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Diagnosis of Human Immunodeficiency Virus Infection Using an Immunoglobulin E-Based Assay

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Immunoglobulin assays that are sensitive and specific for detecting human immunodeficiency virus type 1 (HIV-1) infection are especially important in developing countries where PCR and viral culture may not be readily available. Immunoglobulin E (IgE), which is elevated in HIV-1 infection, is the only antibody that does not cross the placenta, making it potentially valuable for viral detection in both children and adults. This study developed an assay for detection of HIV specific IgE antibodies in adults. A total of 170 serum samples from 170 adults (116 HIV positive and 54 HIV negative) were analyzed. Serum or plasma samples were treated by using the protein G affinity method. The HIV status was determined by using two IgG enzyme-linked immunosorbent assays (ELISAs) and one Western blot evaluation. The IgE enzyme immunoassay test for HIV-1 correctly identified the HIV status in 98.8% of the samples (168 of 170). One false-positive and one false-negative test occurred with the IgE ELISA, as well as with the IgG ELISA test but were correctly identified by the IgE test. Analysis of the data demonstrated a high specificity (99%) and sensitivity (99%) of the IgE test, with 95% confidence intervals. The IgE assay appears to be sensitive and specific, suggesting that IgE-specific antibodies offer an effective method to detect HIV-1 infection in adults.

Reliable and inexpensive tests for human immunodeficiency virus type 1 (HIV-1) detection that can be used in both adults and children are especially important in many developing countries, where PCR and viral culture are not feasible or readily available. The immunoglobulin G (IgG)-based enzyme-linked immunosorbent assay (ELISA) antibody test remains a highly reliable method for establishing HIV infection in adults and older children. Because maternal IgG antibody to HIV is transmitted across the placenta, however, its application in infants is limited.

IgE does not cross the placenta and may provide a method for HIV-1 detection in young children and adults. The potential advantage of an IgE antibody test in HIV disease is supported by previous findings demonstrating specific IgE directed to infectious agents. Certain viral infections are known to produce specific IgE antibodies, to the extent that significant changes in the level of total serum IgE may occur (1, 14, 15, 16, 18, 20, 21, 22, 26). Of importance, during the early stages of HIV-1 disease a significant elevation of total IgE has been reported in children (7, 24, 25). Our earlier studies in HIV-1-infected adults indicate that total IgE is also increased during the early stages of disease, and this elevation appears to be independent of CD4 counts and is not correlated with the levels of other immunoglobulins (13, 20, 29). During later disease stages, the amount of serum IgE in infected individuals appears to parallel the severity of HIV disease and is correlated with a decrease in CD4 lymphocytes (21), suggesting an important role for IgE as a surrogate marker of disease progression (24, 25, 29).

The present study was designed to determine whether IgE-specific antibody to HIV is present in adults and to evaluate its efficacy as a test for the diagnosis of HIV-1 infection. In a simultaneous investigation, we evaluated the presence of IgE-specific antibody to HIV and performed an IgE-based assay for early detection of HIV-1 infection in infants and young children (14).

MATERIALS AND METHODS

Subject samples. A total of 170 serum samples was collected between 1987 and 1993 from HIV-1-infected (n = 116) and HIV-1-seronegative (n = 54) adults being monitored at the University of Miami School of Medicine. All samples were tested in the E. M. Papper Laboratory of Clinical Immunology by using duplicates, and the laboratory investigator was blinded as to the infection status. Blood specimens were collected, and serum or plasma samples were separated and stored at −20°F until used for the analyses.

HIV serostatus determination. All sera were initially screened for HIV-1 IgG antibody by ELISA (Coulter Immunology, Hialeah, Fla.). Repeatedly reactive samples were confirmed by Western blot (Biotech Corp., Rockeville, Md.). Western blots were evaluated according to U.S. Department of Defense (DOD) criteria that conform to the Association of State and Territorial Public Health Laboratory Directors Standards (4, 8). DOD criteria for a positive Western blot are the presence of at least two of the following three major HIV protein bands: gp41, p24, and gp120-160. By DOD standards, Western blots are classified as indeterminate when any bands are present that do not meet the criteria for a positive test.

For the evaluation of HIV-1 infection, the reference standard was either a repeatedly negative ELISA screening assay or a positive Western blot test. The indeterminate specimens were considered negative since most low-risk individuals with sera containing only HIV-1 core antigens, other than p24, are rarely infected or have seroconverted (4, 8).

