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ORIGINAL ARTICLE

Effect of Maternal Immune Status on Responsiveness of Bacillus Calmette-Guérin Vaccination in Mouse Neonates

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Abstract
Objectives: Bacillus Calmette-Guérin (BCG) vaccination has proven to be efficient in immunologically naïve infants; however, it has not been investigated that maternal natural exposure to *Mycobacterium* and/or BCG vaccine could influence the characteristics of immune responses to BCG in newborns. In this study, we analyzed whether the maternal immune status to *M tuberculosis (M tb)* can affect neonatal immunity to BCG using a mouse model.
Methods: Neonates were obtained from mice that were previously exposed to live BCG, to live *M avium*, or to heat-killed *M tb* H37Rv, and from naïve control mothers. One week after birth, the neonates were divided into two subgroups: one group immunized with live BCG via the subcutaneous route and the other group of neonates sham-treated. Interferon-gamma (IFNγ) secretion in response to *in vitro* stimulation with heat-killed BCG or purified protein derivative (PPD) was examined. Protection against *M tb* infection was evaluated by challenging mice nasally with live *M tb* H37Rv followed by counting colonies from spleen and lung homogenates.
Results: BCG-immunized neonates showed increased IFNγ secretion in response to heat-killed BCG or PPD. All mice in BCG-immunized neonates subgroups showed reduced bacterial burden (colony forming unit) in the lungs when compared with control naive neonate mice. However, no statistically significant difference was observed when comparing BCG-immunized mice born from mothers previously exposed to *M avium* or immunized with either heat-killed H37Rv or live BCG and mice born from naïve mothers.
Conclusion: The maternal immune status to *M tb* does not appear to impact on the immunogenicity of BCG vaccine in their progeny in our experimental conditions.

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1. Introduction

Mycobacterium tuberculosis (*M tb*) is an intracellular pathogen that causes severe infectious disease, tuberculosis (TB), which is responsible for high level of mortality and morbidity [1−3]. About 30% of the population is worldwide infected with this infectious agent [1]. Most of them are considered to have latent tuberculosis infection (LTBI) and only 10% of the infection will develop to serious pulmonary TB [2,4]. Nevertheless, new cases of this bacterial infection are still occurring mostly in developing countries, and immunologically immature infants in these regions can be easily exposed to infections with mycobacteria [5].

To prevent from TB infection, Bacillus of Calmette and Guérin (BCG) obtained from a live attenuated strain of *M bovis* (*M bovis*) has been used as an only authorized vaccine since 1921 [6]. Previous study showed outstanding effectiveness of BCG vaccination at birth in control of neonatal miliary TB and meningitis TB as well [7]. However, the vaccination with BCG shows inconsistent responsiveness and relatively low protection efficacy against predominant pulmonary TB in adults [8]. In animal and human studies, neonatal immunization with BCG showed relatively higher protection efficacy than that in adults, which is mediated by Th1-type immune responses [9,10].

Guirado and colleagues [11] reported that antibodies play an essential protective role against mycobacterial infections by passive immunization using sera obtained from mice treated with detoxified *M tb* extracts in B cell-deficient mice, while it is generally accepted that cell-mediated immune response is important in controlling *M tb* infections. While current BCG vaccine has proven to be efficient in immunologically naïve infants, a maternal history of natural exposure to mycobacteria or vaccination against TB has not been studied yet. Therefore, we hypothesized that maternal immune status to mycobacteria may play a critical role in the uptake and subsequent protective immunogenicity of BCG in newborns. In the present study, we analyzed whether the maternal immune status to *M tb* can affect the neonatal immunity to BCG in mouse neonates by assessing *in vitro* IFNγ secretion by stimulated spleen cells [11−13] and by evaluating the protective efficacy after challenge experiments. We found that neonatal BCG immunization can reduce the bacterial load in pulmonary tissues. In addition, we showed that the maternal immune history mediated by either vaccination or pre-exposure to *M tb* was insufficient to elicit the specific immune responses to BCG vaccine in their progeny. This may provide basic insights into importance of immunogenicity through neonatal vaccination and/or maternal vaccination, and it can be considered for further TB vaccine researches.

2. Materials and Methods

2.1. Animals

Six-weeks old female Balb/c mice (OrientBio or Daehan Biolink, Korea) were maintained under specific pathogen-free conditions in the animal facility, International Vaccine Institute (Seoul, Korea), where they were fed with sterilized food and water *ad libitum*. Breeding cages were checked daily for new births, and the pups were kept with the mother until weaning at 3 weeks of age. Animal infections with *M tb* were performed at the Biosafety Level 3 facility of the institute. All experiments described were approved by appropriate institutional animal care and use committees.

2.2. Bacteria

M bovis BCG Pasteur (BCG) [14], *M tuberculosis* H37Rv (*M tb* H37Rv) [15] and *M avium subsp. Hominissuis* (*M avium)*[16] were cultured in suspension in 7H9 Middlebrook media (BD Bioscience, San Diego, CA, USA) supplemented with 10% OADC (v/v, BD, Franklin Lakes, NJ, USA) and 0.05% Tween80.

2.3. Vaccination of mice

One week before mating, mice were immunized with live BCG [subcutaneously, 5×10^5 colony forming units [CFU]), live *M avium* (intranasally, 1×10^5 CFU), or heat-killed *M tb* H37Rv (intranasally, 5×10^5 CFU), respectively. Neonates obtained from individual mothers were divided into two subgroups and vaccinated 1 week after birth: one group immunized with live BCG (5×10^5 CFU) via subcutaneous route and the other group of neonates sham-treated.

2.4. Antibody determination

Four weeks after BCG vaccination, immunoglobulin G (IgG) level in serum obtained from individual mice were analyzed by enzyme-linked immunosorbent assay (ELISA). Briefly, ELISA plates were coated with heat-inactivated BCG (5×10^5 CFU/mL) in phosphate buffered saline (PBS) overnight at 4 °C. Next day, plates were washed four times with PBS-T (v/v, 0.05% Tween20, Sigma-Aldrich, St. Louis, MO, USA) and blocked with 1% bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO, USA) for 1 hour at room temperature. After washings, the samples were applied in serial dilutions and subsequently incubated for 2 hours at room temperature. Following extensive washings with PBS-T, horseradish peroxidase-conjugated goat anti-mouse IgG (Southern Biotech, Birmingham, AL, USA) was diluted in 1% BSA and applied. After 1-hour incubation at room temperature, the plates were washed four times, and the 3,3′, 5,5″-tetramethylbenzidine (TMB) peroxidase substrate (Moss, Inc., Pasadena, MD, USA) was subsequently added to develop the enzymatic color reaction. Optical density was detected