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Immunofluorescence Assay for Detection of Antibodies to Human Immunodeficiency Virus Type 2

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A total of 215 serum samples were tested for antibodies against human immunodeficiency virus type 2 (HIV-2) with an immunofluorescence assay (IFA). Some samples originated from Denmark and some originated from Guinea-Bissau. The IFA results were compared with enzyme-linked immunosorbent assay (ELISA) and Western (immuno-) blot (WB) results. Twenty-nine serum samples were found to be true positive for HIV-2 antibodies as judged from WB and radioimmunoprecipitation results; all of these were also found to be positive in the HIV-2 IFA. Of 80 serum samples originating from HIV-1-infected persons, 60% showed reactivity in the HIV-2 ELISA, and 51% cross-reacted with at least one band in the HIV-2 WB. None of the sera cross-reacted in the HIV-2 IFA. A total of five serum samples (three African and two Danish) gave unspecific results in the HIV-2 IFA. It is concluded that the HIV-2 IFA is more specific and at least as sensitive as a first-generation ELISA and that IFA is superior to WB in discriminating between HIV-1 and HIV-2 infections.

The use of the immunofluorescence assay (IFA) for the detection of antibodies to human immunodeficiency virus type 1 (HIV-1) is well established (17, 18). IFA has been proposed to be useful both as a confirmatory test in connection with testing of sera from low-risk groups and as a screening test for high-risk groups with a limited number of serum samples to screen (1, 3, 7, 8, 11, 13, 20). With the discovery of the second HIV type, HIV-2, there is a need for an easy and specific assay for detection of antibodies to HIV-2.

A number of papers have described the use of first-generation enzyme-linked immunosorbent assays (ELISAs) and Western (immuno-) blot (WB) assays for detection of antibodies to HIV-2 and have pointed out the considerable degree of cross-reactivity with HIV-1-infected sera in these assays (4, 5, 10, 19).

This study was conducted to evaluate the usefulness of an IFA for the detection of antibodies against HIV-2 and to evaluate whether this assay could be useful in discriminating between HIV-1 and HIV-2 infections.

MATERIALS AND METHODS

Sera. Two panels of serum samples were tested. Panel 1 comprised 115 serum samples. Of these, 81 were collected from healthy (Centers for Disease Control group 2-3) HIV-1 antibody-positive Danish patients, and 24 were collected from a transfusion center in Guinea-Bissau; these samples were locally determined to be HIV-2 positive by ELISA. The blood donors were described as healthy. Ten serum samples originated from healthy HIV-2 antibody-positive women from Guinea-Bissau. The clinical statuses of the women were not further elucidated.

Panel 2 comprised 100 serum samples from different blood banks in Denmark. The samples were sent for confirmation in our laboratory and had all been tested positive for HIV-1 by different ELISAs. The sera were selected at random among samples tested negative in the confirmatory HIV-1 WB test.

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Indirect IFA. HIV-2 (LAV-2 ROD)-infected CEM cells and uninfected CEM cells were used as the antigen and control. (CEM cells and LAV-2 ROD were kindly provided by L. Montagnier, Institut Pasteur, Paris, France.)

Cells from each line were washed and suspended in phosphate-buffered saline at a concentration of 5×10^5/ml. Twenty-five microliters of the different cell suspensions was placed on glass slides, dried, and fixed with acetone. Twenty-five microliters of a 1:10 dilution of serum and phosphate-buffered saline with 1% bovine serum albumin was incubated for 30 min with the fixed cell smears and then washed. For detection of antibodies, 25 μl of fluorescein isothiocyanate (FITC)-labeled rabbit antibody to human immunoglobulin G diluted 1:50 (Dakopatt F 202) was added and incubated for 30 min. After being washed, the slides were mounted with glycerol buffer and cover slips and examined under a fluorescence microscope. Each serum was incubated with both virus-infected and noninfected cells. Sera were considered positive if they showed a clear green fluorescence on the infected cell line and no fluorescence on the noninfected cell line. If fluorescence was detected on both cell lines, the sera were considered unspecific. For further elucidation of unspecific results, endpoint titrations were done. Positive and negative control sera were tested with each slide. Reading was done blindly by two individuals.

For HIV-1 IFA, a human T-cell lymphotropic virus type III-infected clone of HUT 78 and an uninfected clone (clone 6D5, kindly provided by the Centers for Disease Control) were used. The procedure described for HIV-2 was followed.

ELISA. For HIV-2 antibody detection, the sera were tested by a commercially available ELISA from Pasteur Diagnostics, ELAVIA HIV-2.

For HIV-1 antibody detection, an in-house indirect ELISA using purified antigen was used. Details have been described previously (15).