IgE testing. Samples of serum or plasma were pretreated by the protein G affinity method (rProtein G Affinity Method; Isolab, Inc., Akron, Ohio). Briefly, after the sample was added to the resin tube and incubated for 10 min, a special disk was then inserted into the tube and pressed down to compress the resin bed,

TABLE 1. Detection of HIV-1-specific IgE and IgG antibodies by Western blot

HIV status (n)	No. of sera		No. of sera	
	IgE HIV (+)	IgE HIV (−)	IgG HIV (+)	IgG HIV (−)
Positive (116)	115	1	115	3
Negative (54)	1	53	1	51

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TABLE 2. Detection of HIV-1-specific IgE and IgG antibodies by HIV assay[a]

Immunoglobulin	% Sensitivity	% Specificity	Positive predictive value	Negative predictive value	Accuracy
IgE	0.991 (0.954–0.999)	0.985 (0.874–0.999)	0.991 (0.952–0.999)	0.982 (0.901–0.999)	0.99
IgG	0.991 (0.954–0.999)	0.944 (0.846–0.988)[b]	0.975 (0.928–0.995)	0.944 (0.846–0.988)[b]	0.97

[a] 95% confidence intervals are given in parentheses.
[b] Comparison of specificity and accuracy between IgE and IgG HIV assay ($P = 0.06$).

and the subsequent supernatant was used for testing. The HIV test was performed by using reagents from the EIA test kit for detection of antibody to HIV-1 (Coulter Immunology). Each well had been coated with HIV-1 and HIV-2 peptides (p53, p24, gp120-160, and p46). The plates were incubated at room temperature with the resin-treated serum (diluted 1/50) for 30 min and then aspirated and washed five times with 300 μl of wash solutions per well. Horseradish peroxidase-conjugated with anti-human IgE was added (Incstar Corp.), and the mixture was incubated for an additional 30 min at room temperature. Plates were reaspirated and rewashed five times with 300 μl of wash solutions per well. Then, 100 μl of fresh substrate solution was added to each well, followed by incubation at room temperature. The reaction was stopped by adding 100 μl of stop solution (1 N H_2SO_4) to each well. The plate was read with bichromatic absorbance at 492 with 620 as a reference marker.

Controls for the specificity of this assay included a blank with only conjugate and substrate added, one serum from an HIV-seronegative individual, and the serum of one known HIV-1-infected person. The effectiveness of the HIV-specific IgG depletion in rProtein G-treated specimens was used as an additional control in each assay. Intraassay precision was determined by comparing 10 replicates of each sample in one assay. Interassay precision was determined by comparing the same 10 samples assayed in five different runs.

Statistical method. Statistical analyses were performed by using SAS software (19), following the examination of distribution, skewness, and presence of outliers. The specific IgE sensitivity, specificity, and predictive value with 95% confidence intervals were computed according to StatXact 4 Windows methodology (Cytel Software Corp., Cambridge, Mass.) by using one serum or plasma sample from each subject. The relative spread of distributions for the inter- and intraassay precision was evaluated by using the coefficient of variation defined as 100% standard deviations/mean. Comparisons of the IgG and IgE analyses for sensitivity, specificity, predictability, and accuracy were evaluated with the Fisher's exact test.

RESULTS

Detection of HIV-1-specific IgE antibody. A total of 170 serum samples from adults were assessed for IgE anti HIV-1 by the enzyme immunoassay (EIA) test. The HIV IgE EIA correctly identified the infection status in 168 (98%) of the specimens. As indicated in Table 1, 115 of the 116 HIV-1-infected adults were identified as HIV IgE and IgG seropositive. One false-negative result occurred, with both the IgE and the conventional ELISA, in an HIV-1-infected adult with a CD4 cell count of less than 50/mm^3.

In the seronegative individuals, 98% of the samples (53 of 54) were correctly identified as HIV IgE seronegative. The false-positive test with both IgE and IgG tests occurred in a single person, who had a positive p24 band in the Western blot. As shown in Table 1, two additional false-positive results occurred with the conventional IgG ELISA test, but these were correctly identified by the IgE test.

The specificity, sensitivity, and predictive values were calculated based on the total group results with 95% confidence intervals and with the Western blot results. The sensitivity and specificity of the IgE test were both 99%. As indicated in Table 2, the specificity and accuracy of the IgE EIA test, compared to the IgG test, tended to be greater ($P = 0.06$). For HIV-1 antibody sensitivity values, both tests were similar.

