NIH Public Access
Author Manuscript
J Acquir Immune Defic Syndr. Author manuscript; available in PMC 2010 March 11.

Published in final edited form as:
J Acquir Immune Defic Syndr. 2009 September 1; 52(1): 17–24. doi:10.1097/QAI.0b013e3181b011f6.

Virologic and Immunologic Correlates With the Magnitude of Antibody Responses to the Hepatitis A Vaccine in HIV-Infected Children on Highly Active Antiretroviral Treatment

Adriana Weinberg, MD[*], **Sharon Huang, MS**[†], **Terence Fenton**[†], **Julie Patterson-Bartlett, BS**[*], **Philimon Gona, PhD**[‡], **Jennifer S. Read, MD, MS, MPH**[§], **Wayne M. Dankner, MD**[||], and **Sharon Nachman, MD**[¶] **the IMPAACT P1008 Team**

[*]Department of Pediatrics, University of Colorado Denver, Aurora, CO

[†]Center for AIDS and Biostatistics Research, Department of Biostatistics, Harvard School of Public Health, Boston, MA

[‡]Statistical Consulting Unit, Department of Mathematics and Statistics, Boston University, Boston, MA

[§]Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD

[||]Pediatric Infectious Diseases, Duke University, Durham, NC

[¶]Department of Pediatric Infectious Diseases, SUNY Health Sciences Center, Stony Brook, NY

Abstract

Background—HIV-infected individuals mount poor antibody responses to vaccines. We sought to identify the immunologic and virologic factors associated with a robust response to hepatitis Avirus (HAV) vaccine in children on highly active antiretroviral treatment.

Methods—One hundred fifty-two pediatric highly active antiretroviral treatment recipients immunized against HAV at weeks 0 and 24 had anti-HAV antibodies, CD4+, CD8+, and CD19+ cell percent assessed at weeks 0 and 32. Subgroups had HIV viremia, B- and T-cell subpopulations, and cell-mediated immunity (CMI) to HAV and other stimulants measured.

Results—Anti-HAV antibodies after complete vaccination correlated positively with CD4+ percent and CD19+ percent and negatively with viremia and CD8+ percent at baseline, but not at 32 weeks. There were no significant correlations between anti-HAV antibodies and B- or T-cell-naïve, memory, or activated subpopulations or non-HAV CMI. Compared with children who remained HAV-CMI-negative, those who mounted HAV-CMI in response to vaccination had higher anti-HAV antibody titers and CD19+ CD21+ CD27+ memory B cell percent at 32 weeks, but no other differences.

Conclusions—In HIV-infected children on highly active antiretroviral treatment, control of viral replication and conserved or reconstituted CD19+ and CD4+ cell numbers and function determine a robust antibody response to anti-HAV primary immunization. Our data support a bidirectional B- and T-cell cooperation in the response to the HAV vaccine.

Copyright © 2009 by Lippincott Williams & Wilkins

Correspondence to: Adriana Weinberg, MD, Professor of Pediatrics, Medicine and Pathology, Director, Clinical Virology Laboratory University of Colorado Denver, Mail Stop 8604, 12700 E. 19th Avenue, Room 11126, Aurora, CO 80045 (adriana.weinberg@ucdenver.edu).

The authors have no conflicts of interest.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Keywords

HIV; hepatitis A virus vaccine; B-cell phenotype; T-cell phenotype; antibody titers; cell-mediated immunity

INTRODUCTION

HIV-infected individuals have notoriously poor antibody responses to microbial antigens and vaccines, including the hepatitis A virus (HAV) vaccine.[1–8] In a previous study, we showed that only a minority of children and adolescents who met AIDS immunologic criteria responded to primary anti-HAV immunization during the first year of effective highly active antiretroviral treatment (HAART).[9] Others have described similarly poor responses to pneumococcal, influenza, and hepatitis B immunizations of HIV-infected individuals.[1]

Although HIV does not infect B cells, it decreases their function[10–13] by diminishing T-cell help[14] and by releasing subvirion particles that directly interfere with B-cell function. [11,12] HIV infection also has been associated with a decrease of total and memory B cells[15–20] and an increase of transitional/immature B-cell subpopulations.[17,21–24] In viremic patients, B cells are more susceptible to CD95L-mediated apoptosis,[18] which may decrease their lifespan and account for the decrease in B cell numbers.[16,25]

HAART improves many aspects of the immune system of HIV-infected patients, including humoral responses to vaccines.[9,25–29] However, not all HAART recipients who reconstitute or preserve their CD4+ T cells mount adequate responses to vaccines. In a previous study, we found that approximately half of the HIV-infected children and adolescents with a CD4+ T cell percentage (CD4%) ≥20% after ≥4 months of HAART had an antibody response to HAV vaccine at least one order of magnitude lower than the one reported in historical studies of healthy children and adults.[30] Two years after immunization, the subjects with low antibody responses to the vaccine were more likely to lose protective anti-HAV antibody titers than those who mounted adequate antibody titers to the vaccine.

To gain more insight into the pathogenic mechanisms that govern immune responses to vaccines in HIV-infected children and to identify immunologic and virologic markers that may help optimize the timing of vaccine administration in these patients, we studied the association between humoral- and cell-mediated immune responses to the HAV vaccine and of each of these anti-HAV responses with the following variables: 1) age; 2) HIV viremia; 3) CD19+ B-cell total as well as naïve, memory, and activated subpopulations; 4) CD4+ T-cell total as well as naïve, memory, and activated subpopulations; and 5) CD8+ T-cell total as well as activated subpopulations.

SUBJECTS AND METHODS

Study Population and Design

HIV-infected children 2 to 21 years of age were enrolled in this Pediatric AIDS Clinical Trials Group 1008 study after obtaining local Institutional Review Board approval and informed consent. Eligibility criteria included receipt of *Pneumocystis pneumoniae* pneumonia prophylaxis for at least 6 months; stable antiretroviral therapy; CD4% ≥25% for children younger than 6 years and ≥20% for subjects older than 6 years; absence of previous hepatitis A vaccination or wild-type disease; and no immune globulin administration for 3 months before enrollment. Children received the first dose of HAV vaccine at enrollment

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

and a second dose 24 weeks later. Doses were 720 and 1440 ELU of HAVRIX (Glaxo-SmithKline, Philadelphia, PA) in children younger and older than 18 years, respectively. T-cell subsets and plasma HIV RNA were measured every 8 weeks. Anti-HAV antibodies were measured at baseline and week 32, corresponding to 8 weeks after the second dose of vaccine. A subset of subjects had T-cell phenotyping and cell-mediated immunity (CMI) measured. These were randomly selected among subjects who were seronegative at baseline, received both doses of the vaccine, and had antibodies measured at week 32.

