Enzyme Immunoassay for Detection of Human Immunodeficiency Virus-Specific Immunoglobulin A Antibodies

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Early diagnosis of human immunodeficiency virus (HIV) infection may be difficult in adults with acute or recent HIV infection and in infants with perinatally acquired HIV. Detection of HIV-specific immunoglobulin A (IgA) antibodies in infant serum by Western blot (immunoblot) has been suggested as a reliable method to identify HIV-infected infants, especially those over the age of 6 months, and as an adjunct to diagnosis of acute HIV infection in adults. We developed a simple enzyme immunoassay for detection of HIV-specific IgA, using standard commercially available reagents. Enzyme immunoassay was comparable to Western blot for detection of HIV-specific IgA in sera from adults ($n = 216$), older children ($n = 49$), and infants born to HIV-infected mothers ($n = 65$). Specificity was 100% and sensitivity ranged from 80 to 92%. IgA-enzyme immunoassay is a simple, highly sensitive method for detection of HIV-specific IgA antibodies and is easily adapted to the standard clinical laboratory.

A number of therapeutic interventions have been shown to be effective in improving the quality and prolonging the duration of life for persons infected with human immunodeficiency virus (HIV) (5, 6, 8, 11, 17, 18, 21). Prophylaxis for *Pneumocystis carinii* pneumonia and zidovudine treatment are now routinely recommended for asymptomatic HIV-infected patients with significant immunodeficiency as well as for symptomatic patients. Optimal clinical application of early intervention strategies is, however, dependent on the availability of accurate methods for early HIV diagnosis.

Although many infants and young children may be eligible to receive early interventions, making a definitive diagnosis of HIV infection by standard immunoglobulin G (IgG)-based serologic testing has been problematic during the first years of life because of the presence of transplacental maternal IgG antibodies. While standard enzyme immunoassay (EIA) for IgG is sufficient to establish the diagnosis of HIV infection in adults, patients recently infected and those with acute HIV syndrome may produce IgA and IgM antibodies initially and have false-negative IgG tests.

A number of significant advances have been made in the early diagnosis of HIV infection in infants. Direct viral detection methods, including HIV culture and polymerase chain reaction (PCR), appear to be highly sensitive (>95% after 3 to 6 months of age) and specific (7, 9, 10, 12-14, 20). However, both of these tests are expensive, neither is widely available, and both require considerable expertise and quality control for optimal clinical use. In addition, it is doubtful that these technologies will be readily adaptable to settings in developing countries, where perinatal HIV infection has become a problem of enormous proportion.

Western blot (immunoblot) methods for detection of anti-HIV IgA antibody have been studied as a tool in early diagnosis of HIV infection in infants and as means of

detecting HIV infection in recently infected blood donors (1, 7, 12, 13, 15, 16, 19, 20, 22-24). Since IgA antibodies do not cross the placenta, detection of specific anti-HIV antibodies of the IgA class signifies infection in the child. Among infants of >6 months of age, the sensitivity of IgA antibody detection appears to be between 77 and 97% (15, 19). While these Western blot techniques hold promise, reagents are expensive and the procedures are time-consuming. Widespread application of the test requires the development of a simple format that can be readily utilized by standard clinical laboratories. We have developed a simple EIA for detection of HIV-specific IgA antibodies, using commercially available reagents. Reported here are the results of a comparison of this new EIA with Western blot, using serum samples obtained from infants (>6 months of age) who were born to HIV-infected mothers as well as samples from older children and adults.

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MATERIALS AND METHODS

Patients and serum samples. Stored (-70°C) serum samples from 330 HIV-infected and uninfected adults and children were tested for the presence of anti-HIV IgA antibodies. Adult samples were randomly chosen from among those obtained from participants in a longitudinal study of heterosexual transmission of HIV and stored in a serum repository. Infant samples were obtained from a longitudinal study of infants born to HIV-infected mothers, and samples from older children were randomly selected from a serum repository obtained from children with HIV infection followed at the Children's Hospital AIDS Program in Newark, N.J.