The performance characteristics of the IgE assay demonstrate nonspecific reactions, i.e., reactivity of the control conjugates. The effectiveness of the HIV-specific IgG depletion in rProtein G-treated specimens was demonstrated in each assay by an absorbance below the value of the negative controls. IgG recovery was less than 1% in the pretreated samples, as has been reported previously (27). In contrast, 88% recovery of IgE was obtained from the samples during the first three runs of the IgE assay. Ten samples were used to evaluate the inter- and intraassay precision. Analyses revealed an interassay variation of less than 7.6 and an intraassay variation of less than 3.1. Table 3 provides specific inter- and intraassay results for a portion of the samples. The accuracy of the IgE assay was 99% (168 of 170).

DISCUSSION

In this study, an immunoglobulin-based assay was developed for the detection of HIV-1-specific IgE antibody. The test was highly sensitive and specific, suggesting that detection of IgE antibody to HIV-1 may be an effective method for the diagnosis of HIV status. The new assay is simple to perform and requires only small amounts of nonhemolyzed serum or plasma. The IgE response is rapid and reaches a peak earlier than the IgG antibodies, suggesting that an IgE-based assay may detect seroconversion earlier than the conventional method. The high sensitivity of the IgE assay is in accord with other reports showing that immunoassays based on IgE antibodies directed to infectious agents are at least as specific and sensitive as those based on other immunoglobulin antibody responses (1, 14, 15, 16, 18, 22, 26). Of particular advantage to laboratories in developing countries, the IgE test can be rapidly performed without complex laboratory equipment and can be run at room temperature, and the same technology may be used for both children and adults (14).

The IgE assay was associated with a high level of accuracy and precision. The reactivity of specific IgE antibodies, expressed as absorbance in our study, was greater than the specific IgG response in approximately 70% of the positive sera from HIV-1-infected individuals. The higher specific reactivity in the IgE assay may be due to background differences in the assays, as well as a larger increase in total IgE (3-fold) than in total IgG (1.4-fold) antibodies (13, 22, 26), probably reflecting higher amounts of specific antibodies. Nonreactivity against the control conjugate and depleted IgG samples during our study suggest nonspecific reactions with the IgE assay and non-cross-reactivity with IgG antibodies. This is consistent with studies based on IgE antibodies for the diagnosis of cytomegalovirus infection (15, 22, 26). The plates used in this assay contained both HIV-1 and HIV-2 peptides. Although the

TABLE 3. Performance characteristics of the IgE assay[a]

Sample	Interassay results		Intra-assay results	
	Mean ± SD	CV (%)	Mean ± SD	CV (%)
1	191 ± 5.22	2.7	192 ± 2.83	1.5
2	742 ± 32.3	4.0	756 ± 19.8	2.6
3	757 ± 15.6	2.1	768 ± 14.1	1.8
4	99.2 ± 7.53	7.6	97 ± 2.83	2.9
5	958 ± 13.4	1.4	936 ± 29.7	3.1

[a] Specific results are shown for a portion of the randomly selected samples. CV, coefficient of variation.

adults were not tested for HIV-2 infection, it should be noted that the incidence of HIV-2 in the U.S. population, including drug abusers, remains quite low (6, 23).

In agreement with previous studies demonstrating total IgE elevation in HIV-1-infected individuals (7, 13, 20, 21, 24, 25, 29), our findings detected IgE-specific antibody response in all of the sera from 115 of 116 HIV-1-infected adults. Whereas IgE elevation has been associated with T-cell dysfunction and a hypergammaglobulinemia phenomenon, the precise cause of IgE elevation during the early stages of HIV disease has not been totally elucidated. The present results suggest that elevation of circulating IgE levels may be due, at least in part, to specific IgE directed to the HIV virus rather than as a result of a nonspecific phenomenon. In support of this proposal, earlier studies have demonstrated specific IgE directed to bacteria and viruses as well as to parasites (1, 15, 16, 18, 22).

Tests for the detection of HIV-1 infection that do not require the complex technology of viral culture or PCR are generally unavailable for adults and children in less-developed countries. Since maternal IgE does not cross the placenta and IgE is produced before any other immunoglobulin in the fetus and in adults, an IgE-based assay may be of particular importance in providing early detection of HIV-1 infection in infants (14) and acute infection in adults.

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