Hepatitis A Antibody Assay

Anti-HAV antibodies were determined using a quantitative enzyme-linked immunosorbent assay. Precoated HAV microtiter plates (Viral Antigens Inc., Memphis, TN) were incubated with serial dilutions of anti-HAV World Health Organization Standards and 100 μL of serum samples diluted 1:21 in assay IgG diluent (Viral Antigens Inc.). After 1 hour of incubation, plates were washed and bound antibodies were revealed with alkaline phosphatase-conjugated goat antihuman IgG and p-NPP chromogenic substrate (Sigma, St. Louis, MO). The antibody titer of each sample was calculated by interpolating its corresponding optical density on the linear regression curve generated with the World Health Organization Standards. This assay was validated using 20 sera from HAV-naïve healthy volunteers and 10 sera with previously determined anti-HAV antibody concentrations, courtesy of Dr. Paul Willems, GlaxoSmithKline Vaccine Division. Titers ≥20 mIU/mL were considered protective against HAV infection and also defined HAV-seropositive or immune status. Responses were further classified as low if <250 mIU/mL and adequate if ≥250 mIU/mL (based on the observation that virtually all healthy individuals immunized against HAV had antibody titers >250 mIU/mL at 4 to 8 weeks after the last dose of HAV vaccine).

Cell Preparation

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized whole blood by Ficoll-Hypaque (Sigma) density gradient centrifugation. Isolated PBMCs were cryopreserved and subsequently thawed as per the International Maternal Pediatric Adolescent AIDS Clinical Trials/Pediatric AIDS Clinical Trials Group cryopreservation consensus protocol (http://impaact.s-3.com/immlab.htm).

Lymphocyte Proliferation Assay

Thawed PBMCs resusupended at a concentration of 2×10^6 cells/mL in RPMI 1640 (CellGro, Manassas, VA) with L-glutamine (Gibco, Carlsbad, CA), 1%penicillin-streptomycin, 4mM HEPES (Gibco), and 10% human AB serum (Gemini, West Sacramento, CA) were stimulated in triplicate wells with medium only, 1650 ELU/mL Hepatitis A antigen (gift of Dr. Martine Wettendorff, Glaxo SmithKline Research Laboratories, Belgium) for 6 days. PBMCs at 10^6 cells/mL of medium as described previously were stimulated with 2.5 μg/mL tetanus toxoid (Connaught, Swiftwater, PA), 50 μg/mL *Candida albicans* antigen (Greer, Lenoir, NC), 5 μg/mL phytohemagglutinin (PHA; Sigma), or no stimulant for 6 days. After stimulation, wells were pulsed with 50 μCi/well ^{3}H-thymidine (Perkin Elmer, Waltham, MA) for 6 hours, harvested, and counted in a Packard scintillation counter. The stimulation index was calculated by dividing the median counts per minute in the stimulated wells by the median counts per minute in unstimulated wells matched by number of cells/well. A stimulation index ≥3 defined a response to HAV, candida, and tetanus and ≥10 to PHA.

For carboxyfluorosuccimide ester (CFSE) dilution assays, PBMCs were labeled with 1.5 μM CFSE (Molecular Probes, Carlsbad, CA) in 0.1% bovine serum albumin phosphate-buffered saline for 10 minutes at 37°C. Staining was quenched with cold 10% human AB

NIH-PA Author Manuscript

serum in RPMI. An aliquot of PBMCs was removed for baseline flow analysis and the remaining cells were stimulated in tissue culture tubes with the same stimulants as described previously except for using pokeweed mitogen (Sigma) instead of PHA to elicit nonspecific proliferation. On the day of the assay, PBMC were washed in phosphate-buffered saline, stained with CD19-PE (Caltag, Carlsbad, CA) surface marker, and analyzed by flow cytometry as described subsequently.

Inducible Cytokines

Supernatants from HAV-stimulated PBMC 6-day cultures and unstimulated controls were used for measuring interleukin (IL)-2, IL-4, IL-5, IL-10, and interferon-γ using Luminex™ kits (Biosource, Carlsbad, CA) according to the manufacturer's instructions. Results were expressed in pg/mL after subtracting the background cytokine concentration in unstimulated wells from that obtained in HAV-stimulated wells.

Flow Cytometry

Thawed PBMCs were resuspended in phosphate-buffered saline containing 1% fetal bovine serum at a concentration of 1×10^6 cells/mL and stained with 3 or 4 combinations of the following fluorochrome-conjugated mAbs: anti-CD8-FITC, anti-CD45RA-FITC, anti-HLA-DRFITC, IgG1-FITC, anti-CD8-PE, anti-CD38-PE, anti-CD95-PE, IgG1-PE, anti-CD4-PE*Cy5, anti-CD8-PE*Cy5, anti-CD8-PE-Alexa700, or anti-CD8-APC (Pharmingen/Becton Dickinson, San Jose, CA); or with a premixed custom formulation containing anti-CD21-FITC, anti-CD27-PE, anti-CD19-PE*Alexa700, and anti-CD95-APC (Caltag Laboratories Inc./Invitrogen, Inc.). After 30 minutes of incubation at 4°C in the dark, the samples were depleted of red blood cells with FACS lysing buffer (Becton Dickinson), washed with phosphate-buffered saline, resuspended in 0.2% paraformaldehyde, and analyzed with FACS Calibur flow cytometer (Becton Dickinson) and CellQuest software (Becton Dickinson). Lymphocytes were gated based on forward versus sidescatter signals (R1) followed by either T- or B-cell selection by combining sidescatter with bright CD4, CD8, or CD19 signals (R2). T- and B-cell subsets were characterized by analyzing 5000 R1*R2 gated events and were expressed as either T- or B-cell percent positivity.