Participants were classified as HIV infected by Centers for Disease Control criteria (2, 3). EIA and Western blot were performed on all samples for detection of anti-HIV IgG,

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TABLE 1. Characteristics of patients whose sera were tested for IgA antibodies against HIV

Patient group	No.	Mean age in yr (range)	Male/female	No. (%) of given race/ethnicity				No. (%) with given HIV status	
				Black	White	Hispanic	Other	Infected	Uninfected
Adults	216	35.6 (21–68)	1.2	55 (26)	101 (47)	53 (24)	7 (3)	134 (62)	82 (38)
Older children	49	5.7 (0.75–17)	1.4	25 (51)	17 (35)	5 (10)	2 (4)	25 (51) ^a	24 (49)
Infants born to HIV-infected mothers	65	0.75 (0.5–1.5)	1.1	60 (92)	1 (2)	4 (6)		12 (18) ^b	53 (82)

^a Centers for Disease Control classification: 2/49 P1, 23/49 P2 (18 AIDS).^b Centers for Disease Control classification: 7/12 P1, 5/12 P2.

using commercially available reagents (Abbott Laboratories, Chicago, Ill.), and results were interpreted by using previously published criteria (4).

Detection of HIV-specific IgA. Each serum sample was tested in a blinded manner for the presence of HIV-specific IgA, using both Western blot and EIA. Prior to each assay, serum was processed (three times) to deplete IgG by using the Quick-Sep IgM:rProtein G affinity method (IsoLab Inc., Akron, Ohio) as described previously (22). Fifty microliters of serum was added to the prefilled tube, which was placed on an orbital shaker at room temperature for 30 min. For the second and third repetitions, the buffer from the prefilled tubes was removed to reduce the amount of serum dilution. The final dilution of absorbed serum was 1:10. A subset of the adult serum samples was also tested for IgA by EIA, using unabsorbed serum for comparison.

Western blot for detection of HIV-specific IgA was performed by using a modification of a commercially available HIV-1 immunoblot kit (Bio-Rad Laboratories, Hercules, Calif.), with an affinity-isolated anti-human IgA conjugate (1:2,000 dilution; TAGO Inc., Burlingame, Calif.) substituted for the anti-human IgG conjugate provided with the kit. Blots with p24, gp41, gp120, or gp160 bands appearing on the strips were considered positive (22).

Detection of anti-HIV IgA by EIA was performed by using a modification of a commercially available IgG kit (HIVAB HIV-1 kit; Abbott Laboratories), with peroxidase-conjugated goat anti-human IgA (Protos Immunoresearch, San Francisco, Calif.) substituted for the peroxidase-conjugated anti-human IgG supplied with the kit. The anti-human IgA conjugate was diluted 1:2,500 with Tris-buffered saline (pH 7.5) containing 8% bovine serum albumin. The final dilution of the test serum sample was 1:100. Each IgA EIA was run with 5 to 10 wells of positive and negative control samples. The EIA cutoff value was determined by calculating the

average optical density (OD) of absorbed sera from 66 HIV-uninfected adult patients plus 3 standard deviations.

Lymphocyte phenotyping. Lymphocyte phenotyping was performed by standard whole-blood analysis in an AIDS Clinical Trials Group-certified laboratory.

Statistical analysis. IgA-EIA results (positive or negative) with sera from adult patients were assessed in relation to CD4 lymphocyte count and CD4 lymphocyte percent, using logistic regression analysis.

RESULTS

Serum samples from 330 individuals were tested for the presence of anti-HIV IgA antibodies by both Western blot and EIA. A single sample was tested for each patient. There were 216 adults, 49 older children (mean age, 8.1 years), and 65 infants (age, 6 to 15 months) who were born to HIV-infected mothers. The characteristics of these patients are given in Table 1.

All sera from HIV-infected older children and adults and sera from infants born to HIV-infected mothers were positive for anti-HIV IgG antibodies by standard EIA and Western blot before absorption with rProtein G. Results of IgA antibody testing are shown in Table 2. Of 171 HIV-infected persons included in this study, 130 (76%) had detectable anti-HIV IgA antibodies by previously published Western blot methodology and 151 (88%) had detectable anti-HIV IgA antibodies by EIA. All uninfected individuals were negative for anti-HIV IgA antibodies by both methods.

