Human Immunodeficiency Virus Status and Delayed-Type Hypersensitivity Skin Testing in Ugandan Children

Anna Maria Mandalakas, MD*; Laura Guay, MD‡; Philippa Musoke, MD§; Cindie Carroll-Pankhurst, PhD*; and Karen N. Olness, MD*

ABSTRACT. *Background.* **In previous studies, delayed-type hypersensitivity (DTH) skin testing has been shown to be affected by several factors including nutritional status, intercurrent infection, host immune status, and previous exposure to the antigen being used.**

Objective. **To determine the effect of human immunodeficiency virus type 1 (HIV-1) status on DTH skin testing in a cohort of HIV-1-infected and noninfected Ugandan children followed prospectively from birth.**

Design. **Nested case-control study.**

Setting. **Primary care clinic serving study participants at Mulago Hospital, Makerere University, Kampala, Uganda.**

Participants. **Thirty HIV-1-infected children and 30 age-matched, HIV-1-noninfected children.**

Methods. **After completion of history and physical, each child underwent Mantoux skin testing with both *Candida* and purified protein derivative (PPD). Results of skin testing were read in 48 to 72 hours. Complete chart reviews were performed on all children. CD4 lymphocyte counts were obtained on all HIV-1-infected children at the time the skin testing was read.**

Results. **The average age of participants was 67 months (range, 51–92 months). HIV-1-infected children (mean CD4 lymphocyte count, 1069 mL^{-1}; range, 86–3378 mL^{-1}), compared with noninfected, age-matched peers, developed significantly smaller PPD reaction size (mean, 1.18 mm ± 4.3 vs 3.6 mm ± 7.6, respectively). *Candida* responses were not different between the two groups of children. Among HIV-1-infected children, there was a larger *Candida* reaction size in children who had recently received chloroquine treatment. There was no significant correlation between *Candida* reactivity and PPD reactivity, progressive HIV-1 disease, or CD4 lymphocyte count. The six children diagnosed clinically with active tuberculosis had lower absolute CD4 lymphocyte counts than children without tuberculosis. Lack of reaction to PPD was associated with lower CD4 lymphocyte counts and progressive HIV-1 disease.**

Conclusions. **In HIV-1-infected Ugandan children, DTH skin testing was influenced by the choice of antigen selected, HIV-1 infection, and recent treatment with chloroquine. Based on these findings, we believe that further prospective, longitudinal investigation into the role of chloroquine in HIV-1-infected children is needed. We emphasize the limitations of DTH skin testing in HIV-infected children as an adjunct in the diagnosis of active tuberculosis.** *Pediatrics* **1999;103(2). URL: http://www.pediatrics.org/cgi/content/full/103/2/e21; *pediatric, human immunodeficiency virus, tuberculosis, purified protein derivative, Candida, chloroquine.***

ABBREVIATIONS. TB, tuberculosis; HIV-1, human immunodeficiency virus type 1; BCG, bacille Calmette-Guérin; DTH, delayed-type hypersensitivity; PPD, purified protein derivative.

Tuberculosis (TB) continues to be a major cause of morbidity and mortality worldwide. The World Health Organization has determined that approximately one-third of the world's population has been infected with *Mycobacterium tuberculosis*.[1,2] TB is now the most frequent cause of death from identifiable infectious pathogens in human immunodeficiency virus type 1 (HIV-1)-infected individuals worldwide.

HIV-1 infection has been identified as the greatest known risk factor for active TB disease. The relationship between HIV infection and TB is now widely accepted.[3–7] Recent pediatric studies have suggested that HIV-positive children are at an eight times higher risk of TB infection than HIV-negative controls.[8]

Based on clinical presentation, the early diagnosis of pediatric TB continues to pose a great challenge. Because children rarely produce sputum when coughing, it is difficult to obtain culture confirmation. Although a myriad of diagnostic methods have been used when studying TB, many of these studies tend to have limited sensitivity (gastric lavage and bronchoscopy) and predictive value (chest radiography and thoracic computed tomography).[9,10]

Tuberculin skin testing is the only readily available test to detect previous infection with *M tuberculosis*. It is widely used in epidemiologic surveys, clinical evaluation of patients with suspected TB, and assessing indications for isoniazid preventive therapy. The degree of sensitization is clearly affected by multiple factors. Tuberculin reactivity is induced by infection with living tubercle bacilli, infection with other mycobacteria, inoculation with dead tubercle bacilli, vaccination with bacille Calmette-Guérin (BCG), and repeated tuberculin delayed-type hypersensitivity (DTH) skin testing.[11] Transient anergy has clearly been shown to occur during rubeola and influenza

From the *Department of Pediatrics, Case Western Reserve University, Cleveland, Ohio; the ‡Department of Pathology, Johns Hopkins University, Baltimore, Maryland; and the §Department of Paediatrics, Makerere University, Kampala, Uganda.

Received for publication Feb 23, 1998; accepted Aug 31, 1998.
No reprints available.

Address correspondence to Anna Maria Mandalakas, MD, Rainbow Center for International Child Health, 11100 Euclid Ave, Cleveland, OH 44106-6038.