Statistical Analysis

Because the immunologic outcome measures did not meet normality assumptions, nonparametric tests were used to examine the associations between antibody responses to HAV vaccine and demographic, immunologic, or virologic characteristics of the study population. The statistical significance of differences in antibody titers across different groups of subjects was assessed by Wilcoxon sum ranks (two groups) or Kruskal-Wallis tests (multiple groups). Spearman's rank order correlation coefficients were used to examine the strength and statistical significance of associations between antibody titers and other continuous variables. When the univariate analyses identified multiple variables positively associated with the primary outcome measure, a multivariate analysis was performed using regression. Statistical significance was defined by $P < 0.05$.

RESULTS

Subject Characteristics and Their Associations With Antibody Responses to Hepatitis A Virus Vaccine

There were 152 subjects who met inclusion criteria for this substudy by virtue of being HAV-seronegative at baseline, having received 2 doses of HAV vaccine, and being tested at 32 weeks (8 weeks after the second dose of vaccine) for antibody responses. The median age was 9 years and 13 children were between 2 and 6 years of age. Demographic characteristics

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

such as age, sex, and race were not associated with antibody responses to the HAV vaccine (Table 1). Subjects with higher plasma HIV RNA concentrations (VL) and those with lower CD4+ T cell percentages (CD4%) at baseline had significantly lower anti-HAV antibody titers after the second dose of the HAV vaccine (*P* values of 0.02 and 0.04, respectively).

B-Cell Phenotypic Characteristics and Antibody Responses to the Hepatitis A Virus Vaccine

To identify the B-cell characteristics associated with robust antibody responses to neoantigens in HIV-infected subjects, we tested correlations between anti-HAV antibody titers at week 32, 8 weeks after the second dose of the HAV vaccine, with baseline and week 32 CD19+% (total B lymphocytes; N = 148) and subpopulations (N = 78) identified by expression of CD21, CD27, and/or CD95 (Table 2). The only parameter significantly associated with the week 32 anti-HAV antibody titers was the baseline CD19+% (N = 148, rho = 0.21, $P = 0.01$).

T-Cell Phenotypic Characteristics and Antibody Responses to the Hepatitis A Virus Vaccine

The antibody response to the HAV vaccine is T cell-dependent and, therefore, it is likely that in the context of HIV infection, anti-HAV antibody production may be limited by deficient T-cell help. To identify the T-cell characteristics associated with robust humoral immune responses to HAV in HIV-infected children on HAART, we performed correlation analyses of week 32 anti-HAV antibody titers with CD4+ and CD8+ T cell% and their subpopulations (Table 3). The anti-HAV antibody titers were weakly but statistically significantly correlated with baseline CD4+% ($r = 0.16$, $P = 0.04$) and inversely correlated with baseline CD8+% ($r = 0.17$; $P = 0.04$). Compared with CD4+ and CD8+% populations, there was a stronger inverse correlation of antibody titers with baseline CD4+ CD45RA-CD95-% central memory T cells (rho = − 0.28, $P = 0.06$). However, this correlation did not reach statistical significance. There were no significant correlations between antibody responses to HAV and baseline naïve or activated CD4+ or CD8+% nor with any of the T-cell phenotypic characteristics at week 32.

Multivariate Analysis of the Effect of Viral Load and Lymphocyte Populations on antibody Responses to Hepatitis A Virus Vaccine

In HIV-infected patients, higher VLs are typically associated with lower percentages of CD4+ and B lymphocytes and higher percentages of CD8+ lymphocytes. To determine which of these variables were independently associated with the magnitude of the antibody response to HAV, we performed a multivariate regression analysis. For this analysis, the antibody titers were log transformed to meet the normal distribution criterion required for the regression analysis. Of the 152 subjects, 97 had complete sets of data, including VL, CD4+, CD8+, and CD19+%. After log transformation of the antibody titers and with the contraction of the sample size, only VL, as a discrete variable, and CD19%, as a continuous variable, remained significantly associated with the anti-HAV antibody titers (*P* values of 0.04 and 0.02, respectively). The backward elimination procedure applied to the regression analysis identified the CD19+% as the strongest predictor of the antibody response to HAV vaccine ($P = 0.02$).

Functional T-Cell Characteristics and Antihepatitis A Virus Antibody Responses to Vaccination

To assess the relationship between antibody and CMI responses to HAV vaccine, we compared the week 32 antibody titers of HAV-CMI-positive (N = 10) with those of HAV-CMI-negative vaccinees (N = 44). Median titers were significantly higher in HAV-CMI-

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

positive children (P = 0.02; Table 4). In contrast, there were no appreciable differences in HAV antibody titers between subjects with positive versus those with negative tetanus or *Candida* CMI at baseline or week 32 (Table 4), indicating that the association of HAV antibodies with HAV CMI was antigen-specific. Only 4 subjects failed to respond to PHA using a stimulation index = 10 as the threshold, which precluded an analysis of potential associations of PHA responses with anti-HAV antibody titers.

Th1 and Th2 characterization of T-cell response to HAV was attempted by measuring IL-2, IL-4, IL-5, IL-10, and interferon-g concentrations in HAV-stimulated PBMC culture supernatants. Median cytokine levels in cultures of PBMC obtained after vaccination varied from 0 pg/mL for IL-2 and IL-4 to 0.09 p/mL for IL-5, 0.47 pg/mL for IL-10, and 0.32 pg/mL for interferon-γ. These very low levels suggested that the assay, which was standardized using PBMC from healthy donors, was not sensitive enough to detect responses of HIV-infected subjects.

Demographic, Virologic, and Immunologic Correlates With Lymphocyte Proliferative Responses to Hepatitis A Virus Vaccine

HAV-CMI is an important component of the protection conferred by the HAV vaccine as indicated by its correlation with antibody titers and by the fact that in the absence of seroconversion after HAV vaccination, the presence of HAVCMI is associated with protection against infection.31 To identify the factors that determine a CMI response to the HAV vaccine, we compared the demographic, virologic, and the T- and B-cell phenotypic characteristics of subjects who developed HAV-CMI at 32 weeks after vaccination versus those who did not. Of the parameters analyzed (same as for antibody responses), only the CD19+ CD21+ CD27+ memory B-cell percent at week 32 was significantly different between subjects with and without detectable HAV-CMI after vaccination: 7 HAV-CMI-positive subjects had a median CD19+ CD21+ CD27+ of 13%, whereas 49 HAV LPA-negative subjects had a median of 9% (P = 0.02, Wilcoxon signed rank test).

