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Short communication

A cross-reacting material CRM_{197} conjugate vaccine induces diphtheria toxin neutralizing antibody response in children and adolescents infected or not with HIV

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ABSTRACT

Anti-diphtheria antibody levels decrease with aging, and frequent booster vaccinations are required to maintain herd immunity. We analyzed the diphtheria toxin neutralizing antibody (DT-Nab) response induced by a conjugate vaccine (meningococcal C polysaccharide-CRM_{197}) in HIV-vertically infected (HI) children and adolescents and healthy controls (HC) with matched age. We report the association of DT-Nab with the bactericidal antibodies to serogroup C meningococcus (MenC). Before vaccination, 21 HI patients (50%) had no protection against diphtheria ($\leq$0.01 IU/ml of antibody) and only 8 (19%) showed complete protection ($\geq$0.1 IU/ml). About half of the HC (56%) had complete protection before immunization and 6 subjects (12%) had no protection against diphtheria. After one and two vaccine injections, 96% of HC and 64% of HI vaccinees, respectively, showed full protection against diphtheria. These data indicate that CRM_{197} was able to induce primary and/or booster response in both groups of individuals.

1. Introduction

Active and efficient immunization programs against diphtheria are based on the use of a formaldehyde-detoxified preparation of diphtheria toxoid to induce protective neutralizing antibody responses [1]. However, anti-diphtheria antibody levels decrease with increasing age, and frequent booster vaccinations are required to maintain herd immunity in the adult population [2–4]. The cross-reacting material (CRM_{197}) of diphtheria toxin is a genetically detoxified preparation of the toxin that does not require detoxification with formaldehyde, causing much less adverse effects in humans [5].

The recommended vaccination schedule against diphtheria in Brazil consists of three doses of diphtheria-tetanus-pertussis (DTP) vaccine in the first year of life, followed by booster doses at 15 months and 4 years of age. After that, a booster dose should be given at every 10 years. Currently, meningococcal serogroup C conjugate vaccines is recommended in a regimen of two doses 8 weeks apart for previously unvaccinated HIV-infected toddlers, children and adolescents, with a booster 5 years later [6].

We have previously reported the bactericidal antibody response to *Neisseria meningitidis* C (MenC) after vaccination (1 injection) of children/adolescents, HIV-vertically infected (HI) and healthy controls (HC), with a conjugate vaccine using CRM_{197} as the carrier protein [7]. The objective of the present study was to analyze the diphtheria toxin neutralizing antibody (DT-Nab) response induced by the conjugate vaccine (C polysaccharide-CRM_{197}) in a sampling of the previously reported study [7]. For the present study, HI vaccinees received a booster dose of vaccine about one year after the first vaccination. We also report the association of DT-Nab

Abbreviations: DT-Nab, diphtheria toxin neutralizing antibody; HI, HIV-infected children/adolescents; HC, healthy controls; MenC, serogroup C meningococcus; CRM_{197}, cross-reacting material; DTP, diphtheria-tetanus-pertussis; HAART, highly active antiretroviral therapy; SBA, serum bactericidal antibody; V1, before immunization; V2, one-two months after first dose; V3, one year after first dose; V4, one-two months after booster; MTT, brometo de [3-(4,5-dimetiltiazol-2yl)-2,5-difenil tetrazolium].

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response with the bactericidal antibody response to MenC, CD4 T cell levels and viral load.

2. Patients and methods

We conducted a prospective cohort study at the *Instituto de Puericultura e Pediatria Martagão Gesteira, Universidade Federal do Rio de Janeiro* (IPPMG/UFRJ), Rio de Janeiro, Brazil, to investigate the seroconversion rate after MenC vaccination in HIV-vertically infected individuals. The study was approved by the IPPMG Institutional Review Board (IRB, number 24/09) and Brazilian Ministry of Health Ethics Commission (*Comissão Nacional de Ética em Pesquisa*, CONEP, number 15578). Details about the inclusion and exclusion criteria, IRB and participant's consent were previously published [7]. Due to a weak antibody response after the first dose of vaccine, HI patients received a booster dose of the vaccine about one year later. HC children with matched age were also included in this study. However, they received only one injection of the vaccine, as per recommendation at the time of this study, in healthy children and youth, aged 1–25 years, a single MenC dose should be given [8]. The conjugate vaccine (Novartis; C Polysaccharide/CRM_{197}) was administered at the recommended dose (10 μg/0.5 ml). Most (93%) of the HI patients of the present study was receiving HAART for about 6.5 years (median).

2.1. Serum samples

For HI, blood samples were collected before (Visit 1) vaccination, 1–2 months after one dose (Visit 2), before booster (10–12 months after first dose, Visit 3) and 1–2 months after boosting (Visit 4) using tubes in the absence of anticoagulant. Serum samples were stored at −20°C.

