



Neonatal BCG vaccination is associated with enhanced T-helper 1 immune responses to heterologous infant vaccines



Daniel H. Libraty^{a,*}, Lei Zhang^a, Marcia Woda^a, Luz P. Acosta^b, AnaMae Obcena^c, Job D. Brion^d, Rosario Z. Capeding^{c,e}

^a Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, MA, USA

^b Department of Immunology, Research Institute for Tropical Medicine, Manila, Philippines

^c Department of Medicine, Research Institute for Tropical Medicine, Manila, Philippines

^d San Pablo City Health Office, San Pablo, Philippines

^e Department of Microbiology, Research Institute for Tropical Medicine, Manila, Philippines

ARTICLE INFO

Article history:

Received 1 October 2013

Revised 18 November 2013

Accepted 24 November 2013

Keywords:

BCG

Vaccines

T-cell

Th1

Neonate

Infant

ABSTRACT

Neonatal Bacille Calmette Guérin (BCG) vaccination has been reported to have beneficial effects beyond preventing infantile tuberculous meningitis and miliary disease. We hypothesized that BCG vaccine given at birth would enhance T-helper 1 (Th1) immune responses to the first vaccines given later in infancy. We conducted a nested case-control study of neonatal BCG vaccination and its heterologous Th1 immune effects in 2–3 months old infants. BCG vaccination at birth was associated with an increased frequency of interferon- γ (IFN- γ) producing spot-forming cells (SFC) to tetanus toxoid 2–3 months later. The frequency of IFN- γ producing SFC to polioviruses 1–3 also trended higher among infants who received BCG vaccination at birth. The frequency of IFN- γ /tumor necrosis factor- α (TNF- α)+CD45RO+CD4+ T-cells upon stimulation with phorbol myristate acetate (PMA)/Ionomycin was higher in 2–3 months old infants who received BCG vaccination at birth compared to those who did not. The circulating frequency of forkhead box P3 (FoxP3)+ CD45RO+ regulatory CD4+ T-cells also trended lower in these infants. Neonatal BCG vaccination is associated with heterologous Th1 immune effects 2–3 months later.

© 2013 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/3.0/>).

Introduction

The Bacille Calmette Guérin (BCG) vaccine is given to neonates in most countries to prevent infantile tuberculous meningitis and miliary disease. It is one of the most widely used vaccines worldwide. The efficacy of neonatal BCG administration has been linked to its ability to effectively induce a T-helper 1 (Th1)-polarized neonatal immune response [1]. Th1 cells are effector and memory CD4+ T-cells polarized to produce interferon- γ (IFN- γ). Neonates and infants generally have reduced Th1 responses to many intracellular pathogens and toxins [1]. Neonatal BCG vaccination has also been reported to reduce neonatal and infant mortality due to diseases other than tuberculosis [2–4]. Beneficial heterologous immune effects on antibody responses to some routine infant immunizations have been reported [5]. When given at age 2 months and at the same time as other routine infant vaccines, BCG has been reported

to enhance the immune responses to some unrelated vaccines [6]. We hypothesized that BCG vaccine given at birth would enhance Th1 immune responses to the first vaccines given later in infancy. In order to examine this hypothesis, we conducted a nested case-control study of neonatal BCG vaccination. This study was within an ongoing clinical study of dengue virus infections during infancy in San Pablo, Laguna, Philippines. We found that BCG vaccination at birth was associated with some heterologous Th1 immune effects 2–3 months later, particularly an increased frequency of IFN- γ producing spot-forming cells (SFC) to tetanus toxoid.

Materials and methods

Ethics statement

The study protocol was approved by the institutional review boards of the Research Institute for Tropical Medicine, Philippines, and the University of Massachusetts Medical School. Mothers and their healthy infants were recruited and enrolled after providing written informed consent.

* Corresponding author. Address: Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Rm S6-862, 55 Lake Ave N, Worcester, MA 01655, USA. Tel.: +1 508 856 4182; fax: +1 508 856 4890.

E-mail address: daniel.libraty@umassmed.edu (D.H. Libraty).

Clinical study

The nested case-control study was drawn from an ongoing clinical study of dengue virus infections during infancy that has been previously described [7]. There were $n = 13$ infants who did not receive BCG vaccine between birth and 2 weeks of age; they received the BCG vaccine after their first infant immunization with the diphtheria/pertussis/tetanus toxoid vaccine (DPT) and oral poliovirus vaccine (OPV) (cases). These infants did not receive neonatal BCG because they were delivered at home in outlying areas. There were $n = 38$ age- and sex-matched infants who received BCG between birth and 2 weeks of age (controls). Vaccination dates were obtained from Expanded Programme in Immunization (EPI) cards. Clinical and epidemiological information were also collected at the study visit. Normalized child growth indicators were determined using World Health Organization (WHO) child growth standards. Peripheral blood mononuclear cells (PBMC) were collected from infants at the first study visit, at approximately 8–12 weeks old. PBMC were isolated using Histopaque® density centrifugation and cryopreserved.