To examine the possibility that HAV-specific B cells proliferated in the LPA, we measured cell divisions by CFSE dye dilution coupled with CD19-surface staining in 4 HAV-vaccinated subjects with positive HAV-specific LPA. Figure 1 shows a typical representation of the results. In HAV-stimulated cultures, the CD19+ B cells represented a median (range) of 5% (0% to 17%) of the CFSE^{lo} lymphocytes, whereas corresponding percentages after pokeweed mitogen stimulation were 21% (3%to 40%). These results indicated that CD19+ B cells did not appreciably contribute to the HAV-specific LPA response.

DISCUSSION

We identified several immunologic and virologic parameters reflective of the stage of the HIV infection and/or immune reconstitution on HAART that determine the ability of HIV-infected children on HAART to mount protective antibody responses after 2 doses of HAV vaccine administered 6 months apart. In the univariate analysis, high CD19+ B lymphocyte percent, high CD4+%, undetectable VL, and low CD8+ T lymphocyte percent at the time of the administration of the first dose of HAV vaccine were associated with an antibody response similar to that of historical healthy controls,32‒35 which was also necessary and sufficient to maintain protective antibody titers 2 years after vaccination in the HIV-infected children.30 In contrast, the same immunologic and virologic parameters measured concurrently with the immune response and closely after the administration of the second dose of the vaccine did not affect the magnitude of the immune response to HAV vaccine. This suggests that the primary immune response to the vaccine depended on the HAART-associated immune preservation or reconstitution, whereas the amnestic response was less

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

affected by HIV disease-specific immunologic and virologic characteristics. Furthermore, a third dose of the HAV vaccine administered 2 years after the primary immunization generated equally high antibody titers in all vaccinees, which did not depend on CD4% or VL at any time. Others have also shown that HIV-infected adults on HAART have lower antibody responses to the first dose of HAV vaccine compared with historical controls.[36] Taken together, these data support the notion that in HAART recipients, anamnestic immune responses are less likely to be affected by the stage of HIV infection than primary responses and, therefore, increasing the number of doses of vaccines may overcome a deficient primary response to the immunogens.

To establish the relative significance of the baseline immunologic and virologic characteristics with respect to antibody responses to HAV vaccine, we performed a multivariate analysis, including the parameters that were significant in the univariate analysis. The multivariate analysis included only participants with complete sets of data, which resulted in a 36% reduction of the sample size originally used to assess the relationship between lymphocyte populations and responses to HAV vaccine. After the reduction of the sample size, the association of CD4+ and CD8+ percent with antibody titers lost significance, but undetectable VL and high CD19+% at the time of the administration of the first dose of the HAV vaccine maintained their association with higher antibody responses by univariate analysis. The multivariate analysis identified the B-cell percent as the most significant factor.

We have previously documented associations between the VL and primary responses to the live attenuated varicella zoster vaccine[37] or to influenza strains that were newly introduced in either the live attenuated or the inactivated seasonal influenza vaccines.[38] Furthermore, this has been observed in individuals who had a diagnosis of AIDS, but also for individuals whose CD4+ cells never decreased below 20%.[1,37–39] The pattern that emerges from these observations is that primary antibody responses of HIV-infected individuals on HAART are more effective when the viral replication is low or undetectable and, therefore, control of the VL should be the an important criterion for when to introduce new vaccines.

Others described associations of deficient antibody production in HIV-infected individuals with the overrepresentation of transitional/immature B-cell subpopulations expressing low CD21 and/or CD27[22,23] or with the underrepresentation of memory B cells expressing adequate levels of CD21, CD27, or immunoglobulins on their surface.[13,15,16,19,40] In this study, we were unable to reproduce these findings, but we identified a strong correlation between the total B-cell lymphocyte percent and the antibody responses to primary HAV immunization. Our finding suggests that for patients who start HAART after undergoing some degree of immunosuppression, it is optimal to initiate primary immunizations when their B-cell percents normalize, which generally occurs with HAART.[25,26]

The anti-HAV antibody production is T-cell-dependent. This may contribute to the association of CD4% at the time of the administration of the first dose of the vaccine with the antibody production. Furthermore, anti-HAV antibody titers were significantly higher in children who had a detectable HAV-specific T-cell response by LPA compared with children with negative HAV-specific LPA. This association was antigen-specific as demonstrated by the lack of association of anti-HAV antibody titers with nonspecific measures of T-cell function such as PHA, *Candida,* or tetanus LPA. In addition, using carboxyfluorosuccimide ester staining and flow cytometry, we found that CD4+, but not CD19+ PBMC, proliferated during the HAV-specific LPA (data not shown). Taken together, these data indicated that HAV-specific T cells boosted the antibody response to the second dose of HAV vaccine. We sought to further explore the nature of the help provided by T cells by measuring IL-2, IL-4, IL-5, IL-10, and interferon-γ secretion in LPA culture

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

supernatants, but the cytokine levels were overall very low and there were no significant associations of Th1 or Th2 cytokines with antibody titers.

T-cell responses to HAV vaccine were found in a much lower proportion of HIV-infected children on HAART compared with historical responses in healthy HAV vaccine recipients. 41 These differences are unlikely to be the result of assay conditions, because the antigens used to test both populations were obtained from the same manufacturer and other assay conditions were not appreciably different. The HAV-specific LPA differences between healthy and HIV-infected individuals are more consistent with the immune deficit of HIV-infected children that persists despite their reconstituting CD4+ cell numbers in response to HAART. We found a significant association between the CD19+ CD21+ CD27+ memory B-cell percent and positive HAV-specific LPA results. This finding underscores the importance of B- and T-cell cooperation in the response of HIV-infected children on HAART to HAV vaccine and suggests that a decrease in number or function of memory B cells may contribute to the attenuation of T-cell-mediated immune responses in HIV-infected individuals on HAART.

