

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 ($\leq 1:243$) 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 zidovudine, lamivudine plus zidovudine, nevirapine plus zidovudine, and lamivudine plus zidovudine.^{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 $\geq 25\%$) 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 (≤ 400 vs >400 copies/mL), baseline reciprocal tetanus titer (<243 vs ≥ 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 log₁₀ 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

TABLE I. Baseline patient characteristics

Variable	Cohort study*	Matched-pairs study†	
	Vaccinated	Vaccinated	Unvaccinated
Patients, n	37	30	30
Median age, y	6.1 (2.9-10.9)	5.7 (2.6-10.0)	7.2 (0.4-16.5)
Male sex, %	41	40	47
Race or ethnicity, %			
White, non-Hispanic	5	7	10
Black, non-Hispanic	59	63	73
Hispanic	35	30	17
Prior antiretroviral therapy, %			
ddI	35	33	40
ZDV/ddI	65	67	53
Other	0	0	7
CD4 cell count			
Median, cells/ μ L	976 (147-2802)	664 (224-2173)	552 (214-3084)
CD4 cell percentage			
Median, %	34 (14-51)	29 (15-49)	27 (10-54)
HIV RNA copy number			
Median, $\log_{10}$	2.60 (2.60-5.65)	4.33 (2.88-6.98)	4.47 (2.60-5.99)
<3 $\log_{10}$, %	76	3	3
3-4 $\log_{10}$, %	8	33	27
4-5 $\log_{10}$, %	11	43	60
5-6 $\log_{10}$, %	5	20	10

Table values for quantitative variables are presented as medians (ranges).

ddI, Didanosine; ZDV, zidovudine.

*Baseline data from date of DTaP vaccination: 16 to 36 weeks after initiation of HAART.

†Baseline data from start of PACTG 377: before initiation of HAART.

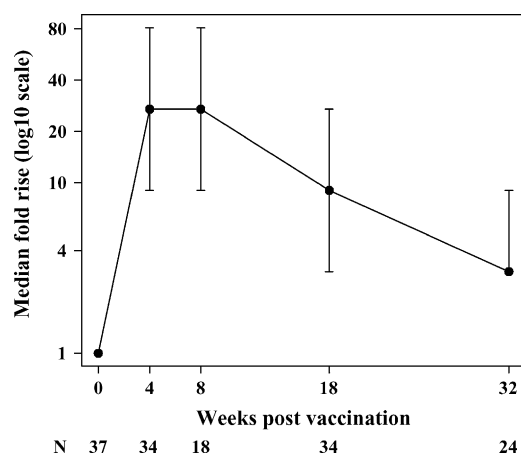


FIG 1. Median tetanus titer response. Bars represent 25th and 75th interquartile ranges. Note: Response is defined as the tetanus titer value after DTaP vaccination divided by the tetanus titer value at baseline (fold increase over baseline).

4 and 8. This response faded to a 9-fold increase and a 3-fold increase at weeks 18 and 32, respectively. Median entry tetanus titer was 1:27, and at 4, 8, 18, and 32 weeks after immunization, it had increased to 1:2187, 1:729, 1:486, and 1:122, respectively ($P < .0001$ for weeks 4, 8, and 18 and $P = .015$ for week 32). Although all 37 children had negative tetanus titers ($\leq 1:243$) at PACTG 377 baseline, 3 (8%) of the 37 children had tetanus titers of

greater than 1:243 at the time of vaccination with DTaP at week 16 or 36 after entry into PACTG 377 (PACTG 727 baseline). These 3 patients were included to ensure reporting of all vaccinated children and to ensure comparability with control patients in the matched-pairs analysis. The proportion of children with positive tetanus titers ($>1:243$) was 74% and 67% at weeks 4 and 8 and decreased to 53% and 38% at weeks 18 and 32, respectively. The proportion of children with at least a 4-fold increase in tetanus titers over baseline values was 82% and 94% at weeks 4 and 8 but had fallen to 56% and 46% at weeks 18 and 32, respectively (Table II).