PEDIATRICS (ISSN 0031 4005). Copyright © 1999 by the American Academy of Pediatrics.

Downloaded from www.aappublications.org/news by guest on September 18, 2021.

infections, after influenza and measles vaccination,[12] and during periods of malnutrition.[13]

Early diagnosis of TB has been shown to be particularly difficult in the HIV-infected population. The immunosuppression that develops with progressive HIV infection has been associated with anergy.[14] Because of this association in HIV-infected individuals, the usefulness of the Mantoux test has been questioned.[15,16] Tuberculin anergy is common in HIV-1-infected adults.[17] In a recent study of HIV-infected adults, the prevalence of tuberculin reactivity varied directly and that of anergy indirectly with the absolute CD4 lymphocyte count.[18] At present, limited information is available concerning DTH skin test responses in HIV-infected children. A recent case series described 12 HIV-infected children with active TB who were tuberculin anergic.[19]

We therefore conducted a study to gather further information about the effects of HIV-1 infection on DTH skin testing in Uganda, a country with a high prevalence of HIV and TB infection. We prospectively examined purified protein derivative (PPD) DTH skin testing in a cohort of HIV-1-infected and noninfected Ugandan children followed from birth. Recently, we evaluated DTH skin testing to both PPD and *Candida* and examined factors associated with anergy including HIV-1 status, nutritional status, acute illness, recent chloroquine treatment, active TB, and stage of HIV-1 infection assessed by CD4 lymphocyte counts.

METHODS

Study Population

In 1988, the AIDS Control Program of the Ugandan Ministry of Health, Makerere University, and Case Western Reserve University began a collaborative study of HIV-1 infection in Ugandan women and their infants. The first cohort, which focused on the natural history of HIV-1 disease, included 589 infants born to enrolled women between January 1989 and September 1990.[20] The second cohort, which focused on the neurodevelopmental aspects of HIV-1 disease, included 530 infants born to enrolled women between November 1990 and April 1992.[21] In the second cohort, only full-term infants without significant birth complications or neurologic or genetic impairments based on newborn physical examination were enrolled. No infants or mothers received zidovudine or other antiretroviral treatments, which were not available in Uganda.

For the current skin testing study, which was conducted during November and December of 1996, both HIV-1-infected and noninfected participants were selected from the previously established cohorts. Of the 154 HIV-1-infected children in the original two cohorts, 34 children had survived to the start of this study. All the living HIV-1-infected children who were traceable were included; 4 children could not be traced. These children were matched by age, within 3 months, to 30 randomly chosen, noninfected controls ($P = .55$). Written informed consent was obtained from the child's parent or legal guardian. In cases of illiteracy, the consent form was read to the guardian. The study protocol was approved by the institutional review boards of Makerere University and Case Western Reserve University.

Immunization Schedules

All infants were immunized shortly after birth according to the United Nations Expanded Program on Immunization guidelines. The immunization schedule included intradermal BCG (0.05 mL) given in the right lower deltoid region. All children received BCG at birth or within the first month of life. The Ugandan Ministry of Health provided all vaccines, which consisted of both Pasteur and Japanese strain.

Physician History and Physical Examination

A history was obtained from each child's parent or primary care provider with regard to any symptoms of acute or chronic illness, exposure to TB, previous TB treatment, and recent chloroquine treatment. Each child received a complete physical examination with special care given to describe any HIV-associated comorbidities.

Assessment of Nutrition and Failure to Thrive

Growth charts plotting height and weight were kept for all children from birth. Failure to thrive was defined as follows: downward crossing of at least two of the percentiles on the weight-for-age chart (excluding the 10th and the 90th percentiles), persistent weight loss of more than 10% of baseline, or less than the 5th percentile on the weight-for-height chart.

Using EPIinfo (USD, Inc, Stone Mountain, GA), weight-for-age and height-for-age Z scores were computed on all the children. Children with Z scores ≤ -2 in either category were considered malnourished.

Assessment of Acute Illness

Children exhibiting two or more of the following were considered acutely ill: fever (>38°C axillary), respiratory distress (respiratory rate of >30 breaths per minute, rib retractions, or nasal flaring), significant cough, gastroenteritis, anemia, and/or dehydration. Cough was considered significant if it was present throughout the day, every day, or recurrent despite medical treatment. Gastroenteritis was defined by the presence of greater than two loose, watery stools per day.

Assessment of Active Tuberculosis

Assessment of active TB disease was based on a modification of the World Health Organization criteria for the diagnosis of TB in children.[22] The scoring system outlined by the WHO includes the following criteria; duration of illness, nutrition, family history of TB, tuberculin test, malnutrition, unexplained fever and night sweats, and symptoms consistent with disseminated disease. Because the tuberculin test was one of our outcome measures, we excluded skin test results. Children were screened for significant cough of greater than 1-month duration, unexplained fever for more than 1 month, and evidence of a failure to thrive and/or a history of household exposure to a TB-infected individual. All children with two or more of the symptoms were evaluated by chest radiograph. Active TB disease was defined as the presence of two or more of these symptoms and evidence on chest radiograph consistent with pulmonary TB or physical examination findings consistent with disseminated TB. A panel of three pediatricians reviewed all chest radiographs. Chest radiographs were considered positive if they displayed hilar and/or paratracheal lymphadenopathy or infiltrates consistent with TB. Children found to have active TB disease were started on anti-TB treatment.