There was a negative association between CD8+ T-cell percent at the time of the administration of the first dose of the vaccine and antibody titers generated by the vaccine. However, there was no correlation between CD8+ T-cell subpopulations and low antibody response to the vaccine. Because CD8+ T-cell percent automatically increases with lower CD4+ and CD19+, T- and B-cell percent, respectively, it is conceivable that the association between CD8+ T cells and antibody responses to the HAV vaccine was indirect and did not reflect a pathogenic mechanism. There was also an inverse association between antibody titers and CD4+ CD45RACD95-memory T-cell percent at baseline. The significance of this correlation needs to be further explored.

In conclusion, our data indicate that antibody responses to primary immunization in HIV-infected children on HAART are primarily dependent on VL and B-cell percent and secondarily on T-cell percent. The recent World Health Organization recommendation to start HAART in all vertically infected children as soon as the diagnosis of HIV infection is established is likely to also result in improved responses to childhood vaccines, because this new recommendation should then result in administering vaccines to children with low or undetectable VL and before they experience appreciable B-cell losses.

Acknowledgments

We thank Dr. Susan Moir (National Institute of Allergy and Infectious Diseases) for scientific advice, and Dr. Jerome Bill and Rebecca Peters from the University of Colorado Denver, Eric Riedel and Nancy Raftery from the Children's Hospital of Philadelphia, and Diane Smith from the University of California in Los Angeles for technical support. We thank the patients and their caregivers for participation. The participants were as follows: Paul Palumbo, MD, Richard Stephens, PhD, MD, Arry Dieudonne, MD, and Juliette Johnson, RN (University of Medicine and Dentistry of New Jersey/University Hospital, Newark, NJ); Abeer Khayat, MD, Saniyyah Mahmoudi, ARNP, MSN, Melissa Scites, BSN, RN, CCRC, and Sharon Cusic, RN (University of Florida Health Sciences Center, Jacksonville, FL); Vincent Bonagura, MD, Susan Schuval, MD, Constance Colter, PNP, and Lorraine Campbell, RN (Schneider Children's Hospital, New Hyde Park, NY); Joseph A. Church, MD, Theresa M. Dunaway, RN, CCRP, MBA, and Cathy Salata, RN (Children's Hospital Los Angeles, Los Angeles, CA); Kathleen M. Mohan, ARNP, Ann J. Melvin, MD, MPH, Lori Van Slyke, MSW, and Lisa M. Frenkel, MD (University of Washington and Children's Hospital & Regional Medical Center of Seattle); Margaret Khoury, MD, Andrea Kovacs, MD, and James Homans, MD (University of Southern California Medical Center, Los Angeles, CA); Irma Febo, MD, Licette Lugo, MD, Ibet Heyer, RN, and Ruth Santos, RN (University of Puerto Rico, San Juan, PR); Margaret A. Keller, MD, Nasser Redjal, MD, ChrisAnna Mink, MD, and Mary Colmenar-Flores, LVN (Harbor-UCLA Medical Center, Torrance, CA); Barbara W. Stechenberg, MD, and Maripat Toye, RN, MS (Baystate Medical Center Children's Hospital, Springfield, MA); Denise Ferraro, RN, Silvia Muniz, and Jane Perillo, MS (Stony Brook University, Stony Brook, NY); Stephen A. Spector, MD, Rolando Viani, MD, MTP, Mary Caffery, RN, and Lisa Stangl, PNP (University of California, San Diego, La Jolla, CA); Audra Deveikis, MD, and Karen Elkins, PNP (Miller Children's Hospital, Long Beach, CA); Mary E. Paul, MD, William T. Shearer, MD, PhD,

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Valerie A. Nichols, BSN, RN, and Chivon D. Jackson, BSN, RN (Baylor Texas Children's Hospital, Houston, TX); Anne Gershon, MD, Marc Foca, MD, Alice Higgins, RN, and Marie Donahue, PNP (Columbia Presbyterian Medical Center, New York, NY); Eleanor Jimenez, MD, Midnela Acevedo, MD, Milagros Gonzalez, MD, and Isos Moraima Burgos, RN (San Juan City Hospital, Rio Piedras, PR); Steven D. Douglas, MD, Richard M. Rutstein, MD, Caroll A. Vincent, CRNP, MSN, Nancy B. Tustin, MLT(ASCP)(HEW), and Donald E. Campbell, PhD (Children's Hospital of Philadelphia, Philadelphia, PA); Elaine J. Abrams, MD, Susan Champion, MD, Delia Calo, BA, Maxine Frere, RN, and Champion, MD, MPH (Harlem Hospital, New York, NY); Hannah Gay, MD, and Sondra Sadler, RN (University of Mississippi Medical Center, Jackson, MS); Diane W. Wara, MD, Deborah Trevithick, RN, MS, Audrey Kamrin, RN, MSN, PNP, and Susan Farrales, RN, MS, FNP (University of California, San Francisco, Moffitt Hospital, San Francisco, CA); Elizabeth J. McFarland, MD, Myron J. Levin, MD, Emily Barr, PNP, and Suzanne Paul, FNP (Children's Hospital/University of Colorado, Denver, CO); Kenneth McIntosh, MD, Nancy P. Karthas, CPNP, MS, RN, Catherine Kneut, RN, MS, CPNP, and Lynne Lewis, MS, RN, CPNP (Children's Hospital of Boston, Boston, MA); Stephen Ira Pelton, MD, Ellen Rae Cooper, MPH, MD, and Anne Marie Regan, MEd, PNP, MSN (Boston Medical Center, Boston, MA); Ann Petru, MD, Teresa Courville, RN, MN, Karen Gold, RN, MA, and Lauren Poole, RN, FNP (Children's Hospital, Oakland, CA); Francis Gigliotti, MD, Geoffrey Weinberg, MD, Barbra Murante, PNP, and Susan Laverty, RN (University of Rochester, Rochester, NY); Edward Handelsman, MD, Hamid Jack Moallem, MD, Denise Swindell, and JeanMarie Kaye, RN (Children's Hospital at Downstate, Brooklyn, NY); Ellen Chadwick, MD, Debbie Cloutier, RN, Lynn Heald, RN, and Amy Talsky (Chicago Children's Memorial Hospital, Chicago, IL); Andrew Wiznia, MD, Jacob Abadi, MD, Margaret Chin, PNP, and Karen Dorio, RN (Jacobi Medical Center, Bronx, NY); and Russell B. VanDyke, MD, Thomas Alchediak, MD, Margarita Silio, MD, and Cheryl Borned, RN (Tulane University, New Orleans, LA).