There was a corresponding modest increase in lymphoproliferative assay measurements at week 8 associated with tetanus toxoid stimulant at 1.25 μ g/mL and 2.5 μ g/mL (data not shown). However, no changes were found for the other lymphoproliferative assay measurements.

Table III presents the results of the matched-pairs study, comparing the tetanus titer values for 30 vaccinated children and 30 unvaccinated children matched for baseline reciprocal tetanus titer and RNA value at week 12. The matched-pairs analysis showed that the tetanus titer values in the vaccinated children were a median of 3-fold larger at week 44 compared with those of the unvaccinated children ($P = .0005$). Tetanus titer response was a median 9-fold increase from baseline to week 44 for the vaccinated children compared with a median 3-fold increase from baseline to week 44 for the unvaccinated children in the matched-pairs study. The corresponding tetanus

TABLE II. Tetanus titer response after DTaP vaccination

Week	Patients, n	Tetanus titer	Percentage positive	Fold increase over baseline	Percentage fold increase over baseline [§]		
		Value*	>243 [†]	Response [‡]	≥3	≥4	≥5
0	37	27 (9, 81)	8%	—	—	—	—
4	34	2187 (243, 2187)	74%	27 (9, 81)	97%	82%	82%
8	18	729 (243, 2187)	67%	27 (9, 81)	94%	94%	94%
18	34	486 (27, 729)	53%	9 (3, 27)	79%	56%	56%
32	24	122 (23, 729)	38%	3 (3, 9)	79%	46%	46%

From the cohort study of 37 children vaccinated with DTaP.

*Median of the reciprocal tetanus titer values; 25th and 75th percentiles are shown in parentheses.

[†]Percentage of children with a reciprocal tetanus titer value of greater than 243.

[‡]Median fold increase over baseline tetanus titer; 25th and 75th percentiles are shown in parentheses.

[§]Percentage of children in each category with the indicated fold increase over baseline tetanus titer.

TABLE III. Tetanus titer response from the matched-pairs study

	Vaccinated (n = 30)	Unvaccinated (n = 30)	Ratio of vaccinated/unvaccinated	P value [‡]
Baseline tetanus titer*	27 (9, 243)	27 (9, 243)	1.00 (0.33, 3.00)	.11
Week 44/48 tetanus titer*	243 (81, 729)	243 (27, 729)	3.00 (0.33, 9.00)	.0005
Week 44/48 tetanus titer response [†]	9 (3, 27)	3 (1, 9)	3.00 (0.33, 9.00)	.00004

All children received HAART. Tetanus titer comparisons are not presented for time points between baseline and week 44/48 because extensive tetanus titer monitoring was not conducted for the unvaccinated children. Data presented are only for the 30 children in the matched-pairs analysis. Values are presented as medians, with the 25th and 75th percentiles shown in parentheses.

*Reported values are the reciprocal tetanus titers.

[†]Response is defined as the fold increase over baseline tetanus titer.

[‡]Signed-rank test of a ratio equal to 1.

titer response ratio at week 44 was 3 times larger for the vaccinated children when compared with that of the matched unvaccinated children ($P = .00004$).

Predictors of tetanus titer response

We investigated the correlation between tetanus titer response and a number of baseline factors, including age, lymphocyte subsets, and 3-color flow cytometric parameters, which have been previously shown to be correlated with HAART outcome.¹¹ Tetanus titer response was greater for children with higher ($\geq 68\%$) CD4⁺/CD62L⁺/CD45RA⁺ percentages at weeks 4 ($P = .04$), 18 ($P = .04$), and 32 ($P = .03$).

Correlations with HIV-1 RNA and CD4

Potential associations between vaccination with DTaP or tetanus titer response and subsequent HIV-1 RNA and CD4 values were studied by using both the cohort and matched-pairs analyses. No significant associations with HIV-1 RNA or CD4 were found (data not shown).