Delayed-Type Hypersensitivity Skin Testing

All children underwent DTH skin testing by using PPD (Tubersol [5 TU/0.1 mL], Connaught, Swiftwater, PA) and *Candida* antigen (Candin, Allermed, San Diego, CA). By using standardized procedures, 0.1 mL of PPD was administered intradermally on the volar aspect of the left forearm. In a similar fashion, 0.1 mL of *Candida* antigen was administered to the right forearm. *Candida* antigen was administered undiluted. In 48 to 72 hours, the transverse diameter of induration at each injection site was measured by using the ''ballpoint-pen'' method.[23,24] DTH skin testing and reading were performed by highly experienced personnel blinded to the subjects HIV status. Throughout the study, investigators were blinded to HIV-1 status. A positive PPD reaction was defined as greater than or equal to 5 mm induration in HIV-1 infected children and greater than or equal to 10 mm induration in HIV-1-noninfected children.[25] A positive *Candida* reaction was considered any induration ≥5 mm.[26] The presence or absence of a BCG scar was noted on all subjects. Data on previous PPD skin testing were obtained from each subject's clinic chart.

The use of *Candida* antigen (Candin, Allermed) has been previously studied in adults from this same population. When compared with another commonly used preparation of *Candida* antigen, Candin was found to elicit a significantly larger reaction

Downloaded from www.aappublications.org/news by guest on September 18, 2021

TABLE 1. Human Immunodeficiency Virus Type 1 (HIV-1) Clinical Staging According to the 1994 CDC Pediatric HIV Classification System

	N (No Symptoms)	A (Mild Symptoms)	B (Moderate Symptoms)	C (AIDS)
1. No immunosuppression	1	6	2	4
2. Mild to moderate immunosuppression	0	2	4	4
3. Severe immunosuppression	0	1	0	3

Abbreviations: CDC, Centers for Disease Control; AIDS, acquired immunodeficiency syndrome.

size.[27] An additional unpublished study demonstrated that anergy to Candin was associated with 22 deaths per 100 person-years compared with 10 deaths per 100 person-years in nonanergic individuals. Based on these previous findings, we were confident of our choice of Candin as our control antigen.

Lymphocyte Characterization

Peripheral blood absolute CD4 lymphocyte counts and percent CD4 lymphocytes were determined on all HIV-1-infected children by flow cytometry, using a Coulter EPIC Profile II flow cytometer (Coulter Electronics, Hialeah, FL). Assays were completed within 48 to 72 hours of skin testing, using the laboratory facilities on site at Makerere University.

HIV Serology

HIV antibody testing was performed on all infants at 12 and 18 months of age, with all positive results confirmed by western blot. HIV antibody was tested by using an enzyme-linked immunosorbent assay (Recombigen HIV-1 enzyme immunoassay, Cambridge Bioscience, Worcester, MA). Confirmatory western blots (Novapath Immunoblot Assay, Bio-Rad, Hercules, CA) were interpreted as positive if they contained any two of the following bands: gp 120/160, gp 41, or p24.

Statistical Methods

Data analysis included univariate, two-tailed significance testing with χ^2, and Fisher's exact test, when indicated by small sample size, t tests for differences in mean induration between groups, and Pearson's correlation coefficient. Forward stepwise multivariate regression analysis also was performed to test for the combined effects of presumed predictor variables on size of skin test reaction. In all regression models, no more than four variables were tested simultaneously because of the limited sample size. Also, based on sample size, a more liberal inclusion criteria (P = .1) was used so that no indicative variables would be overlooked. All data were analyzed by using SPSS (SPSS Inc, Chicago, IL) for MS Windows, Release 6.1.

RESULTS

Description of Sample

Thirty HIV-1-infected and 30 age-matched, noninfected children were studied. All the children completed the study. Of the 30 seronegative children, 23 were seroreverters, born to seropositive mothers. The mean age was 67 months, with a standard deviation of 10.7 months and a range of 51 to 92 months.

Sixty percent of the HIV-infected and 43% of the noninfected children were females. Males and females did not differ with regard to the incidence of acute illness, malnutrition, chloroquine exposure, or active TB disease.

Based on maternal history and chart review, no child had undergone skin testing in the 36 months before enrollment. Thirteen of the children had no previous history of DTH skin testing. Of the remaining 47 children, 15 children had one previous DTH skin test, 31 children had two previous DTH skin tests, and 1 child had three previous DTH skin tests. Despite documentation of receipt in all children, BCG scars were absent in 6 HIV-1-infected and 6 HIV-1-noninfected children.

Among the HIV-1-infected children, the clinical stages varied greatly (Table 1). Lymphocyte subsets were available for 27 HIV-infected children; the parents of 2 children refused phlebotomy and 1 sample was inadequately processed.