Across all age groups, EIA compared favorably to Western blot for detection of anti-HIV IgA antibodies. While both Western blot and EIA were highly specific for HIV infection, the sensitivity of these tests in the whole group was only modest (76 and 88%, respectively). Western blot for IgA was negative in approximately one-quarter of all HIV-infected

TABLE 2. Sensitivity, specificity, and positive (PPV) and negative predictive (NPV) values of Western blot (WB) and EIA for detection of anti-HIV-specific IgA^a

Patient group (n)	Test	No. of infected pts	No. of infected pts with positive test	% Sensitivity (95% CI)	No. of uninfected pts	No. of uninfected pts with positive test	% Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Adults and older children (265)	WB	159	120	75 (69.8–80.2)	160	0	100 (97.0–100)	100 (97.0–100)	73 (67.7–78.3)
	EIA		140	88 (84.1–91.9)		0	100 (97.0–100)	100 (97.0–100)	85 (80.7–89.3)
Infants (65)	WB	12	10	83 (73.9–92.0)	53	0	100 (94.5–100)	100 (94.5–100)	96 (91.2–100)
	EIA		11	92 (85.4–98.6)		0	100 (94.5–100)	100 (94.5–100)	98 (94.6–100)
Total (330)	WB	171	130	76 (71.4–80.6)	159	0	100 (97.0–100)	100 (97.0–100)	79 (74.6–83.4)
	EIA		151	88 (84.5–91.5)		0	100 (97.0–100)	100 (97.0–100)	89 (85.6–92.4)

^a pts, patients; 95% CI, 95% confidence interval.

patients; EIA was negative in 12%. A total of 21 samples from HIV-infected patients were positive for IgA antibodies by EIA but negative by Western blot. These sera were tested (following absorption) for HIV-specific IgG. Nineteen (90%) had no detectable IgG, suggesting that the positive EIA was not the result of a nonspecific reaction due to remaining IgG antibodies.

IgG depletion appears to be an important step in the EIA method, as is the case with Western blot methods for HIV-specific IgA. A total of 111 serum samples from HIV-infected adults and 66 samples from uninfected adults were tested by IgA-EIA without IgG absorption, and results were compared with those from serum depleted of IgG. Absorption was necessary to optimally discriminate HIV-infected from uninfected patients. For unabsorbed serum samples, there was considerable overlap of OD readings between patients who were HIV infected and HIV-uninfected controls, with the mean OD (0.01) for the latter approximating the OD cutoff value (0.11).

CD4 lymphocyte parameters were used to assess the relationship between degree of immunosuppression and the finding of false-negative results on EIA. Results of CD4 lymphocyte phenotyping from the same day as serum was collected for HIV-specific IgA studies were available for 131 of the 134 HIV-infected adult patients. Neither CD4 lymphocyte count ($P = 0.66$) nor CD4 lymphocyte percent ($P = 0.83$) was a significant predictor of IgA-EIA results.

HIV-exposed infants. Sera from 65 infants who were born to HIV-infected mothers were studied. Serum was obtained from these infants between ages 6 and 15 months (mean age, 9 months). All were positive for anti-HIV IgG antibodies by EIA and Western blot at that time. All 65 infants were followed beyond 18 months of age, and 12 (18%) had persistently positive EIAs and Western blots for IgG antibodies and a positive culture for HIV and/or repeatedly positive PCR (HIV infected). Fifty-three infants (82%) had repeatedly nonreactive EIAs and Western blots for anti-HIV IgG on serial evaluations after their initial positive values and negative HIV cultures and PCRs; they were clinically well (HIV uninfected).

EIA was comparable to Western blot for detection of anti-HIV IgA among HIV-exposed infants. On the other hand, both Western blot and EIA failed to identify a few truly infected infants. The sensitivities of Western blot and EIA were 83 and 92%, respectively. Of the 12 children with proven HIV infection, 10 had detectable IgA antibodies by both methods. One child was positive for IgA by EIA but negative by Western blot and one child was negative by both assays (at 8 months of age). Specificity for both Western blot and EIA for IgA was 100%; none of the true negatives tested positive.

DISCUSSION

Detection of HIV-specific IgA antibodies by EIA is highly specific (100%) for HIV infection in all age groups, including HIV-exposed infants 6 to 15 months of age. The test is simple and less expensive than standard Western blot methodology and can easily be adapted to the clinical laboratory setting. Overall, the sensitivity of this test was 88%.

The primary application of EIA-IgA is to infants born to infected mothers; the sensitivity appears to be high enough to make it potentially helpful in identifying HIV-infected infants. However, EIA-IgA may also be useful in specific circumstances in adults. Since IgA and IgM responses may occur early in the course of HIV seroconversion prior to the detection of IgG antibody, their detection could be a useful

adjunct to diagnosis of acute or early HIV infection in adults. For example, recent studies have suggested that IgA detection may be useful in improving the sensitivity of tests used in blood banks by identification of infection among recently infected blood donors with weakly reactive or false-negative IgG EIA results (23). EIA-IgA may be useful for examination of adult samples with low reactivity in EIA-IgG tests and indeterminate Western blot results.