WB assay. For confirmation of HIV-1 antibody-positive ELISA results of both panels 1 and 2, an in-house WB assay using commercially available antigen from Du Pont Co. (Wilmington, Del.) was used. Details have been described previously (12). Briefly, the viral antigen was subjected to

TABLE 1. ELISA, IFA, and WB results from panel 1

Panel 1 group[a] (no. of samples)	No. with ELISA result for:				No. with IFA result for:						No. with WB result for:					
	HIV-1		HIV-2		HIV-1			HIV-2			HIV-1			HIV-2		
	+	−	+	−	+	−	U[b]	+	−	U	+	−	D[c]	+	−	D[c]
1 (81)	81	0	48	33	80	1	0	1	79	1	81	0	0	5	39	37
2 (24)	11	13	24	0	0	24	0	18	3	3	0	14	10	18	6	0
3 (10)	6	4	10	0	0	10	0	10	0	0	0	6	4	10	0	0

[a] Group 1, HIV-1-infected Danish patients; group 2, blood donors from Guinea-Bissau; group 3, HIV-2-infected persons from Guinea-Bissau.
[b] U, Unspecified.
[c] D, Dubious; only p24 reactivity could be demonstrated.

sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 5 to 15% gradient gel. The proteins were electroblotted onto nitrocellulose sheets, which were cut into strips. Serum diluted 1:500 in Tris hydrochloride (pH 10.2)–0.5 M NaCl with 0.1% Tween 20 and 1% skim milk was incubated with the strips overnight. Rabbit anti-human immunoglobulin G was used as the conjugate (p214; Dakopatt, Copenhagen, Denmark). Finally, a color reaction was done.

According to the recommendations made by the Consortium for Retrovirus Serology Standardization (6), sera with reactivity with at least p24 in combination with gp41 and/or gp120/160 were considered positive. The in-house WB was evaluated according to the proficiency control program run by the Centers for Disease Control and correlates with the commercially available WB tests used by other laboratories.

For confirmation of HIV-2 antibody positivity of groups 2 and 3 in panel 2, a commercially available WB from Pasteur Diagnostics (LAV-BLOT-2) was used. The positivity criteria were in accordance with the instructions of the manufacturer.

For confirmation of HIV-2 antibody positivity of the rest of the sera, an in-house WB was performed. Density gradient-purified virus from the culture supernatant of the LAV-2 ROD-infected 6D5 clone of HUT 78 was used as the antigen. The immunoblot assay was run as described previously (12), except that a 10% polyacrylamide gel was used and enhancement with biotin avidin was performed. Sera reacting with p24 as well as with gp105/140 were considered positive. As a control, groups 2 and 3 from panel 1 were retested by the in-house WB for HIV-2; complete agreement with the LAV-BLOT-2 results was seen.

RESULTS

The ability of the HIV-2 IFA to identify sera from HIV-2-infected individuals was evaluated by examination of samples from 34 individuals from Guinea-Bissau with suspected or known HIV-2 infection. The results are shown in Table 1 (groups 2 and 3). The 10 samples from known HIV-2-infected persons were all positive in the HIV-2 IFA as well as in HIV-2 ELISA and WB. The 24 samples of group 2 from blood donors from Guinea-Bissau with positive HIV-2 ELISA screening results were also found to be positive in the HIV-2 ELISA applied in this study. Eighteen samples were positive in the HIV-2 IFA, while three samples were negative and three samples reacted nonspecifically. The 18 IFA-positive samples were likewise found to be positive in the HIV-2 WB, while the remaining 6 samples were negative.

The possible cross-reaction of sera from HIV-1-infected persons in the HIV-2 IFA was evaluated by examination of serum samples from 81 individuals from Denmark with previously diagnosed HIV-1 infection. The results were compared with those obtained by ELISA and WB and with the results from the corresponding assays for HIV-1 antibody detection. The results are shown in Table 1 (group 3). All sera were positive by ELISA as well as WB for HIV-1 antibodies. There was one serum sample which reacted negatively in the HIV-1 IFA, and this sample was also the only sample from group 1 which gave a positive reaction in the HIV-2 IFA. Further analysis by comparative radioimmunoprecipitation assay (kindly performed by L. Montagnier, Institut Pasteur, Paris, France) indicated that the serum was from an individual infected with HIV-2 rather than HIV-1. It was subsequently realized that the serum originated from an immigrant from the Ivory Coast, where HIV-2 infection is known to be endemic. Thus, none of the sera from the HIV-1-infected Danish individuals cross-reacted in the HIV-2 IFA. Of the remaining 80 HIV-1 antibody-positive serum samples, 47 (60%) gave a positive reaction in HIV-2 ELISA and 41 (51%) cross-reacted with at least one band in the HIV-2 WB. Four samples (5%) fulfilled the criteria for a positive HIV-2 WB reaction, although the antibody titers against HIV-2 envelope antigen were much lower than the HIV-1 envelope-specific antibody titers, as judged from the intensities of the WB bands.