Mantoux Skin Test Reactions

Mantoux skin test reactions to PPD and *Candida* for all children are shown in Fig 1. Six children were anergic, ie, 2 HIV-1 noninfected and 4 HIV-1 infected.

Ten children were PPD-positive, ie, 8 HIV-1 noninfected and 2 HIV-1 infected children. The mean PPD size was 1.2 ± 4.3 mm among HIV-1-infected children and 4.6 ± 7.6 mm among HIV-1-noninfected children (P = .035). To accommodate the high proportion of children who had no PPD reaction, dichotomous analyses were also performed by using the previously defined guidelines for anergy. This approach yielded similar, but only marginally significant, differences between HIV-1-infected and noninfected children (Fisher's exact test, P = .08). There was no significant difference in PPD size because of acute illness, malnutrition, chloroquine exposure, BCG scar presence, BCG scar size (data not shown), or active TB disease (Table 2). There was no correlation between PPD reaction size and CD4 lymphocyte count or *Candida* reaction size.

Two HIV-1-infected children mounted reactions to PPD that were less than 5 mm. One of these children had no history of TB exposure and an HIV clinical stage of N. No CD4 count was obtained. Although he was diagnosed with malnutrition, he had no other

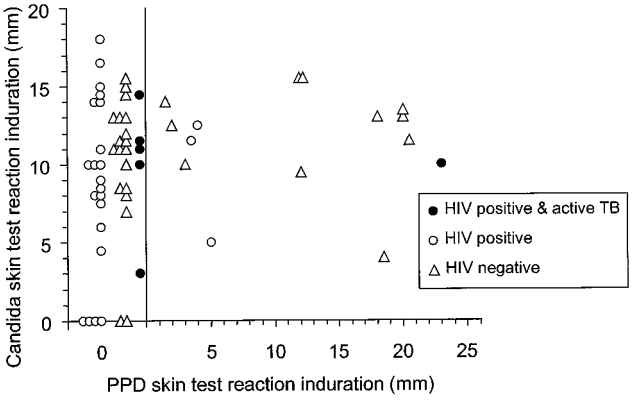

Fig 1. Purified protein derivative and *Candida* reaction size in 30 human immunodeficiency virus type 1 (HIV-1)-infected and 30 HIV-1-noninfected children

Downloaded from www.aappublications.org/news by guest on September 18, 2021

TABLE 2. Difference in Mean Induration

	PPD (mm)	*Candida* (mm)
HIV infection	3.5*	1.8
Active TB	1.0	0.0
Acute illness	2.7	0.0
Malnutrition	1.5	0.9
Recent chloroquine exposure	1.6	3.9†

Abbreviations: PPD, purified protein derivative; HIV, human immunodeficiency virus; TB, tuberculosis.
* $P < .05$; † $P < .01$.

active clinical problems. The second child had been diagnosed and treated for TB 5 years before study enrollment. He was also malnourished with an HIV clinical stage of C2 and a CD4 count of 352.

When comparing HIV-1-infected versus HIV-1-noninfected children, there was no significant difference in mean *Candida* reaction size (9.1 ± 5.0 vs 10.9 ± 3.9, respectively; $P = .14$). The same dichotomous strategy noted above was used to examine reactions to *Candida.* This analysis produced similar results. There was no significant difference in *Candida* reaction based on HIV-1 status ($P = .47$). There was no significant difference in *Candida* size because of acute illness, malnutrition, or active TB (see Table 2). There was no correlation between CD4 lymphocyte count and size of *Candida* reaction.

When examining the total sample (n = 60), children who had received chloroquine within the 6-week period before study enrollment had significantly larger *Candida* reactions than those who had not recently been treated with chloroquine (12.9 vs 9.0 mm, respectively; $P = .004$) (see Table 2). By using a stratified approach to explain the potential confounding by HIV-1 status, we demonstrated that among the HIV-1-infected children there was a significantly larger *Candida* reaction in children who had been treated with chloroquine in the 6-week period before study enrollment (12.8 vs 7.8 mm; $P = .012$). Conversely, the HIV-1-noninfected children had no significant difference in *Candida* reaction size with regard to chloroquine exposure. There was no significant difference in the incidence of chloroquine exposure ($P = .77$) associated with HIV-1 infection. Within the HIV-1-infected children, there was no significant difference in mean CD4 lymphocyte count associated with recent chloroquine treatment.

Between the HIV-1-infected and HIV-1-noninfected children, there were no significant differences in the incidence of concomitant acute illnesses or malnutrition. Within the group of HIV-1-infected children, there were no significant associations between mean CD4 lymphocyte count and malnutrition, acute illness, or chloroquine treatment. Examining the entire group of children revealed no significant association between size or presence of BCG scar and *Candida* or PPD reactions.

