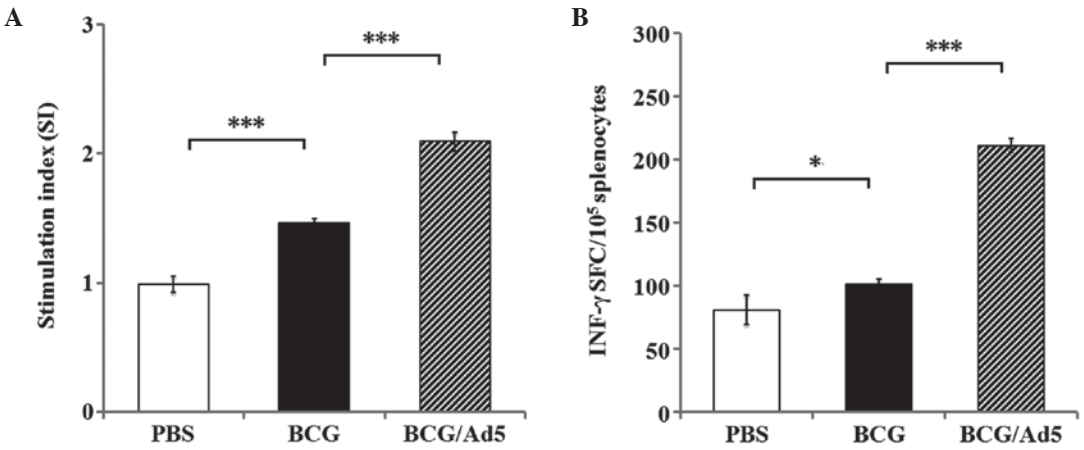

Figure 1. Antigen-specific T cell responses in mice. Splenic T cell responses to the Mtb antigens were determined using splenic mononuclear cells isolated from immunized mice by lymphocyte proliferation assay, IFN-γ-enzyme-linked immunospot assay and flow cytometric analysis. (A) Stimulation index of T cells in response to Mtb antigens. (B) Frequencies of antigen-specific IFN-γ-secreting splenic T cells in the indicated groups. Values are expressed as the mean ± standard deviation of three independent triplicate experiments (n=8 per group). *P<0.05; ***P<0.001. Mtb, *Mycobacterium tuberculosis*; IFN-γ, interferon-γ; PBS, phosphate-buffered saline group; BCG, Bacillus Calmette Guerin group; BCG/Ad5, BCG with recombinant adenovirus Ad5-CEAB group; SFC, spot forming cells.

anti-mouse CD8a (1 μg/mouse; 553033) and peridinin chlorophyll (PerCP) hamster anti-mouse CD3e (1 μg/mouse; 553067) antibodies (diluted 1:500; BD Biosciences) for 30 min at 4˚C. The APC rat immunoglobulin (Ig)G2aκ, PE rat IgG2aκ and PerCP hamster IgG1κ isotype controls were included for isotype control staining (diluted 1:500; BD Biosciences).

Antigen-specific lymphocyte proliferation test. A total of $5x10^5$ isolated splenocytes from individual mice were seeded into 96-well plates and stimulated in triplicate, with or without Mtb CEAB antigen mixture, in 5% FCS RPMI-1640 medium at 37˚C in an atmosphere of 5% CO_2 for 72 h. T cell proliferation was evaluated using the CellTiter 96® Aqueous One Solution Cell Proliferation Assay (Promega Corp., Madison, WI, USA), which is a sensitive fluorescence based microplate assay, according to the manufacturer's protocol. The proliferative responses were expressed as stimulation index (SI) that was calculated using the following formula: SI = mean optical density (OD) value of antigen-stimulated cells/mean OD value of control cells.

Enzyme-linked immunospot (ELISPOT) assays for interferon (IFN)-γ. The frequency of splenic antigen-specific IFN-γ-secreting spot forming cells (SFC) was determined by ELISPOT using a Mouse IFN-γ ELISPOT Ready-SET-Go! Reagent set (eBioscience, San Diego, CA, USA) according to the manufacturer's instructions with minor modifications. Briefly, isolated splenocytes were seeded at a density of $1x10^5$cells/well in a 96-well filter plate (MSIPS4510; Millipore, Billerica, MA, USA) pre-coated with mouse IFN-γ antibodies overnight. Cells were then incubated with or without the stimulator (Mtb CEAB antigen mixture) for 40 h at 37˚C. Visible spots were counted under a light microscope (SZX16; Olympus, Tokyo, Japan).