Supported by contract N01-HD-33162 (AW) and by the Colorado Center for AIDS Research Grant P30 AI054907. Overall support for the International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT) was provided by the National Institute of Allergy and Infectious Diseases (U01 AI068632) and by the Eunice Kennedy Shriver National Institute of Child Health and Human Development. This work was supported by the Statistical and Data Analysis Center at Harvard School of Public Health under the National Institute of Allergy and Infectious Diseases cooperative agreement #5 U01 AI41110 with the Pediatric AIDS Clinical Trials Group (PACTG) and #1 U01 AI068616 with the IMPAACT Group. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases or the National Institute of Child Health and Human Development.

REFERENCES

1. Abzug MJ, Pelton SI, Song LY, et al. Immunogenicity, safety, and predictors of response after a pneumococcal conjugate and pneumococcal polysaccharide vaccine series in human immunodeficiency virus-infected children receiving highly active antiretroviral therapy. Pediatr Infect Dis J. 2006; 25:920–929. [PubMed: 17006288]
2. Hess G, Clemens R, Bienzle U, et al. Immunogenicity and safety of an inactivated hepatitis A vaccine in anti-HIV positive and negative homosexual men. J Med Virol. 1995; 46:40–42. [PubMed: 7623005]
3. Kemper CA, Haubrich R, Frank I, et al. Safety and immunogenicity of hepatitis A vaccine in human immunodeficiency virus-infected patients: a double-blind, randomized, placebo-controlled trial. J Infect Dis. 2003; 187:1327–1331. [PubMed: 12696015]
4. Mount AM, Mwapasa V, Elliott SR, et al. Impairment of humoral immunity to Plasmodium falciparum malaria in pregnancy by HIV infection. Lancet. 2004; 363:1860–1867. [PubMed: 15183624]
5. Neilsen GA, Bodsworth NJ, Watts N. Response to hepatitis A vaccination in human immunodeficiency virus-infected and -uninfected homosexual men. J Infect Dis. 1997; 176:1064–1067. [PubMed: 9333168]
6. Rodriguez-Barradas MC, Alexandraki I, Nazir T, et al. Response of human immunodeficiency virus-infected patients receiving highly active antiretroviral therapy to vaccination with 23-valent pneumococcal polysaccharide vaccine. Clin Infect Dis. 2003; 37:438–447. [PubMed: 12884170]
7. Santagostino E, Gringeri A, Rocino A, et al. Patterns of immunogenicity of an inactivated hepatitis A vaccine in anti-HIV positive and negative hemophilic patients. Thromb Haemost. 1994; 72:508–510. [PubMed: 7878624]
8. Wallace MR, Brandt CJ, Earhart KC, et al. Safety and immunogenicity of an inactivated hepatitis A vaccine among HIV-infected subjects. Clin Infect Dis. 2004; 39:1207–1213. [PubMed: 15486846]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

9. Rigaud M, Borkowsky W, Muresan P, et al. Impaired immunity to recall antigens and neoantigens in severely immunocompromised children and adolescents during the first year of effective highly active antiretroviral therapy. J Infect Dis. 2008; 198:1123–1130. [PubMed: 18752430]
10. Jiang W, Lederman MM, Mohner RJ, et al. Impaired naive and memory B-cell responsiveness to TLR9 stimulation in human immunodeficiency virus infection. J Virol. 2008; 82:7837–7845. [PubMed: 18524824]
11. Qiao X, He B, Chiu A, et al. Human immunodeficiency virus 1 Nef suppresses CD40-dependent immunoglobulin class switching in bystander B cells. Nat Immunol. 2006; 7:302–310. [PubMed: 16429138]
12. Viau M, Veas F, Zouali M. Direct impact of inactivated HIV-1 virions on B lymphocyte subsets. Mol Immunol. 2007; 44:2124–2134. [PubMed: 17134757]
13. De Milito A, Nilsson A, Titanji K, et al. Mechanisms of hyper-gammaglobulinemia and impaired antigen-specific humoral immunity in HIV-1 infection. Blood. 2004; 103:2180–2186. [PubMed: 14604962]
14. Moir S, Ogwaro KM, Malaspina A, et al. Perturbations in B cell responsiveness to CD4+ T cell help in HIV-infected individuals. Proc Natl Acad Sci U S A. 2003; 100:6057–6062. [PubMed: 12730375]
15. De Milito A, Morch C, Sonnerborg A, et al. Loss of memory (CD27) B lymphocytes in HIV-1 infection. AIDS. 2001; 15:957–964. [PubMed: 11399977]
16. Hart M, Steel A, Clark SA, et al. Loss of discrete memory B cell subsets is associated with impaired immunization responses in HIV-1 infection and may be a risk factor for invasive pneumococcal disease. J Immunol. 2007; 178:8212–8220. [PubMed: 17548660]
17. Moir S, Ho J, Malaspina A, et al. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. J Exp Med. 2008; 205:1797–1805. [PubMed: 18625747]
18. Moir S, Malaspina A, Pickeral OK, et al. Decreased survival of B cells of HIV-viremic patients mediated by altered expression of receptors of the TNF superfamily. J Exp Med. 2004; 200:587–599.
19. Morrow M, Valentin A, Little R, et al. A splenic marginal zone-like peripheral blood CD27+B220-B cell population is preferentially depleted in HIV type 1-infected individuals. AIDS Res Hum Retroviruses. 2008; 24:621–633. [PubMed: 18426338]
20. Titanji K, De Milito A, Cagigi A, et al. Loss of memory B cells impairs maintenance of long-term serologic memory during HIV-1 infection. Blood. 2006; 108:1580–1587. [PubMed: 16645169]
21. Berberian L, Valles-Ayoub Y, Sun N, et al. AVH clonal deficit in human immunodeficiency virus-positive individuals reflects a B-cell maturational arrest. Blood. 1991; 78:175–179. [PubMed: 2070051]
22. Ho J, Moir S, Malaspina A, et al. Two overrepresented B cell populations in HIV-infected individuals undergo apoptosis by different mechanisms. Proc Natl Acad Sci U S A. 2006; 103:19436–19441. [PubMed: 17158796]
23. Malaspina A, Moir S, Ho J, et al. Appearance of immature/transitional B cells in HIV-infected individuals with advanced disease: correlation with increased IL-7. Proc Natl Acad Sci U S A. 2006; 103:2262–2267. [PubMed: 16461915]
24. Moir S, Malaspina A, Ogwaro KM, et al. HIV-1 induces phenotypic and functional perturbations of B cells in chronically infected individuals. Proc Natl Acad Sci U S A. 2001; 98:10362–10367. [PubMed: 11504927]
25. D'Orsogna LJ, Krueger RG, McKinnon EJ, et al. Circulating memory B-cell subpopulations are affected differently by HIV infection and antiretroviral therapy. AIDS. 2007; 21:1747–1752. [PubMed: 17690573]
26. Moir S, Malaspina A, Ho J, et al. Normalization of B cell counts and subpopulations after antiretroviral therapy in chronic HIV disease. J Infect Dis. 2008; 197:572–579. [PubMed: 18240953]
27. Chen MY, Hung CC, Fang CT, et al. Reconstituted immunity against persistent parvovirus B19 infection in a patient with acquired immunodeficiency syndrome after highly active antiretroviral therapy. Clin Infect Dis. 2001; 32:1361–1365. [PubMed: 11303273]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

