Tetanus immunity after diphtheria, tetanus toxoids, and acellular pertussis vaccination in children with clinically stable HIV infection

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Background: HIV infection often impairs the immune response to childhood vaccines.

Objective: We sought to study the ability of HIV-infected children receiving highly active antiretroviral therapy (HAART) to generate a booster response to immunization with a recall antigen to which they had lost humoral immunity.

Methods: Diphtheria, tetanus toxoids, and acellular pertussis (DTaP) vaccination was given at either 16 or 36 weeks after initiation of HAART to 37 HIV-infected children 2 to 9 years of age with a history of DTaP or diphtheria-tetanus-pertussis receipt who had negative tetanus antibody titers (≤1:243) at baseline.

Results: There was a clear increase in tetanus titers after vaccination, with an increase of 27-fold over the baseline values at weeks 4 and 8. The effect on tetanus titers faded to a 9-fold and 3-fold increase over baseline values at weeks 18 and 32, respectively. DTaP vaccination did not affect HIV-1 RNA viral load or CD4 percentage or cell count. There was no increase in either acute or long-term adverse events associated with the DTaP vaccination.

Conclusion: Although children with stable HIV infection receiving HAART can mount antigen-specific responses to tetanus immunization, the durability of these responses might be limited. Long-term monitoring of specific immune function in such children is indicated. (J Allergy Clin Immunol 2005;116:698-703.)

Key words: *Antiretroviral therapy, highly active; child; diphtheria-tetanus-acellular pertussis vaccines; HIV infections; immune response*

Current standard treatment of children with HIV disease is the combination of 3 or more antiretroviral drugs from at least 2 different classes of drugs, including either a protease inhibitor or a nonnucleoside reverse transcriptase inhibitor. Such combinations, usually referred to as highly active antiretroviral therapy (HAART), effectively suppress viral replication in plasma and permit immune reconstitution in HIV-infected children.[1,2]

Immune responses to childhood vaccines are often impaired by HIV infection. This study (Pediatric AIDS Clinical Trials Group [PACTG] 727) was initiated to assess tetanus-specific immune reconstitution in HIV-infected children treated with HAART who had lost their tetanus immunity. PACTG 727 investigated the degree and durability of humoral and cell-mediated response to tetanus after diphtheria, tetanus toxoids, and acellular pertussis (DTaP) vaccination of children 2 to 9 years of age entered into a randomized clinical trial comparing 4 different HAART regimens (PACTG 377).[3,4]

METHODS

Study design and patient population

PACTG 377 was a multicenter randomized clinical trial that compared change from current nucleoside analogue therapy with one of 4 stavudine-containing drug regimens: nevirapine plus ritonavir, lamivudine plus nelfinavir, nevirapine plus nelfinavir, and lamivudine with both nevirapine and nelfinavir.[3,4] All subjects were HIV infected, were aged 4 months to 17 years, had stable CD4 cell numbers or percentages, remained in Centers for Disease Control Immune Category 1 (no immunologic suppression; CD4 ≥25%) or 2 (moderate

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Abbreviations used
DTaP: Diphtheria, tetanus toxoids, and acellular pertussis vaccination
HAART: Highly active antiretroviral therapy
NIAID: National Institute of Allergy and Infectious Diseases
PACTG: Pediatric AIDS Clinical Trials Group

immunologic suppression; CD4 15% to 24%) during the 4 months before study entry,[5] and were receiving the same antiretroviral therapy for the 16 weeks before study entry. All were naive to stavudine, lamivudine, all protease inhibitors, and all nonnucleoside reverse transcriptase inhibitors. Exclusion criteria included current grade 3 or 4 (severe or life threatening) laboratory test abnormalities (as judged by protocol-specified, standard pediatric toxicity criteria), active opportunistic and/or serious bacterial infection, and current diagnosis of malignancy or pregnancy. One hundred ninety-three children from 50 sites entered the study between December 1997 and September 1998. The duration of study treatment for each child was initially planned to be 48 weeks but was extended to 96 weeks for children who were still receiving their initial study treatment.