Cytokine induction and quantification. Splenocytes at a concentration of $5x10^5$/well were seeded into 24-well plates and cultured in 5% FCS RPMI-1640 medium containing Mtb CEAB antigen mixture for 72 h at 37˚C in an atmosphere of 5% CO_2. Culture supernatants were then harvested by centrifugation at 500 x g for 10 min and the concentrations of IFN-γ, tumor necrosis factor (TNF)-α and interleukin (IL)-2 were determined using an enzyme-linked immunosorbent assay (ELISA) cytokine detection system (RayBiotech, Inc., Norcross, GA, USA) according to the manufacture's instructions. All experiments were performed in triplicate.

ELISA assay for antigen-specific secretory (s)IgA and IgG. For sIgA measurement, bronchoalveolar lavage (BAL) samples were collected according to a method previously described (37). SIgA in the supernatant of BAL fluid was determined using an ELISA kit (eBioscience) according to the manufacturer's protocol. ELISA plates were pre-coated with Mtb CEAB antigen mixture (5 mg/ml) at 4˚C overnight.

For IgG measurement, mouse peripheral blood (~600 ml) was collected 2 weeks following the final immunization and the concentration of serum antigen-specific IgG was ascertained using a mouse ELISA Ready-SET-Go! kit (eBioscience) according to the manufacturer's instructions with minor modifications. ELISA plates were customized by pre-coating with Mtb CEAB antigen mixture at 4˚C overnight, rather than directly coated with the capturing antibodies provided in the kits.

Statistical analysis. Experimental data were expressed as the mean ± standard deviation. Differences between groups were analyzed using a one-way analysis of variance followed by Tukey's post-hoc test with SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA).

Results

Antigen-specific T cell responses. It is widely accepted that T cell responses have important roles in the host defense against Mtb infection (16). In the present study, the Mtb antigen-specific T cell response was analyzed *in vitro* by assessing the ability