28. Deayton JR, Sabin CA, Britt WB, et al. Rapid reconstitution of humoral immunity against cytomegalovirus but not HIV following highly active antiretroviral therapy. AIDS. 2002; 16:2129–2135. [PubMed: 12409733]
29. Resino S, Galan I, Perez A, et al. HIV-infected children with moderate/severe immune-suppression: changes in the immune system after highly active antiretroviral therapy. Clin Exp Immunol. 2004; 137:570–577. [PubMed: 15320908]
30. Weinberg A, Gona P, Nachman SA, et al. Antibody responses to hepatitis A virus vaccine in HIV-infected children with evidence of immunologic reconstitution while receiving highly active antiretroviral therapy. J Infect Dis. 2006; 193:302–311. [PubMed: 16362896]
31. Prevention of hepatitis A through active or passive immunization: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 1999; 48:1–37.
32. Horng YC, Chang MH, Lee CY, et al. Safety and immunogenicity of hepatitis A vaccine in healthy children. Pediatr Infect Dis J. 1993; 12:359–362. [PubMed: 8392163]
33. Sandman L, Davidson M, Krugman S. Inactivated hepatitis A vaccine: a safety and immunogenicity study in health professionals. J Infect Dis. 1995; 171 Suppl 1:S50–S52. [PubMed: 7876649]
34. Van Damme P, Thoelen S, Cramm M, et al. Inactivated hepatitis A vaccine: reactogenicity, immunogenicity, and long-term antibody persistence. J Med Virol. 1994; 44:446–451. [PubMed: 7897379]
35. Wiens BL, Bohidar NR, Pigeon JG, et al. Duration of protection from clinical hepatitis A disease after vaccination with VAQTA. J Med Virol. 1996; 49:235–241. [PubMed: 8818971]
36. Launay O, Grabar S, Gordien E, et al. Immunological efficacy of a three-dose schedule of hepatitis A vaccine in HIV-infected adults: HEPAVAC study. J Acquir Immune Defic Syndr. 2008; 49:272–275. [PubMed: 18845961]
37. Levin MJ, Gershon AA, Weinberg A, et al. Immunization of HIV-infected children with varicella vaccine. J Pediatr. 2001; 139:305–310. [PubMed: 11487761]
38. Levin MJ, Song LY, Fenton T, et al. Shedding of live vaccine virus, comparative safety, and influenza-specific antibody responses after administration of live attenuated and inactivated trivalent influenza vaccines to HIV-infected children. Vaccine. 2008; 26:4210–4217. [PubMed: 18597900]
39. Overton ET, Sungkanuparph S, Powderly WG, et al. Undetectable plasma HIV RNA load predicts success after hepatitis B vaccination in HIV-infected persons. Clin Infect Dis. 2005; 41:1045–1048. [PubMed: 16142673]
40. Jacobsen MC, Thiebaut R, Fisher C, et al. Pediatric human immunodeficiency virus infection and circulating IgD+ memory B cells. J Infect Dis. 2008; 198:481–485. [PubMed: 18582200]
41. Schmidtke P, Habermehl P, Knuf M, et al. Cell mediated and antibody immune response to inactivated hepatitis A vaccine. Vaccine. 2005; 23:5127–5132. [PubMed: 16054733]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