IFN- γ ELISPOT

Briefly, cryopreserved PBMC ($2-4 \times 10^5$ cells per well) were thawed and seeded onto polyvinylidene difluoride membrane 96-well plates (Millipore) precoated with $5 \mu\text{g/ml}$ anti-IFN- γ monoclonal antibody (clone D1K; Mabtech). Stimuli were tetanus toxoid (EMD Calbiochem, $150 \mu\text{g/ml}$), inactivated poliovirus vaccine (Sanofi Pasteur SA, 1/3.3 dilution), recombinant hepatitis B surface antigen (Genway Biotech, $27 \mu\text{g/ml}$), phytohemagglutinin (PHA) (Sigma-Aldrich, $5 \mu\text{g/ml}$), or media control (complete RPMI 1640/10% fetal calf serum). After 48 h of incubation (except PHA, 18–24 h incubation), cells were removed by washing with phosphate-buffered saline plus 0.05% Tween 20. Secondary biotinylated anti-IFN- γ monoclonal antibody (clone 7-B6-1; Mabtech) was added at $2 \mu\text{g/ml}$ and the plates were incubated for 2 h at room temperature. Plates were washed again and IFN- γ was detected with avidin-peroxidase (3420-2H, Mabtech) and substrate kit (NovaRed, Vector Laboratories). The frequency of IFN- γ -producing cells was determined by using the ImmunoSpot S4 Pro Analyzer and the ImmunoSpot Academic V.4 Software (Cellular Technologies Ltd.). Experiments were performed in triplicate wells.

Flow cytometry

IFN- γ and TNF- α secreting, and FoxP3+, CD4+ T-cells in infant PBMC were identified by ICS (intracellular cytokine staining). PBMC were washed with media, and then left unstimulated or stimulated for 4 h with PMA/Ionomycin (BD Biosciences). The stimulations were done in the presence of $1 \mu\text{l}$ Brefeldin A (BD Biosciences). Cells were first stained with surface Ab to CD45RO (clone UCHL1), fixed and permeabilized with the FoxP3 buffer set (BD Biosciences), and then stained with Abs to CD3 (clone UCHT1), CD4 (clone SK3), CD8 (clone SK1), IFN- γ (clone B27), TNF- α (clone 6401.1111), and FoxP3 (clone 259D/C7) (all Abs from BD Biosciences). Cells were analyzed using a FACSAria flow cytometer (BD Biosciences). LIVE/DEAD® Fixable Dead Cell Stain Kit (LDA) (Invitrogen) was used to exclude nonviable cells from analysis. Relevant cells were identified as LDA- /CD3+ /CD4+ /CD8- /CD45RO+ or CD45RO- /IFN- γ + or TNF- α + or FoxP3+ cells (Supplementary Fig. 1). Data was analyzed using FlowJo® software (Treestar).

Statistical analysis

The SPSS software package (version 20.0) was used for statistical analyses. Comparisons between two continuous variables were

performed using the non-parametric Mann-Whitney U test. Comparisons between categorical variables were performed using the χ^2 test. P -values <0.05 were considered significant. P -values ≥ 0.05 and <0.10 were considered a trend.

Results

Case-control study of neonatal BCG vaccination

We conducted a nested case-control study of the heterologous Th1 immune effects of neonatal BCG vaccination. The timing of vaccinations, age at the time of PBMC collection, and characteristics of infants in the case-control study are shown in Table 1. There were no significant differences in any measured variable between infants in the case and control groups.

Neonatal BCG vaccination is associated with an increased frequency of IFN- γ producing cells to tetanus toxoid and polioviruses in 2–3 months old infants

We performed IFN- γ ELISPOT assays to tetanus toxoid, polioviruses 1–3, hepatitis B surface antigen, and phytohemagglutinin (PHA) in the PBMC from 2–3 months old infants. Neonatal BCG vaccination was associated with an increased frequency of IFN- γ SFC to tetanus toxoid. The frequency of IFN- γ SFC to polioviruses 1–3 (inactivated poliovirus vaccine) also trended higher among infants who received BCG vaccination at birth (Fig. 1). There were no differences in the frequencies of IFN- γ SFC to hepatitis B surface antigen or PHA between the infants who received neonatal BCG vaccination and those who did not (Fig. 2). The frequencies of IFN- γ SFC to tetanus toxoid and polioviruses 1–3 were correlated (Spearman $r = 0.61$, $p < 0.001$). The frequencies of IFN- γ SFC to tetanus toxoid or polioviruses 1–3 did not correlate with IFN- γ SFC to either hepatitis B surface antigen or PHA.

Neonatal BCG vaccination is associated with an increased frequency of IFN- γ + /TNF- α + CD45RO+CD4+ T-cells upon PMA/Ionomycin stimulation in PBMC from 2–3 months old infants

The gating strategies for flow cytometry and intracellular cytokine staining (ICS) are shown in Supplementary Fig. 1. We examined IFN- γ and TNF- α production in CD4+ T-cells upon PMA/Ionomycin stimulation in the PBMC from 2–3 months old infants. BCG vaccination at birth was associated with an increased frequency of PMA/Ionomycin-stimulated IFN- γ + /TNF- α + CD45RO+CD4+ T-cells during early infancy. There was no difference in the frequencies of PMA/Ionomycin-stimulated IFN- γ + /TNF- α + CD45RO-CD4+ T-cells between infants who received BCG vaccination at birth and those who did not (Fig. 3). Essentially all infant CD4+ T-cells that produced IFN- γ upon PMA/Ionomycin stimulation also produced TNF- α .

Neonatal BCG vaccination is associated with a decreased trend of circulating FoxP3+CD45RO+ regulatory CD4+ T-cells in 2–3 months old infants

BCG vaccination at birth was associated with a decreased trend in the circulating frequency of FoxP3+CD45RO+CD4+ T-cells 2–3 months later. FoxP3+CD4+ T-cells are considered to be mainly regulatory T-cells (Tregs). There was no difference in the circulating frequencies of FoxP3+CD45RO- CD4+ T-cells between infants who received BCG vaccination at birth and those who did not (Fig. 4). The vast majority of all CD4+ T-cells, including FoxP3+ CD4+ T-cells, in the infants were CD45RO- (approximately 90%).

Download English Version:

<https://daneshyari.com/en/article/2474398>

Download Persian Version:

<https://daneshyari.com/article/2474398>

[Daneshyari.com](https://daneshyari.com)