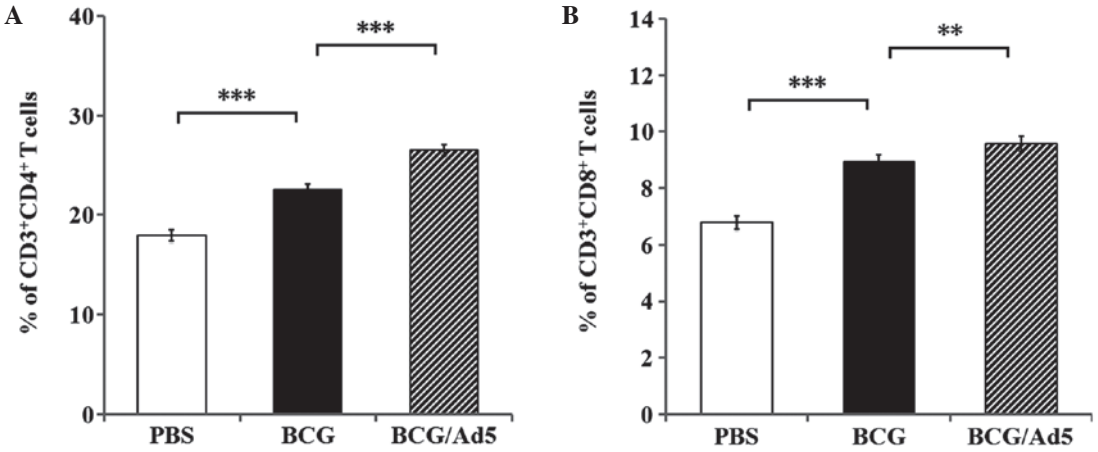

Figure 2. Frequencies of $CD4^+$ and $CD8^+$ T lymphocytes in immunized mice as a percentage of the total number. At 2 weeks following the final immunization, mice were sacrificed and their lymphocytes were isolated and exposed to *Mycobacterium tuberculosis* antigens for 36 h. $CD4^+$ and $CD8^+$ T cells were determined by staining with CD3e/CD4 and CD3e/CD8a antibodies, respectively. (A) Percentage of $CD3^+CD4^+$ T lymphocytes subsets. (B) Percentage of $CD3^+CD8^+$ T lymphocyte subsets. Values are expressed as the mean ± standard deviation of three independent triplicate experiments (n=8 per group). $^{**}P<0.01$; $^{***}P<0.001$. PBS, phosphate-buffered saline group; BCG, Bacillus Calmette Guerin group; BCG/Ad5, BCG with recombinant adenovirus Ad5-CEAB group.

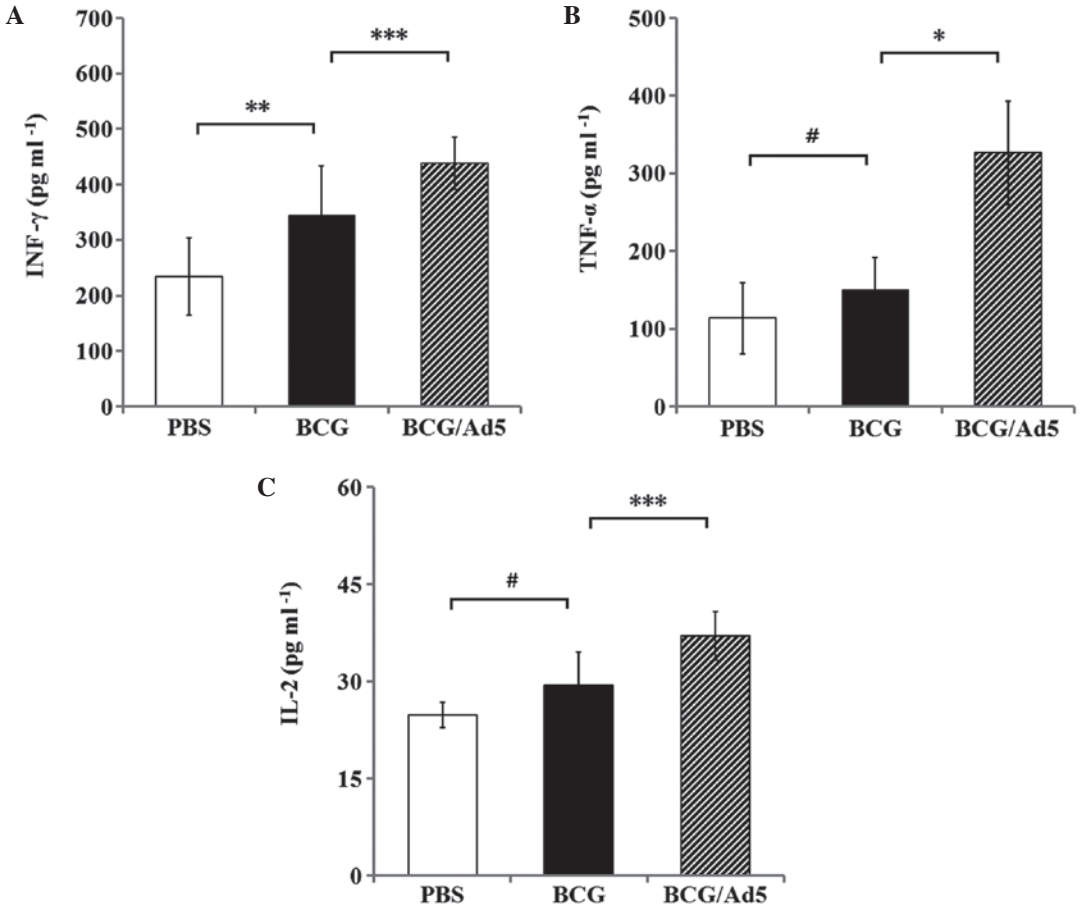

Figure 3. Levels of antigen-stimulated cytokines production in immunized mice. At 2 weeks following the final immunization, mice were sacrificed and splenic mononuclear cells from individual mice were stimulated with the *Mycobacterium tuberculosis* CEAB antigen mixture and the supernatants were harvested. Subsequently, the concentrations of (A) INF-γ, (B) TNF-α and (C) IL-2 were quantitatively analyzed using enzyme-linked immunosorbent assays. Values are expressed as the mean ± standard deviation of three independent triplicate experiments (n=8 per group). $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; $^{\#}P>0.05$. IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α; IL-2, interleukin-2; PBS, phosphate-buffered saline group; BCG, Bacillus Calmette Guerin group; BCG/Ad5, BCG with recombinant adenovirus Ad5-CEAB group.