By using multiple-regression analysis to simultaneously control for the variables of interest (malnutrition, acute illness, chloroquine treatment, CD4 lymphocyte count, and HIV-1 status), we showed similar results. There was a significance association between HIV-1 infection and a smaller PPD reaction size ($P = .01$) while controlling for acute illness, which was the only marginally significant variable ($P = .08$). As shown by the same analysis strategy for evaluating the size of the *Candida* reaction, the only variable that predicted induration size was chloroquine treatment. Chloroquine exposure was associated with a larger mean induration to *Candida* ($P = .003$) while controlling for HIV-1 status, which was not significant at the 0.05 level ($P = .09$). Within each group, HIV-1 infected and HIV-1 noninfected, there was no significant association between any of the factors.

TABLE 3. Clinical Findings in Children With Active Pulmonary Tuberculosis

Cough >1 Month	Failure to Thrive	Household TB Exposure	Fever >1 Month	Chest Radiograph
+	−	+	−	Infiltrates
+	−	−	+	Infiltrates
+	+	−	−	Infiltrates
−	+	+	−	Infiltrates
+	−	+	−	Infiltrates
−	+	+	+	Infiltrates

Abbreviation: TB, tuberculosis.

Outcome Relative to Active Tuberculosis

The incidence of active TB disease was found to be higher in the HIV-1-infected children. Six HIV-1-infected children had active TB compared with none of the HIV-1-noninfected children ($P = .01$). The clinical description of the children diagnosed with TB is summarized in Table 3. There were no cases of disseminated TB diagnosed. Of these 6 children, 3 had previously been diagnosed with active TB disease from 12 to 24 months before the current study (Table 4). One child had completed an adequate course of TB treatment. The families of the remaining 2 children were unsure of treatment completion. Five of 6 children with active TB were nonreactive to PPD. Only 1 of these 5 children was nonreactive to *Candida.* The single child who was reactive to both *Candida* and PPD had the least advanced clinical stage (B1) of the children with active TB. Within the HIV-1-infected children, the mean absolute CD4 lymphocyte count of the children diagnosed with active TB disease was significantly lower than the children with no active TB disease (624 ± 448 and 1196 ± 815, respectively; $P = .04$) (see Table 4). The proportion of children with severe, moderate, or no evidence of immunosuppression was not significantly different between the children with active TB and those without ($P = .16$), reflecting the small sample size.

DISCUSSION

When examining DTH skin testing responses in HIV-1-infected and noninfected Ugandan children, we found that the response mounted by the immune system varied significantly based on the antigen used. The difference in the mean PPD reaction size in HIV-1-infected and HIV-1-noninfected groups (1.18 and 4.65, respectively) was statistically significant ($P < .035$), whereas *Candida* reaction size was not different statistically. With regard to *Candida,* these findings are similar to the study completed by Raszka et al[28] in which analysis was completed on

Downloaded from www.aappublications.org/news by guest on September 18, 2021

TABLE 4. Children With Active Pulmonary Tuberculosis

HIV-1 Status	PPD (mm)	*Candida* (mm)	Absolute CD4	CD4 (%)	HIV Stage	Previous History of TB
Positive	0	11	726	13.2	C2	Yes
Positive	0	14.5	196	8.9	C3	No
Positive	23	10	1325	28.2	B1	No
Positive	0	10	624	26.0	B2	Yes
Positive	0	3	86	1.9	C3	No
Positive	0	11.5	790	14.1	C2	Yes

Abbreviations: HIV-1, human immunodeficiency virus type 1; PPD, purified protein derivative; TB, tuberculosis.

both the number of positive skin reactions and the size of mean induration. In their study of 27 HIV-infected and 14 noninfected children, no significant difference was found in the number of positive skin reactions to a different *Candida albicans* antigen at a dilution of 1:10 or in the size of mean induration to *C albicans* at dilutions of both 1:10 and 1:100. There was a significant difference in the number of positive reactions to *C albicans* at a dilution of 1:100.

Our study is the first to be completed in HIV-1-infected children from sub-Saharan Africa, comparing DTH skin testing of *Candida* and PPD. Our results clearly show that HIV-1-infected children have a greater reaction elicited by *Candida* than 5-TU PPD. In addition, there was no statistical difference in *Candida* reaction size between HIV-1-infected and noninfected children. HIV-1-infected children have a higher incidence of mucocutaneous candidal infections. Theoretically, this increases *Candida* sensitization. This is one potential explanation for the larger reaction size to *Candida* versus PPD and a *Candida* reaction size that is not statistically different based on HIV-1 status.

There is no definitive way to distinguish positive tuberculin reactions caused by BCG from those caused by *M tuberculosis* infection. BCG was administered to 56 participants at birth. The remaining 4 participants received BCG within the first month of life. In children vaccinated at or near birth, postvaccination tuberculin sensitivity wanes rapidly over the first 5 years of life.[29] Because the mean age of children in our study was 67 months, it is unlikely that any of our tuberculin-sensitive children were exhibiting postvaccine tuberculin sensitivity.

