Vaccine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Immune response to a Tdap booster in vertically HIV-infected adolescents

Fernanda Garcia Spina [a], Aída Gouvea [a], Regina Célia de Menezes Succi [a], Flavia Calanca [a], Lily Yin Weckx [a], Maria Teresa Terreri [a], Maria Aparecida Sakauchi Takano [b], Maria Isabel de Moraes-Pinto [a,*]

[a] Department of Pediatrics, Federal University of São Paulo, São Paulo, SP, Brazil
[b] Bioindustrial Division, Butantan Institute, São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 17 April 2018
Received in revised form 15 July 2018
Accepted 17 July 2018
Available online xxxx

Keywords:
Tdap
Vertical HIV infection
Immunization
Cytokines
Antibodies

ABSTRACT

Background: Pertussis cases have increased worldwide and knowledge on immune response and cytokine profile after Tdap vaccine in immunodeficient adolescents is scarce.
Objective: To evaluate the immune response after Tdap in HIV-infected (HIV) and in healthy adolescents (CONTROL).
Methodology: Thirty HIV adolescents with CD4 cell counts >200 and 30 CONTROLs were immunized with Tdap, after a prior whole-cell DTP vaccine primary scheme. Blood samples were collected immediately before and after vaccine. Lymphocyte immunophenotyping was performed by flow cytometry; tetanus, diphtheria and pertussis toxin antibodies were assessed by ELISA; whole blood was stimulated with tetanus toxoid and *Bordetella pertussis* and supernatants were assessed for cytokines by xMAP.
Results: Mean age of HIV and CONTROL groups were 17.9 e 17.1 years, respectively. Pain at injection site was more intense in CONTROL group. HIV group had similar increase in tetanus antibodies at 28 days (geometric mean concentration, GMC, 15.6; 95% CI, 7.52–32.4) than CONTROL group (GMC, 23.1; 95% CI, 15.0–35.5), but lower diphtheria antibodies at 28 days (GMC, 2.3; 95% CI, 0.88–6.19) than CONTROL group (GMC, 16.4; 95% CI, 10.3–26.2); for pertussis, the percentage of individuals who seroconverted was lower in HIV than CONTROL group (HIV, 62.1% versus CONTROL, 100%; p = .002). Both groups built a cellular immune response to tetanus, with a Th2 (IL-4, IL-5 and IL-13) and Th1 (IFN-γ) response, with lower cytokine levels in HIV than in CONTROL group. Especially for pertussis, cellular and humoral responses were less intense in HIV adolescents, with a lower Th1 and Th17 profile and higher IL-10 levels. HIV-infected adolescents on viral suppression showed an enhanced immune response to all the three vaccine antigens, although still at lower levels if compared to CONTROL group.
Conclusions: Both groups tolerated well and built an immune response after Tdap. However, HIV-infected adolescents would probably benefit from more frequent booster doses.

1. Introduction

Although the incidence of diseases such as tetanus, diphtheria and pertussis has declined after routine vaccination [1], outbreaks still occur worldwide [2–4], even in places with high vaccination coverage [5].

Many vertically HIV-infected children are now able to reach adolescence [6]. However, they still develop a lower humoral immune response after vaccination [7] and a faster decay of antibodies is observed despite the successful use of combined antiretroviral therapy (cART) [8]. After revaccination [9] or following booster doses [10], the majority of HIV-infected youngsters reach protective antibody levels.

Due to the immunological alterations that persist in these individuals despite the use of cART, it is extremely important to assess serum antibodies over time as well as response to vaccine boosters. No study has assessed the immune response of vertically HIV-infected adolescents to Tdap so far. This study evaluated the humoral and cellular response after a Tdap booster dose in HIV-infected and in healthy adolescents.

* Corresponding author at: Rua Pedro de Toledo, 781, 9° andar, São Paulo, SP 04039-032, Brazil.
E-mail address: m.isabelmp@uol.com.br (M.I. de Moraes-Pinto).

https://doi.org/10.1016/j.vaccine.2018.07.043
0264-410X/

2. Materials and methods

This is a prospective longitudinal experimental study in which 30 HIV-infected (HIV) and 30 healthy (CONTROL) adolescents and young adults were evaluated between June 2015 and October 2016.

Individuals from HIV group were recruited at the Pediatric AIDS Outpatient Clinic of the Universidade Federal de São Paulo, in São Paulo, Brazil. Individuals from CONTROL group were recruited at the Adolescent Outpatient Clinic of the same university and among undergraduate students and adolescents.

Subjects from both groups needed to meet the following criteria: aged between 10 and 24 years; to have had 3 doses of diphtheria-tetanus-whole-cell pertussis vaccine (DTwP) and at least one DTwP or tetanus-diphtheria (Td) booster dose with a minimum of a 3 year-interval from study entry. Pregnancy was an exclusion criterion for both groups, as well as moderate or severe acute infection diagnosed in the previous 15 days.

Individuals from HIV group had to be infected by vertical transmission and to have CD4+ T cells above 200 cells/mm^3 on the last assessment.

Individuals from CONTROL group should not present any chronic, autoimmune, allergic or neoplastic disease, or use of immunosuppressive medication.

This study was approved by the Ethics Committee of the Universidade Federal de São Paulo and all parents/guardians signed the informed consent prior to enrollment (protocol number 777.149).

2.1. Clinical data collection

Clinical parameters and previous CD4+ T cell counts, HIV viral load and antiretroviral regimen were obtained from patients' medical records. Vaccination data were obtained from medical records and/or the patients' vaccination card on admission to study.