of the prime-boosted strategy to induce T cell responses. The results of Mtb CEAB antigen-specific splenic T cell responses revealed a significantly elevated splenic T cell proliferation in immunized mice in the BCG and BCG/Ad5 groups compared with the PBS-treated group (Fig. 1A). In addition, the IFN-γ ELISPOT assay revealed an increased frequency

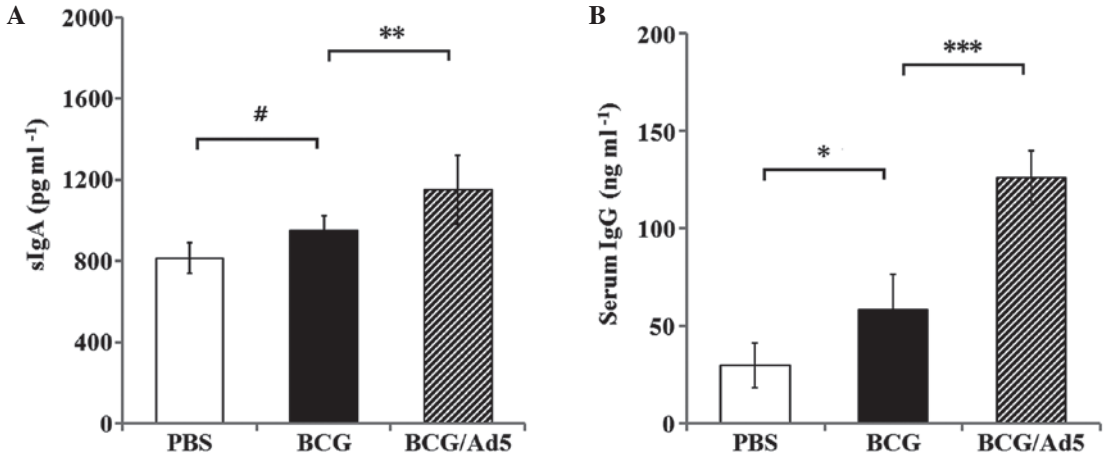

Figure 4. Antigen-specific antibody responses against *Mycobacterium tuberculosis* antigens in immunized mice. (A) Bronchoalveolar lavage fluid from mice was analyzed for antigen-specific sIgA concentrations using ELISAs. (B) Sera samples of mice were measured for antigen-specific IgG concentrations using ELISAs. Values are expressed as the mean ± standard deviation of three independent triplicate experiments (n=8 per group). *P<0.05; **P<0.01; ***P<0.001; $^{\#}$P>0.05. sIgA, secretory immunoglobulin A; IgG, immunoglobulin G; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline group; BCG, Bacillus Calmette Guerin group; BCG/Ad5, BCG with recombinant adenovirus Ad5-CEAB group.

of Mtb antigen-specific IFN-γ-secreting splenic T cells in the mice immunized with BCG (P<0.05) and BCG/Ad5 (P<0.001) compared with the PBS-treated group (Fig. 1B). Furthermore, higher frequencies of $CD4^+$ (Fig. 2A) and $CD8^+$ (Fig. 2B) T cell populations were observed in mice immunized with BCG (P<0.001) and BCG/Ad5 (P<0.001) compared with the PBS-treated group. Of note, all of the above examined indexes of immune response in mice immunized with BCG/Ad5 were significantly increased compared with those of the BCG group. These results clearly demonstrated that the subcutaneous BCG prime mucosal Ad5-CEAB-boosted strategy was capable of stimulating a more potent antigen-specific T cell response in mice compared with that of the BCG group.