Safety of DTaP vaccination

A matched-pairs analysis compared the rates and severity of adverse events between the vaccinated and unvaccinated children. There was no evidence of additional adverse events, acute or long-term, associated with DTaP vaccination ($P = .78$ for adverse events within 30 days of vaccination and $P = .41$ for adverse events within 6 months, data not shown). The most common adverse events seen for children after DTaP vaccination were

associated with ear, nose, and throat illnesses (21%); skin rashes (15%); and fever (10%). However, these adverse events were typical of the adverse events seen 4 to 8 months after initiation of HAART.

DISCUSSION

Since the introduction of HAART in the late 1990s, studies of children with moderate-to-advanced HIV infection have demonstrated the potential of immune reconstitution after HAART and have attempted to boost immune response. Melvin and Mohan¹² studied the response to immunization with tetanus, measles, and Hib conjugate vaccine in 19 children with HIV infection and well-preserved immune function at the time of initiation of HAART. These children had undetectable plasma HIV-1 RNA levels and a median of 26% CD4⁺ T cells before receiving HAART. At the time of immunization, these children had a median CD4 of 35%. Antibody levels were measured to measles, tetanus, and Hib before and after immunizations with each of these vaccines. After immunizations in these children, 10 of 11 had detectable antibody levels to tetanus; 83% and 75% had no conversions to measles and Hib, respectively. Moreover, these authors demonstrated detectable antibody levels as long as 1 year after immunization. Although detectable at 1 year, the tetanus antibody levels had decreased to 25% of their peak value 4 weeks after immunization. Contrary to the rapid decrease in tetanus antibody levels, immunization

with measles vaccine resulted in a 4-week postimmunization antibody level that did not decrease significantly over a year. This suggests that persistence of antigen in the live viral vaccine enhanced the durability of the immune response to reimmunization.

A recent study of children with advanced HIV disease on lymphoproliferative and antibody responses to recall and neoantigens in children (PACTG 1006) reported 53 virologic responders who had been immunized with either tetanus or hepatitis A vaccine at 8, 16, and 24 weeks after initiation of therapy.¹³ Subsequently, these subjects were immunized with the other vaccine at weeks 32, 40, and 48. Lymphoproliferative responses to tetanus were observed in approximately two thirds of the subjects receiving preimmunizations. Results did not differ whether the subjects received the neoantigen or recall antigen first. Good serologic responses to tetanus in these subjects were observed after preimmunization.

Children in our study had less immunosuppression at the time of vaccination than PACTG 1006 children. Despite 16 to 36 weeks of HAART and less advanced disease, only 8% of our children had preimmunization titers greater than 1:243, a value considered to be protective. The excellent initial responses to a single immunization with tetanus in our subjects might have been related to relative preservation of total CD4 counts, as well as preservation of memory T cells capable of rapid expansion on reimmunization. Despite these initial responses, the duration of antibody level and lymphoproliferative response was disappointing in our patients.

Volk et al¹⁴ reported the change in tetanus titer after a booster dose of tetanus toxoid in 45 children uninfected with HIV aged 2 to 15 years. They observed a 188-fold increase in geometric mean tetanus titer relative to booster baseline after 2 weeks (0.13 to 24.50 geometric mean), with a decrease to a 48-fold change at 6 months and a 29-fold change from booster at 12 months. The tetanus titer response was clearly less strong and of shorter duration in our study of HIV-infected children of comparable ages.

Studies of HIV-infected children suggest that even in children with relatively well-preserved immune function who were started on HAART, only partial reconstitution can be expected. Fortunately, these subjects appear to respond well to reimmunization with recall antigens. Further studies correlating viral load and specific T-cell subsets will be important in defining those immune characteristics that are predictive of successful immunization and focus its use as an effective tool in achieving immune reconstitution in HIV-infected individuals.

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