2.1.1. Blood collection

A ten-milliliter peripheral blood sample was collected on the day (day 0), after 14 (day 14) and 28 days of vaccination (day 28).

Lymphocyte immunophenotyping was performed on day 0; follicular T cells were assessed on days 0 and 14; cellular immunity to tetanus and pertussis as well as tetanus, diphtheria and pertussis serology were assessed on days 0, 14 and 28; HIV viral load was assessed on days 0 and 28.

2.2. Vaccine

After blood sample collection on day 0, 0.5 mL of the diphtheria, tetanus, acellular pertussis (Tdap) adsorbed vaccine (ADACEL® Sanofi Pasteur, Toronto, Canada, batches: C4590AG and C4869BA) was administered intramuscularly. No other vaccine was administered during the previous 30 days or the following 30 days of Tdap.

2.3. Vaccine tolerability

All individuals were observed for 30 min after vaccination. They were given a thermometer and a questionnaire on adverse events, which was completed by adolescents and/or their caregivers during the first 7 days after vaccination. An axillary temperature of 37.8 °C or higher was considered as fever.

2.4. Laboratory evaluation

Pregnancy test: Confirme® pregnancy test (Alamar, Diadema, Brazil) in urine was performed prior to vaccine administration on all female participants.

HIV serology: Individuals from CONTROL group were tested for HIV at study entry using Determine™ HIV-1/2 kit rapid test (Abbott, Tokyo, Japan).

HIV viral load was assessed using the RealTime HIV-1 06L18 kit (Abbott, Illinois, CA) and the m2000 RealTime System (Abbott, Illinois, CA).

HIV infected individuals with HIV viral load below 40 copies/mL (detection limit of viral load test) were considered on virologic suppression and those with ≥40 copies/mL, as without virologic suppression.

A *blip* was considered after the vaccine challenge when, for those patients who had undetectable HIV viral load on day 0, a single viral load increase was observed between 50 and 1000 copies/mL on day 28, which was followed by a viral load below detection limit [11].

2.4.1. Tetanus, diphtheria and pertussis antibody assessment

Tetanus and diphtheria antibodies were tested with an in-house double antigen enzyme-linked immunosorbent assay, as previously described [12]. The reference sera used in the ELISA were obtained from the National Institute for Biologicals and Control (NIBSC, Hertfordshire, UK).

Tetanus and diphtheria antibody levels greater than or equal to 0.1 IU/mL were considered protective [13,14]. Seroconversion rate was also evaluated as fold increase of antibody levels.

Pertussis toxin antibodies were tested using SERION ELISA classic *Bordetella pertussis*-IgG kit (Virion-Serion, Würzburg, Germany).

As there is no pertussis correlate of protection, the cut-off of 62.5 IU/mL was considered as suggestive of *B. pertussis* infection in the previous 12 months (80% sensitivity and 95% specificity) [15,16].

Individuals with pertussis toxin antibodies above 62.5 IU/mL on day 0 were excluded from seroconversion analysis. For individuals who had pertussis toxin antibody levels on day 0 below 62.5 IU/mL, seroconversion was considered when they were above 5 IU/mL starting from below this level; if baseline values were higher than 5 IU/mL, seroconversion was considered if values were at least twice the initial levels [17].

2.4.2. Immunophenotyping of peripheral blood lymphocytes

CD4+ T, CD8+ T, B and NK cell quantification was performed by flow cytometry using Trucount tubes (Becton Dickinson, San Jose, CA).

Immunophenotypic subset analyses were performed on fresh blood. At least 20,000 events were acquired for each sample. Each sample was stained with the following fluorochrome conjugated monoclonal antibodies (BD Biosciences, San Jose, CA): CD3-APC-Cy7, CD4-V500, CD8-APC, CD10-PE-Cy7, CD16-PE, CD19-FITC, CD21-APC, CD27-PE, CD38-PE, CD45-PerCP, CD45RA-FITC, CD56-PE, HLA-DR-PerCP, CCR7-PECF594, CCR6-PerCPCy5.5, ICOS-PE, CXCR5-Alexafluor 647, PD-1-PE-Cy7, CXCR3-BV-421 and isotype controls. The markers used to assess CD3+CD4+ and CD3+CD8+ T lymphocyte subsets were: Naıve cells: CD45RA+CCR7+; Central memory cells: CD45RA-CCR7+; Effector memory cells: CD45RA-CCR7-; Effector cells: CD45R+CCR7-. Activated CD4+ or CD8+ T cells were assessed as HLA-DR+CD38+ using isotype controls.

B cells were identified as CD3-CD19+. Transitional B cells: CD10+; Mature B cells: CD10-. Naive cells CD21+CD27-; Resting memory: CD21+CD27+; Exhausted memory CD21-CD27-; Activated memory: CD21-CD27+ [18].

NK cells were assessed as CD45+CD3-CD56+/CD16+.

Follicular (Tfh) memory T cells were identified as CD4+CD45RA-CXCR5hi. Subsets were assessed as CCR6+CCR3- for Tfh17, CCR6-CXCR3-, for Tfh2 and CCR6-CXCR3+, for Tfh1. Activated cells were identified as ICOS+PD1+CCR7low; Quiescent cells, as ICOS- PD1+CCR7int and ICOS- PD1-CCR7hi [19].

Please cite this article in press as: Spina FG et al. Immune response to a Tdap booster in vertically HIV-infected adolescents. Vaccine (2018), https://doi.org/10.1016/j.vaccine.2018.07.043