Antigen-specific cytokines responses. Cytokines were previously suggested to have important roles in the host defense against Mtb (16). In the present study, concentrations of cytokines INF-γ, TNF-α and IL-2 were detected using ELISA analysis of the culture supernatant of lymphocytes stimulated with Mtb antigens *in vitro* (Fig. 3). The results showed that the levels of all the tested cytokines were significantly higher in the BCG prime-Ad5-CEAB-boosted group compared with the PBS-treated group. Of note, significantly elevated levels of antigen-induced cytokines INF-γ (P<0.001) (Fig. 3A), TNF-α (P<0.05) (Fig. 3B) and IL-2 (P<0.001) (Fig. 3C) were reported in the BCG prime-Ad5-CEAB-boosted group compared with the BCG group. This therefore indicated that the BCG prime-Ad5-CEAB boost strategy had a greater potency to enhance antigen-specific immunity in mice compared with BCG alone.

Antigen-specific antibody responses. Mucosal immunity is known to have important roles against Mtb infection and sIgA is the most abundant antibody isotype produced in mucosal tissues (38); therefore, sIgA production was examined in BAL fluid of mice. As shown in Fig. 4A, sIgA levels in BAL were markedly elevated in mice immunized with the BCG/Ad5 compared with the BCG group (P<0.01). However, no statistically significant difference was observed between the BCG group and PBS-treated group (P>0.05) (Fig. 4A). These data suggested that the prime-boost strategy was able to potently augment mucosal immune responses *in vivo*.

Humoral immunity has also been demonstrated to have a protective role in mycobacterial infections (39). In order to evaluate the IgG antibody response in immunized mice in the BCG or BCG prime Ad5-CEAB-boosted groups, the titers of IgG in mice were examined at 2 weeks following the final immunization. As shown in Fig. 4B, mice immunized with BCG or BCG/Ad5 elicited significantly higher titer of antigen-specific IgG compared with the group treated with PBS. Furthermore, IgG levels in mice immunized with BCG/Ad5 were significantly higher compared with those of the BCG group (P<0.001), which indicated that the prime-boosted strategy may elicit more efficient antibody responses.

Discussion

It has been reported that repeated vaccination with the same vaccine induces increased levels of antibody production compared with a single vaccination. However, such homologous boosts with the same vaccine may not be sufficient for protection against intracellular pathogens, such as Mtb (7). Studies in humans have demonstrated that revaccination with BCG does not confer additional protection against TB (40,41) and certain studies in humans and animals even reported negative effects associated with BCG revaccination (40-43). However, heterologous prime-boost strategies using BCG and a novel anti-TB vaccine may elicit robust immune responses, which are more efficacious than BCG alone. Since BCG is the only commonly used anti-TB vaccine in most developing countries, employment of a second vaccine to boost BCG-primed immunity may be the most practical novel strategy. In accordance with the heterologous prime-boost strategy, the present study investigated the safety and efficacy of a novel recombinant vaccine candidate, Ad5-CEAB, using the BCG-prime-boost strategy in mice. To the best of our knowledge, the recombinant adenovirus Ad5-CEAB used as booster in the present study was the first attempt for

the co-expression of four Mtb antigens as a mixture of individual proteins. The results demonstrated that the adenovirus vector may be a promising novel vaccine platform capable of boosting BCG-induced immunity.

Vaccines against intracellular infections are dependent on the induction of cell-mediated immunity (44). As an intracellular pathogen, Mtb localizes to the vacuole of the host's macrophages and cellular immunity has a crucial role in the immune response against Mtb infection. The cellular immune response is primarily composed of $CD4^+$, $CD8^+$ and other subsets of T cells. $CD4^+$ T cells were reported to contribute to the initial resistance to Mtb via the production of IFN-γ and other cytokines in order to induce macrophage activation (45). However, $CD8^+$ T cells produce IFN-γ and cytokines in addition to producing perforin and granulysin, which act to directly kill Mtb-infected cells and attack the invaded Mtb bacilli (46). In the present study, significantly increased frequencies of antigen-specific $CD4^+$, $CD8^+$ and INF-γ-secreting T cells were detected in the splenocytes of mice boosted with Ad5-CEAB compared with those primed with BCG alone. In addition to its ability to induce antigen-specific T cell responses, the prime-boost strategy also displayed a capacity to augment antigen-specific T helper type-1 cytokine production, including the secretion of INF-γ, TNF-α and IL-2. Cytokines have also been demonstrated to have important roles in host defense against Mtb infection. For example, IFN-γ was reported to activate infected macrophages and directly inhibit intracellular replication and growth of Mtb (47,48). By contrast, TNF-α was demonstrated to be essential for the initiation of the immune response against Mtb infection (49).