As postvaccination tuberculin sensitivity diminishes, induration associated with PPD is more likely to be representative of TB infection or active TB disease. TB is highly endemic in eastern Africa. In an earlier national survey in 1970, 58% of Ugandan adults older than 15 years were tuberculin positive.[30] The rate of reported TB cases in Uganda in 1995 was 119.6 per 100 000 population.[31] In our study, there was no significant difference in reported TB exposure between the HIV-1-infected and noninfected children. Although it is expected that HIV-1-infected children have a higher prevalence of TB exposure based on the parental immunocompromised status, 76% of our HIV-noninfected children were seroreverters. Thus, in our study the prevalence of TB exposure was comparable between HIV-1-infected and noninfected groups of children. As previously stated, both groups of children mounted statistically similar *Candida* reactions. If the HIV-1-infected and noninfected children were able to mount reactions similar to 5-TU PPD, we anticipate similar PPD reaction size because of natural TB infection. This was not the case. In the HIV-1-infected group of children, there was a statistically smaller mean PPD induration and a greater proportion of children nonreactive to PPD.

In contrast to previous studies, we were unable to show an association between a lack of reaction to *Candida* and the degree of HIV-related immunosuppression. Among HIV-1-infected children, there was no significant association between the size of *Candida* reaction and the absolute CD4 lymphocyte count. We found no correlation between a lack of reactivity to *Candida* and a lack of reactivity to PPD. Although five HIV-1-infected children with TB had no reaction to PPD, all but one of these children displayed reactions to *Candida* (see Table 4).

When comparing the characteristics of the HIV-1-infected children with TB (see Table 4), we found that a lack of reaction to PPD was associated with progressive HIV-1 disease and lower CD4 lymphocyte counts. When comparing HIV-1-infected children with active TB disease with those without active TB disease, the mean CD4 lymphocyte counts were significantly lower in those with active TB disease ($P = .04$). These findings are consistent with those of several studies that have shown a significant association between anergy and advanced HIV-related immunosuppression. In a large multicenter US study involving 1353 adults, Markowitz et al[18] found that patients with HIV infection and less than 400 CD4 lymphocytes/mm^3 had a lower prevalence of PPD reactivity than HIV-1-noninfected controls. In the study by Raszka et al,[28] absence of any induration of more than 5 mm to a panel of at least four antigens was shown to be associated with symptomatic disease, evidence of clinically advanced HIV infection, or a CD4 percentage of 15% or less.

In our study, PPD reactivity proved to be a poor predictor of TB in HIV-1-infected children. Ten children were PPD positive; 9 of these children were asymptomatic with normal chest radiographs and no signs of disseminated TB on physical examination. Of the 9 PPD positive children without TB, 1 was HIV-1 infected (see Fig 1). Six children were diagnosed with active TB disease and 1 of these children had a positive PPD (see Table 4). Of note, all children with active TB were HIV-1 infected. The lack of reaction to PPD in HIV-1-infected children with active TB is consistent with the cases described by

Downloaded from www.aappublications.org/news by guest on September 18, 2021

Chan et al.[19] Chan and his colleagues describe 12 HIV-infected children with active TB who were tuberculin anergic. The lack of active TB in the HIV-1-noninfected children most likely reflects our small sample size.

In many countries where endemic malaria exists, such as Uganda, it is common practice to self-medicate with chloroquine as a treatment for nonspecific fever. The effects of chloroquine on DTH skin testing in HIV-1-infected children have never been previously examined. Assuming that study participants have had equal *Candida* exposure within their respective HIV-1 status groups, any significant difference in *Candida* reaction size was caused by host factors. In our study, HIV-1-infected children recently treated with chloroquine had significantly larger *Candida* reaction size than HIV-1-infected children without recent chloroquine exposure. These findings are consistent with studies completed in HIV-1-infected adult populations. After treatment of HIV-1-infected cells with chloroquine, there was a significant reduction in cell size, virus associated with glycoprotein, gp 120 of HIV-1, and total virus yield. Also, most virions released were noninfectious.[32] In a randomized, double-blind, placebo-controlled study, hydroxychloroquine or placebo was given to 40 HIV-1-infected adults for 2 months. Not only was chloroquine administration associated with a significant decrease in the total plasma levels of HIV-1 RNA, but there was also a significant increase in the proliferative responses to *Candida* in the chloroquine group versus the placebo group.[33]

Chin et al[15] associated transient anergy in HIV-infected adults with baseline CD4 lymphocyte counts. In our study, there was no significant difference in CD4 lymphocyte counts between children with and without recent chloroquine treatment. We believe that further prospective, longitudinal investigation into the role of chloroquine in HIV-1-infected children is needed.

Our study sample was derived from the long-term survivors of previous cohorts in an area with high *M tuberculosis* transmission rates. Thus, sample size was limited by survival. The significance of survivor bias on the outcome of this study is unclear. The risk of active TB in anergic, HIV-1-infected children is affected by the community prevalence and attack rate. When generalizing our conclusions to other HIV-infected pediatric populations, these factors must be considered.