Weiblen and associates at the Massachusetts Department of Public Health developed and tested immunoblot methods for HIV-specific IgA detection, using infant serum that had been depleted of interfering IgG by absorption with recombinant protein G (22, 24). Detection of specific IgA antibodies by immunoblot was highly specific for HIV infection, but sensitivity was age dependent, with the highest sensitivity in infants of >12 months of age. Subsequent studies have confirmed the utility of IgA detection in HIV-exposed infants (15, 19). Rare, if any, false-positive results have been identified. The sensitivity of the test appears low (6 to 17%) in the newborn period but increases to approximately 80 to 97% after 6 months of age.

Studies performed to date have utilized immunoblot as the standard methodology for detection of HIV-specific IgA antibodies (15, 16, 19, 22, 23). Compared with Western blot, EIA is less time-consuming and considerably less expensive and requires less technical expertise. The IgA-EIA described here utilizes standard commercially available reagents and equipment and can be performed in standard clinical laboratories. The estimated total cost of EIA (including labor and absorption steps) is approximately \$11.00, about one-third of the cost of the IgA Western blot. Recently, a dot blot method which also reduces the cost and complexity of IgA detection has been developed by Martin et al. (16).

Early reports of EIA for use in IgA detection were disappointing. Weiblen et al. reported previously on experience with the use of a recombinant gp160-based EIA and found that this assay was less sensitive than Western blot (22). In contrast, the EIA described here, using commercially available reagents, was more sensitive than standard Western blot methods (88 versus 76%). This may be related to differences in the antigens used. According to the manufacturer, primarily anti-p24 and anti-gp41 antibodies are detected by the commercial kits adapted for our assay. In this initial study, we chose to include only infants who were over the age of 6 months, since IgA detection by Western blot is known to have low sensitivity below this age. Further studies are needed in larger numbers of children and in children of younger age to assess the IgA-EIA method, since overall its sensitivity appears to be greater than that of the Western blot.

The primary disadvantage of the EIA-IgA methodology is the need to deplete the samples of IgG antibodies, as required for Western blot testing (24). Absorption of IgG has been an important step when commercially available blotting reagents are used to reduce interference (cross-reactivity) by IgG (24). In our EIA, optimal resolution of positive and negative samples also required IgG absorption. This, however, adds time to sample preparation and processing. For the comparative study reported here, sera were absorbed three times to assure high-level depletion of IgG from the sample. Preliminary data from studies performed recently in our laboratory suggest that there is little difference in EIA OD readings following one, two, or three absorptions. Ongoing studies of the clinical application of IgA-EIA in our laboratory currently use only a single absorption step. Mar-

tin et al., using a dot blot with a mixture of recombinant proteins (gp160, gp120, p66, and p24), suggest that this method can be performed without absorption as a screening test but that confirmation of positive results, using IgG depleted serum, is still necessary (16).

The relatively low cost and rapidity with which IgA results are available make this test a potentially useful adjunct to early diagnosis of HIV infection, especially for infants of >6 months of age. However, despite refinements in methodology, the sensitivity of IgA detection in HIV infection remains below 90%. Thus, a negative result does not exclude the diagnosis of HIV, and further testing or follow-up is necessary. IgA-EIA may be useful as a screening test for HIV-exposed infants, with additional virus-specific tests utilized as confirmation of infection. Considering the cost of HIV culture and PCR, IgA-EIA may be a useful alternative in some settings (such as developing countries), where HIV culture and PCR are not available. This approach would reduce the age at which a diagnosis can be made serologically from 18 months to approximately 6 months for the majority of infants.

One proposed explanation for the fact that approximately 10% or more of HIV-infected patients have negative IgA antibody tests is the failure of patients with advanced immunodeficiency to mount an IgA antibody response. In our study, among 131 HIV-infected adult patients, there was no significant relationship between CD4 lymphocyte count and negative results on HIV-IgA-EIA. Among infants born to HIV-infected mothers studied by Landesman et al., there was no difference in the ability of the HIV-IgA immunoblot assay to identify asymptomatic individuals versus symptomatic individuals, but the sample size was small (15).

EIA appears to be a useful and perhaps better alternative to Western blot for detection of HIV-specific IgA in all age groups. Additional studies are necessary to confirm our findings, and larger groups of infants need to be studied to validate the sensitivity and specificity of this assay. Further longitudinal studies are warranted to assess the clinical application of IgA-EIA for early diagnosis of HIV infection in infants born to HIV-infected mothers and adults with acute or early HIV infection.

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