For HC group, that received only one vaccine injection, we collected blood samples before (visit 1), 1–2 months after vaccination (visit 2) and one year later (visist 3).

In total, we analyzed 168 blood samples from 42 HI patients, with median age of 12 years, and 150 blood samples from 50 HC individuals with median age of 10 years.

2.2. Bactericidal assay

Serum bactericidal antibody (SBA) titers were measured as previously described [7,9], using human complement source.

2.3. Diphtheria toxin (DT) neutralization test

The DT neutralization test in Vero cells (green monkey renal episerum) was used. The concentration of serum diphtheria antitoxin in cell culture was estimated according to the procedure described by Miyamura et al. [10], with some modifications. Serial 2-fold dilutions (25 μL) of serum were mixed with 25 μL DT equivalent to four times the minimal cytotoxic dose (0.26 ng/mL) (Instituto Nacional de Controle de Qualidade em Saúde, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil) and incubated for 1 h at 37°C. Then, 50 μL suspension containing 2.5×10^5 Vero cells/mL in modified Eagle's medium supplemented with 10% fetal calf serum was added to each well. The plates were gently shaken, covered and incubated at 37°C/5%CO_2 for 6 days. On the basis of concurrent testing with a reference serum (equine antiserum from Instituto Nacional de Controle de Qualidade em Saúde, Fundação Oswaldo Cruz, Rio de Janeiro Brazil; 10 IU/ml antibody to DT), the antibody titer is reported as IU/mL. An antitoxin-positive control serum, a toxin control, a test serum control, and a cell control were run for every plate. Neutralizing antibody titer was defined as the highest dilution of serum neutralizing toxin that killed 100% Vero cells. Cell viability was determined by the MTT assay [11]. Neutralizing antibody levels were categorized according to internationally accepted ranges: <0.01 IU/mL (non-protective), 0.01–0.09 IU/mL (basic protection), and ≥0.1 IU/mL (full protection) [12–14].

2.4. Statistical analysis

The levels of significance of the differences between groups were examined by, either the Mann-Whitney test (unpaired samples) or the Wilcoxon matched-pair test (paired samples), as non-parametric data were obtained. The correlation between different measurements of immune response was analyzed using Spearman rank test, after graph analyses. These analyses were performed with the GraphPad-Prism software, version 7 (GraphPad Software, Inc., USA). The differences between the percentage of seroconversion and seroprotection were analyzed by software Epi Info™ 6.04d, CDC.

3. Results and discussion

3.1. CRM_{197} activates antibody response against DT

Fig. 1A shows the DT-Nab response in HI individuals. We observed a significant ($P < 0.0001$) increase of specific antibodies after the first immunization (median of 0.31 IU/ml) compared with pre-immunization levels (median of 0.01 IU/ml). One year after immunization, there was a significant decrease of DT-Nab levels (median of 0.03 IU/ml; $P < 0.0001$) but it was still higher than pre-immunization levels (0.01 IU/ml).

Vaccine boosting induced a marked increase of DT-Nab levels (median of 0.32 IU/ml; $P < 0.0001$), reaching similar levels as detected after the first dose.

Fig. 1B shows the DT-Nab response for HC group. There was a huge ($P < 0.0001$) increase of antibody levels against DT at visit 2 (median of 5) when compared to pre-vaccination levels (median of 0.16). One year after vaccination (V3), it was detected a significant drop in the level of DT-Nab (median of 0.62, $P < 0.0001$). Notice that, although the median levels of V1 and V3 antibodies were low, the median of V3 (0.62) was higher ($P < 0.0001$) than V1 (median = 0.16). The DT-Nab response of HC vaccinees was significantly ($P < 0.0001$) higher than the response of HI group at all time points analyzed. All together, these data indicate that CRM_{197} protein was able to activate the memory response induced by the diphtheria toxoid administered during childhood especially in HC group and to a less extension in HI group. CRM_{197} also primed the immune system of HI patients since we detected a booster response after the second conjugate vaccine administration.

3.2. Protection status to diphtheria differs between HI and HC groups

Table 1 shows the protection status to diphtheria based on DT-Nab levels for HI and HC groups. Before vaccination, 21 HI patients (50%) had no protection against diphtheria (≤0.01 IU/ml of antibody), 13 (31%) had basic protection (above 0.01–0.09 IU/ml) and only 8 (19%) showed complete protection (≥0.1 IU/ml) against the disease. Therefore, based on DT-Nab levels, most of the individuals infected with HIV (81%) were not fully protected against diphtheria before vaccination with the conjugate vaccine.

In contrast, for HC group, about half of the individuals (56%) had complete protection (≥0.1 IU/ml) before immunization, 16 (32%) had basic protection (up 0.01–0.09 IU/ml) and 6 subjects (12%) had no protection against diphtheria (≤0.01 IU/ml). The frequency of HC patients with protective DT-Nab levels was significantly

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