In our study of HIV-1-infected children, multiple factors affected DTH skin testing response, including antigen selection, HIV-1 infection, and recent chloroquine treatment. No significant difference in *Candida* reaction size was associated with HIV-1 infection. A lack of reaction to PPD was associated with HIV-1 infection even in the face of active TB. There was no association between *Candida* and PPD reactivity. Recent chloroquine treatment was associated with larger *Candida* reaction size. PPD positivity was a poor predictor of active TB disease. Based on these findings, we emphasize the limitations of DTH skin testing in HIV-1-infected children. To identify anergic children, reactivity to *Candida* DTH skin testing alone cannot be used as a correlate to PPD DTH skin testing. As recommended by the most recent CDC guidelines,[34] which were published after the completion of our study, use of the two Food and Drug Administration-approved Mantoux method skin tests (mumps and *Candida*), or a panel of several antigens, may provide a greater predictive value when testing for anergy. As an adjunct to the diagnosis of active TB in HIV-1-infected children, PPD skin testing is most useful when positive. A nonreactive PPD skin test gives little conclusive information.

ACKNOWLEDGMENTS

Funding for this research was provided by the Center for AIDS Research at Case Western Reserve University.

We thank the Center for AIDS Research at Case Western Reserve University and individual friends who supported this project.

We acknowledge the participation of and extend our appreciation to the staff of Ward 11 at Mulago Hospital, Makerere University, Kampala, Uganda. Special thanks are given to John L. Johnson, MD (Case Western Reserve University), and Sam Nyole for their contributions to this study.

REFERENCES

1. World Health Organization.*Childhood Tuberculosis and BCG Vaccine. EPI Update (Supplement).* Geneva, Switzerland: World Health Organization; 1989
2. Starke JR, Jacobs RF, Jereb J. Resurgence of tuberculosis in children. *J Pediatr.* 1992;120:839–855
3. Chaisson RE, Shecter GF, Theuer CP, Rutherford GW, Echemberg DF, Hopewell PC. Tuberculosis in patients with acquired immunodeficiency syndrome: clinical features, response to therapy, and survival. *Am Rev Respir Dis.* 1987;136:570–574
4. Barnes PF, Bloch AB, Davidson PT, Sneider DE Jr. Tuberculosis in patients with human immunodeficiency virus infection. *N Engl J Med.* 1991:1644–1649
5. Tomlinson DR, Moss F, McCarty M, et al. Tuberculosis in HIV seropositive individuals: a retrospective analysis. *Int J STD AIDS.* 1992;3:38–41
6. Sudre P, ten Dam HG, Kochi A. Tuberculosis: a global overview of the situation today. *Bull WHO.* 1992;70:149–159
7. Perriens JH, St Louis ME, Mukadi YB, et al. Pulmonary tuberculosis in HIV-infected patients in Zaire. *N Engl J Med.* 1995;332:779–784
8. Bhat GJ, Diwan VK, Chintu C, Kabika M, Masona J. HIV, BCG and TB in children: a case control study in Lusaka, Zambia. *J Trop Pediatr.* 1993;39:219–223
9. Schaaf HS, Beyers N, Gie R, et al. Respiratory tuberculosis in childhood: the diagnostic value of clinical features and special investigations. *Pediatr Infect Dis J.* 1995;14:189–194
10. Delacourt C, Mani TM, Bonnerot V, et al. Computed tomography with normal chest radiograph in tuberculosis infection. *Arch Dis Child.* 1993; 69:430–432
11. Sepulvida RL, Ferrer X, Latrach C, et al. The influence of Calmette-Guerin bacillus immunization on the booster effect of tuberculin testing in healthy young adults. *Am Rev Respir Dis.* 1990;142:24–28
12. American Thoracic Society. The tuberculin skin test. *Am Rev Respir Dis.* 1981;124:356–363
13. Sinha DP, Bang FB. Protein and caloric malnutrition, cell mediated immunity, and BCG vaccination in children from rural west Bengal. *Lancet.* 1976;2:531–534
14. Jason J, Murphy J, Sleeper L, et al. Immune and serologic profiles of HIV-infected and non-infected hemophilic children and adolescents. *Am J Hematol.* 1994;46:29–35
15. Chin DP, Osmond D, Page-Shafer K, et al. Reliability of anergy skin testing in persons with HIV infection. *Am J Respir Crit Care Med.* 1996; 153:1982–1984
16. Sears SD, Fox R, Brookmeyer R, Leavitt R, Polk BF. Delayed hypersensitivity skin testing and anergy in a population of gay men. *Clin Immunol Immunopathol.* 1987;45:177–183
17. Okwera A, Eriki PP, Guay LA, Ball P, Daniel TM. Tuberculin reactions in apparently healthy HIV-seropositive and HIV-seronegative women–Uganda. *MMWR.* 1990;39:638–639, 645–646
18. Markowitz N, Hansen N, Wilcosky T, et. al. Tuberculin and anergy