In addition to T cell responses, the prime-boost strategy exhibited a capacity to evoke antibody responses in the present study. Antibody responses have a protective role in preventing mycobacterial infections, particularly the mucosal antibodies. For example, sIgA, the most abundant naturally-produced antibody isotype in mucosal tissue, has an indispensable role in preventing primary Mtb infection at the mucosal surfaces; in addition, sIgA was reported to prevent the adsorption of pathogens at the mucosal epithelium (50,51). A murine study demonstrated that sIgA may act to prevent the entrance of mycobacterial bacilli into the lungs (52). The results of the present study revealed that the BCG prime and mucosal Ad5-CEAB boost strategy was able to significantly augment mucosal immune responses *in vivo*.

Antigen-specific IgG antibodies are commonly used as biomarkers to confirm the expression of Mtb antigens in animal models; however, the role of serum antibodies in the pathogenesis and control of TB has been controversial for a long time. Previous studies have demonstrated that serum antibodies may have protective effect in animal models of tuberculosis (39,53). In addition, analysis of the isotypic distribution of immunoglobulin may offer an insight into the possible immunological mechanisms involved in cellular immunity (54). Together with the observation of increased $CD4^+$ and $CD8^+$ T cell populations in BCG/Ad5-CEAB-immunized mice, the results of the present study clearly demonstrated that the BCG prime mucosal Ad5-CEAB boost vaccination strategy effectively evoked the immune system for T cell- and antibody-mediated antigen-specific immune responses in mice.

The mucosal immune response is the first line of defense against infectious agents and is crucial for the immune response against Mtb infection. Increasing evidence has indicated the effectiveness of vaccination at the mucosal site compared with vaccination via other routes for inducing protection from mucosal infectious diseases (15,55). Numerous studies have verified that mucosal immunity may provide unique advantages for protection against Mtb infection (34,56-58). Therefore, any vaccines or vaccination strategies that are able to elicit the mucosal immune response may enhance the efficacy of protection against Mtb infection. Great efforts have been made to improve the protective efficacy of TB vaccines and various types of vaccine candidates or vectors have been developed, including the recombinant BCG (rBCG), DNA vaccines, nanoparticle vaccines, recombinant modified vaccinia virus Ankara and recombinant adenoviral-based vaccines (12,15,32,59,60). Among them, the adenoviral-based TB vaccines have gained increased attention, as they were first evaluated as mucosal TB vaccine candidates (15). An increasing number of studies have thus focused on mucosal immunity; these studies have suggested that intranasal/intrapulmonary vaccination with recombinant adenoviral vaccines may induce an antigen-specific mucosal immune response (15,61-66). In line with these findings, the results of the present study demonstrated that intranasal boost with the recombinant adenovirus Ad5-CEAB was able to enhance the BCG-primed immune response. In addition, the present study reported that levels of antigen-specific sIgA in BAL fluid were significantly increased in the Ad5-CEAB-boosted group, indicating that intranasal boost with Ad5-CEAB may induce a potent antigen-specific mucosal immune response in mice.

In conclusion, the results of the present study demonstrated that the heterologous prime-boost strategy of subcutaneously primed BCG-intranasal boost with recombinant adenovirus Ad5-CEAB was able to elicit an enhanced antigen-specific immune response in mice compared with that conferred by homologous prime-boost immunization with BCG. These results provided evidence for the effectiveness of TB vaccines from recombinant adenoviral vectors and novel anti-TB vaccination strategies. The BCG prime Ad5-CEAB boost vaccination strategy appears promising as an anti-TB vaccination strategy, thus we aim to evaluate this in mice infected with Mtb in future studies.

Acknowledgements

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