Children 2 to 9 years of age who had completed their primary vaccination series of either DTaP or diphtheria-tetanus-pertussis and who had negative tetanus titers ($\leq$1:243) at PACTG 377 study entry were eligible to concurrently enroll in PACTG 727 and receive DTaP vaccine at week 16 or 36 on the basis of a randomized assignment. Thirty-nine children entered this substudy and continued to receive one of the 4 HAART regimens. Two of these children are not included in the following analyses. One child had no tetanus titer values recorded, and the other child had only a baseline tetanus titer. Serum specimens were taken and analyzed for tetanus titers at PACTG 377 baseline (before initiation of HAART) and subsequently at weeks 16 and 36 (dates of DTaP vaccination) and weeks 44 and 48. The primary endpoint for this substudy was a tetanus titer response, defined as the tetanus titer value after DTaP vaccination divided by the tetanus titer value at the time of vaccination—the fold increase over baseline value.

The objectives of this investigation were to study the reconstitution of humoral immune response to recall antigen DTaP among children seronegative for tetanus despite a full primary immunization series, the reconstitution of cell-mediated immune response by comparing baseline entry lymphoproliferative response with post-immunization lymphoproliferative response, and the differences in degree and duration of humoral or cell-mediated responses between subjects immunized after 16 versus 36 weeks of HAART therapy. There was no difference between the subject groups vaccinated at 16 versus 36 weeks with respect to any primary or secondary endpoint. The remainder of this article will focus on the combined group of vaccinated subjects.

Of additional interest was the determination of whether receiving a DTaP vaccination was beneficial to these HIV-infected children. PACTG 377 children who did not receive a DTaP vaccination were used as a comparison group for a matched-pairs analysis. Thirty subjects enrolled in PACTG 727 who received DTaP vaccine were compared with 30 matched control subjects selected in a blinded fashion from the PACTG 377 patients who did not receive an additional DTaP vaccination. The pairs were matched on RNA value at week 12 ($\leq$400 vs >400 copies/mL), baseline reciprocal tetanus titer (<243 vs $\geq$243), and, to the extent possible, age at baseline and CD4 percentage values at week 12. The institutional review boards of all the sites participating in PACTG 377/727 approved this study. Written informed consent was obtained from all subjects or their legal guardians.

Tetanus antibody titers

Tetanus antibody levels were performed at a central laboratory (Dr Marc Golightly, State University of New York at Stony Brook, Stony Brook, NY). The standard sensitized RBC agglutination assay used a sensitizing antigen and standards from the New York State Department of Health. Control ranges were determined from age-matched healthy children at the central laboratory.

Lymphocyte subset measurements

Lymphocyte surface markers (CD3, CD4, CD8, and CD19), using 2- or 3-color flow cytometry, complete blood cell count, and differentials, were determined by using standard methods in local laboratories at the enrolling sites. All the laboratories were participating in the National Institute of Allergy and Infectious Diseases (NIAID) Flow Cytometry Quality Assurance Program.[6]

Three-color flow cytometric measurements

Lymphocyte phenotyping was performed by using a panel of fluorochrome-conjugated mAbs formulated and pretitered by the supplier for these studies. Flow cytometry was performed in 7 immunology core laboratories by using a consensus whole blood lysis protocol and common lots of pretitered monoclonal reagents produced commercially (PharmMingen, San Diego, Calif). All core flow laboratories participated in the NIAID quality assurance program noted above. The extended 3-color flow panel consisted of the following combination of markers: CD4/CD45RA/CD62L, CD4/CD38/HLA-DR, CD4/CD28/CD95, CD8/CD45RA/CD62L, CD8/CD38/HLA-DR, and CD8/CD28/CD95.[7]

Viral load

HIV-1 RNA copy number was assessed with the Roche Amplicor Monitor Assay (Roche Diagnostics Corp, Indianapolis, Ind)[8] by a single laboratory at The Johns Hopkins University, Baltimore, Maryland, which was certified as proficient for this assay by the NIAID Virology Quality Assurance Program.[9] The lower limit of assay quantification for RNA was 400 copies/mL.

Statistical analysis

Comparisons among treatment groups used Fisher's exact test for categorical variables and the Wilcoxon/Kruskal-Wallis test for continuous variables.[10] All P values were 2 sided and were not adjusted for multiple comparisons.

RESULTS

Study population

For the 37 children who received DTaP vaccination, the median age was 6.1 years, the median CD4 cell count was 976 cells, and the median HIV RNA was 2.60 $\log_{10}$ copies/mL (Table I). Baseline patient characteristics were similar between the 37 children in PACTG 727 and the 2 matched-pairs groups (no statistically significant differences) formed from PACTG 727 and the parent study, PACTG 377.

Tetanus titer response

Median tetanus titer response for the 37 children in the cohort study is shown in Fig 1. A median 27-fold increase in tetanus titers over baseline values was seen at weeks