Downloaded from www.aappublications.org/news by guest on September 18, 2021

testing in HIV-seropositive and HIV-seronegative persons. *Ann Intern Med*. 1993;119:185–193
19. Chan SP, Birnbaum J, Rao M, Steiner P. Clinical manifestations and outcomes of tuberculosis in children with acquired immunodeficiency syndrome. *Pediatr Infect Dis J*. 1996;15:443–447
20. Mmiro P, Ndugwa C, Guay L, et al. Effect of human immunodeficiency virus-1 infection on the outcome of pregnancy in Ugandan women. *Pediatr AIDS HIV Infect*. 1993;4:67–73
21. Drotar D, Olness K, Wiznitzer M, et al. Neurodevelopmental outcomes of Ugandan infants with human immunodeficiency virus type 1 infection. *Pediatrics*. 1997;100(1). URL: http://www.pediatrics.org/cgi/content/full/100/1/e5
22. Harries AD, Maher D. *TB/HIV: A Clinical Manual*. Geneva, Switzerland: World Health Organization; 1996:63–64
23. Sokol JE. Measurement of delayed skin test responses. *N Engl J Med*. 1975;293:501–502
24. Jordan TJ, Sunderam G, Thomas L, Reichman LB. Tuberculin reaction size measurements by the pen method compared to traditional palpation. *Chest*. 1987;92:234–236
25. Committee on Infectious Disease, 1993–1994. Screening for tuberculosis in infants and children. *Pediatrics*. 1994;93:131–134
26. Wright PW, Crutcher JE, Holiday DB. Selection of skin tests to evaluate PPD anergy. *J Fam Pract*. 1995;41:59–64
27. Johnson JL, Nyole S, Shepardson L, et al. Simultaneous comparison of two commercial tuberculin skin test reagents in an area with a high prevalence of tuberculosis. *J Infect Dis*. 1995;171:1066–1067
28. Raszka W, Moriarty R, Ottolini M, et. al. Delayed-type hypersensitivity skin testing in human immunodeficiency virus-infected pediatric patients. *J Pediatr*. 1996;129:245–250
29. Joncas JH, Robitaille R, Gauthier T. Interpretation of the PPD skin test in BCG-vaccinated children. *Can Med Assoc J*. 1975;113:127–128
30. Stoff H, Patel A, Sutherland I, et al. The risk of tuberculosis infection in Uganda, derived from the findings of national tuberculin surveys in 1958 and 1970. *Tubercle*. 1973;34:1–22
31. World Health Organization. *Global Tuberculosis Control: WHO Report*. Geneva, Switzerland: World Health Organization, Global Tuberculosis Programme; 1997:225
32. Tsai W, Nara P, Kung H, Oroszlan S. Inhibition of human immunodeficiency virus infectivity by chloroquine. *AIDS Res Hum Retroviruses*. 1990;6:481–489
33. Sperber K, Louie M, Kraus T, et.al. Hydroxychloroquine treatment of patients with human immunodeficiency virus type 1. *Clin Ther*. 1995; 17:622–636
34. Centers for Disease Control and Prevention. Anergy skin testing and preventive therapy for HIV-infected persons: revised recommendations. *MMWR*. 1997;46:5–6

Downloaded from www.aappublications.org/news by guest on September 18, 2021

Human Immunodeficiency Virus Status and Delayed-Type Hypersensitivity Skin Testing in Ugandan Children

Anna Maria Mandalakas, Laura Guay, Philippa Musoke, Cindie Carroll-Pankhurst and Karen N. Olness

Pediatrics 1999;103;e21
DOI: 10.1542/peds.103.2.e21

Updated Information & Services	including high resolution figures, can be found at: http://pediatrics.aappublications.org/content/103/2/e21
References	This article cites 28 articles, 3 of which you can access for free at: http://pediatrics.aappublications.org/content/103/2/e21#BIBL
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): **Infectious Disease** http://www.aappublications.org/cgi/collection/infectious_diseases_sub **HIV/AIDS** http://www.aappublications.org/cgi/collection/hiv:aids_sub **International Child Health** http://www.aappublications.org/cgi/collection/international_child_health_sub
Permissions & Licensing	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: http://www.aappublications.org/site/misc/Permissions.xhtml
Reprints	Information about ordering reprints can be found online: http://www.aappublications.org/site/misc/reprints.xhtml

American Academy of Pediatrics
DEDICATED TO THE HEALTH OF ALL CHILDREN®

Downloaded from www.aappublications.org/news by guest on September 18, 2021

PEDIATRICS®
OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

Human Immunodeficiency Virus Status and Delayed-Type Hypersensitivity Skin Testing in Ugandan Children

Anna Maria Mandalakas, Laura Guay, Philippa Musoke, Cindie Carroll-Pankhurst and Karen N. Olness

Pediatrics 1999;103;e21
DOI: 10.1542/peds.103.2.e21

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://pediatrics.aappublications.org/content/103/2/e21

Pediatrics is the official journal of the American Academy of Pediatrics. A monthly publication, it has been published continuously since 1948. Pediatrics is owned, published, and trademarked by the American Academy of Pediatrics, 345 Park Avenue, Itasca, Illinois, 60143. Copyright © 1999 by the American Academy of Pediatrics. All rights reserved. Print ISSN: 1073-0397.

American Academy of Pediatrics
DEDICATED TO THE HEALTH OF ALL CHILDREN®

Downloaded from www.aappublications.org/news by guest on September 18, 2021