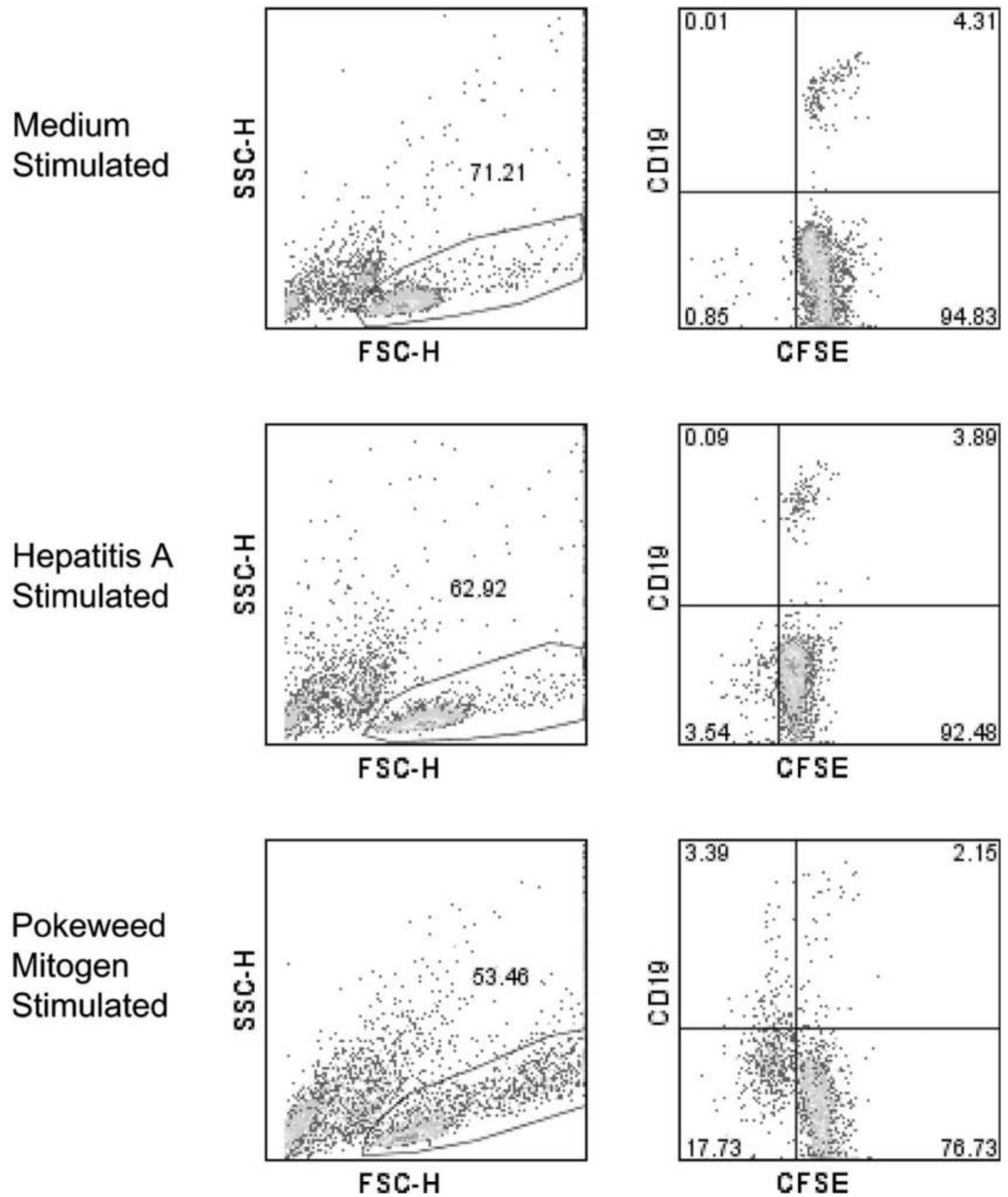

FIGURE 1.
Carboxyfluorosuccimide ester-measured proliferation of CD19+ B lymphocytes in response to hepatitis A virus and pokeweed mitogen stimulation.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

TABLE 1

Baseline Characteristics and Antibody Responses to Hepatitis a Virus Vaccine in HIV-Infected Children on Highly Active Antiretroviral Treatment

	Distribution	Median (mean) Ab Titer	Spearman Correlation Coefficient	*P* Value
Age (years)				
Mean (median)	9.11 (9.22)	NA	−0.04	0.60
	(N = 152)			
Sex				
Male	71	249 (473)	NA	0.17*
Female	81	373 (619)		
Race				
White	25	249 (632)	NA	0.59†
Black	77	310 (507)		
Hispanic	49	466 (587)		
Asian	1†			
Plasma HIV RNA (copies/mL)				
<400	45 (45%)	572 (765)	NA	0.02*
≥400	54 (55%)	273 (453)		
	(N = 99)			
CD4+%				
Median (mean)	31 (32)	NA	0.16	0.04
	(N = 151)			

*Wilcoxon signed rank test.

†Kruskal-Wallis test.

NA, not applicable.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

TABLE 2

Baseline B-Cell Phenotypic Characteristics and Antibody Responses to Hepatitis a Virus Vaccine in HIV-Infected Children on Highly Active Antiretroviral Treatment

	Number of Subjects	Spearman Correlation Coefficient	*P* Value
CD19+%	148	0.21	0.01
CD19+ CD21+%	78	0.05	0.67
CD19+ CD27+%	78	0.14	0.22
CD19+ CD95−%	78	0.01	0.93
CD19+ CD21+ CD27+%	78	0.15	0.19
CD19+ CD21+ CD27−%	78	−0.05	0.64
CD19+ CD21+ CD95+%	78	0.03	0.77
CD19+ CD21+ CD95−%	78	0.05	0.67

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

TABLE 3

Baseline T-Cell Phenotypic Characteristics and Antibody Responses to Hepatitis a Virus Vaccine in HIV-Infected Children on Highly Active Antiretroviral Treatment

	Number of Subjects	Spearman Correlation Coefficient	*P* Value
CD4+%	151	0.16	0.04
CD4+ CD45RA-% (memory)	47	−0.01	0.97
CD4+ CD45RA-CD952% (central memory)	47	−0.28	0.06
CD4+ CD45RA+ CD952% (naïve)	47	−0.03	0.84
CD4+ CD38+ HLADR+% (activated)	52	0.15	0.27
CD8+%	151	−0.17	0.04
CD8+ CD38+ HLADR+% (activated)	53	−0.002	0.99

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

TABLE 4

Baseline and Week 32 Functional T-Cell Characteristics and Antibody Responses to Hepatitis a Virus Vaccine in HIV-Infected Children on Highly Active Antiretroviral Treatment

	Study Week	Number of Subjects	Median (mean) Ab Titers	*P* Value*
Hep A LPA				
Negative (SI < 3)	32	44	289 (490)	0.02
Positive (SI ≥ 3)		10	895 (834)	
Candida LPA				
Negative (SI < 3)	0	9	448 (597)	0.71
Positive (SI ≥ 3)		42	342 (489)	
Candida LPA				
Negative (SI < 3)	32	5	738 (899)	0.58
Positive (SI ≥ 3)		43	363 (515)	
Tetanus LPA				
Negative (SI < 3)	0	17	423 (576)	0.42
Positive (SI ≥ 3)		24	304 (499)	
Tetanus LPA				
Negative (SI < 3)	32	13	216 (483)	0.45
Positive (SI ≥ 3)		28	413 (503)	

*Wilcoxon signed rank test.

LPA indicates; SI, stimulation index.