Of the 24 donor serum samples from Guinea-Bissau, 18 could be confirmed positive by HIV-2 WB, which means that the total number of samples from HIV-2-infected persons was 29 (Table 1). Sixteen (55%) of these cross-reacted in the HIV-1 ELISA, and thirteen (45%) were also reactive in the HIV-1 WB, giving rise to dubious results because of cross-reaction with the HIV-1 p24 polypeptide. None of the sera from HIV-2-infected persons cross-reacted in the HIV-1 IFA.

To evaluate the specificity of the IFA method in comparison with the HIV-2 antibody ELISA, 100 serum samples from Danish blood donors were examined. These sera were selected from donor sera with nonspecific reactions in blood bank ELISA screening for HIV-1 antibodies. Only sera without bands in the confirmatory HIV-1 WB were included. Four samples were reactive in the HIV-2 ELISA, and these four samples were further analyzed by HIV-2 WB, in which three samples were negative and one specimen gave a dubious reaction (single p24 reaction). None of the sera were positive in the HIV-2 IFA, but the single HIV-2 WB-dubious sample reacted nonspecifically in HIV-2 IFA.

Further analyses by control cell preabsorption and parallel endpoint titration on HIV-2-infected and control cells of the five serum samples displaying nonspecific reactions in HIV-2 IFA did not indicate the presence of HIV-2-reactive antibodies in any of these sera.

DISCUSSION

Previous studies have shown that a significant number of HIV-1-positive sera cross-react in HIV-2 ELISAs (5, 7, 10,

19). These difficulties can to some extent be resolved by the use of second- or third-generation ELISAs (2, 9, 16) or the use of competitive ELISAs (19). In this study, we found 60% of the HIV-1-positive sera to be reactive in the HIV-2 ELISA, while only one sample tested positive in the HIV-2 IFA. This shows that the use of HIV-2 IFA as a screening test will resolve the problems due to cross-reactivity.

This study shows that not only the ELISA but also the WB assay has problems with cross-reactivity. Five of the serum samples originating from WB-confirmed HIV-1-positive persons showed reactivities with the core and envelope proteins when tested in the HIV-2 WB. Four of these showed only weak reactivities to the HIV-2 envelope proteins compared with the reactivities to the HIV-1 envelope proteins. However, the fifth serum sample was strongly reactive in both the HIV-1 WB and the HIV-2 WB. This serum sample was HIV-2 IFA positive but HIV-1 negative, and the results obtained by radioimmunoprecipitation assay showed that it originated from an HIV-2-infected person. This indicates that the HIV-2 IFA can be of value in the differential diagnosis between HIV-1 and HIV-2 infections in cases in which the WB assays show reactivity for both viruses.

The panel of Danish donor sera comprised samples with false-positive reactions in different HIV-1 ELISAs; therefore, these samples can be regarded as problem serum samples. The fact that only one sample reacted unspecifically in the HIV-2 IFA while four samples were nonspecifically reactive in the HIV-2 ELISA indicates that the HIV-2 IFA is very specific or at least more specific than the HIV-2 ELISA.

Of the 215 serum samples tested by HIV-2 IFA, 5 showed nonspecific reactions, and 2 of these presented a 24-kilodalton band in the HIV-2 WB. Control cell preabsorption and endpoint titration of the five serum samples did not indicate the presence of virus antibodies. However, low-titer virus-specific antibodies in samples with nonspecific reactions may not be detected in this kind of analysis if the titer of cell-specific antibodies is greater than the virus-specific antibody titer. This might be the case with the two IFA-unspecific and WB p24-reactive samples, which might be samples from persons in the process of early HIV-2 seroconversion. However, this is not likely to be the explanation, as these two serum samples originated from a Danish blood donor and an HIV-1-infected Danish male.

The high frequency of unspecific reactions in the African sera (10%) compared within the Danish sera (1%) is in accordance with results of previous studies (14, 21).

The results obtained with these two selected panels of sera do not allow exact estimates of the sensitivity and specificity of the HIV-2 IFA to be made. Nevertheless, our results indicate that the HIV-2 IFA is at least as sensitive and probably more specific than the first-generation ELISA for the detection of HIV-2 antibodies and that there is a very good correlation between the HIV-2 IFA and HIV-2 WB.

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