JOURNAL OF CLINICAL MICROBIOLOGY, June 1994, p. 1449–1456
0095-1137/94/$04.00+0

Vol. 32, No. 6

Enzyme Immunoassay Using Native Envelope Glycoprotein (gp160) for Detection of Human Immunodeficiency Virus Type 1 Antibodies

B. C. NAIR,[1]* G. FORD,[1]† V. S. KALYANARAMAN,[1] M. ZAFARI,[1]‡ C. FANG,[2]§ AND M. G. SARNGADHARAN[1]

Advanced BioScience Laboratories, Inc., Kensington, Maryland 20895,[1] and American Red Cross, Rockville, Maryland 20850[2]

Received 29 November 1993/Returned for modification 3 February 1994/Accepted 3 March 1994

An enzyme immunoassay using the purified native gp160 for the detection of human immunodeficiency virus type 1 (HIV-1) antibody was developed. This assay was determined to be highly specific, since (i) 157 serum samples that were confirmed negative by Western blot (immunoblot) (WB) were negative, (ii) 41 serum samples from populations with medical conditions that might cause nonspecific assay reactivity were all negative, and (iii) all 15 serum samples that showed false-positive reactions in one or more commercial HIV-1 screening tests were negative. The assay gave 100% specificity with a randomly selected and unlinked panel of 1,000 serum samples from healthy blood donors. The sensitivity of the assay was assessed by testing 238 samples confirmed as HIV-1 antibody positive by a standardized WB assay. All 238 serum samples (100%) were reactive in the native gp160 assay. In a dilution panel of 14 weakly WB-positive serum samples, 7 samples reacted two- to fivefold more strongly in the gp160 assay than in a virus lysate-based assay; the remaining 7 samples gave comparable reactivities in the two tests. The reactivities of 13 of these 14 serum samples in the gp160 assay were higher than in a commercial enzyme immunoassay that uses a recombinant envelope protein as the antigen. The native gp160 assay was more sensitive to identify seroconversion. In a well-characterized panel of sequential blood samples from a seroconverter, the new assay detected antibodies at least one sample ahead of the other commercial assays tested.

The identification of human immunodeficiency virus type 1 (HIV-1) and the establishment of a culture method for the continuous production of HIV-1 (6, 18, 37) have led to the development of a series of serological tests for the diagnosis of HIV-1 infection. These diagnostic tests employed solubilized virus harvested from infected-cell cultures as the antigen in enzyme immunoassays (EIAs) for the detection of HIV-1 antibody (10, 42). The major problem associated with the use of solubilized virus is the nonspecific reaction caused by the contaminating host antigens (23, 25, 31). Substituting the viral lysate with recombinant antigen or synthetic peptides in EIA has reduced the number of false-positive reactions, but such assays are limited to recognizing only antibodies directed against chosen portions of the HIV proteins (4, 11, 19, 29, 45, 49). Use of the entire molecule of antigen in its native configuration may be of great diagnostic importance, especially for the recognition of antibodies against the conformational epitopes which may be crucial in the early detection of the infection (1, 34, 40).

Antibodies to envelope glycoproteins (gp160, gp120, and gp41) of HIV constitute a major proportion of the humoral immune response in HIV-infected individuals (2, 30, 32), and the transmembrane portion of the envelope glycoprotein (gp41) has been identified as the antigen most consistently recognized by the serum antibodies (12, 15, 16, 20, 35, 41, 42). Since it is impractical to purify gp41 or its precursor polyprotein (gp160) from virus-infected cells in quantities required for serological screening tests (50), recombinant or synthetic peptides corresponding to highly antigenic regions of the envelope glycoproteins were used for the development of sensitive and specific tests for detecting HIV-1 antibodies (4, 19, 49). We have described a culture system capable of producing large quantities of native envelope proteins of HIV-1 by using a clone of the HUT78 cell line chronically infected with the HIV-1_{451} isolate (26). This cell line secreted unprocessed envelope glycoprotein, gp160, as well as mature gp120 into the extracellular medium. Under serum-free conditions, these cells released approximately five times the amount of viral glycoproteins released by cultures in normal medium. This facilitated the purification of gp160 and gp120 in large quantities (28). In this paper we report the development of a highly sensitive and specific EIA using gp160 as the antigen and demonstrate the distinct advantage of using the purified native antigen for HIV-1 serodiagnosis. This assay is not intended to detect HIV-2 antibodies.

MATERIALS AND METHODS

Serum specimens. The following groups of serum specimens were used to assess the diagnostic characteristics of the EIA.

(i) Three hundred ninety-five serum samples had been characterized by Western blot (immunoblot) analysis for their HIV-1 antibody status in a previous study (15). Two hundred thirty-eight of these samples were classified as HIV-1 antibody positive and 157 samples were classified as anti-HIV-1 negative on the basis of the Western blot analysis.

(ii) Forty-four plasma samples representing a variety of serological conditions that might interfere in specific antibody tests were provided under code by David Anderson of Food

* Corresponding author. Mailing address: Advanced BioScience Laboratories, Inc., 5510 Nicholson La., Kensington, MD 20895. Phone: (301) 881-5600. Fax: (301) 468-9466.
† Present address: Sienna Biotech, Inc., Columbia, MD 21046.
‡ Present address: Biogen, Inc., Cambridge, MA 02142.
§ Present address: Saliva Diagnostics Systems, Singapore.

and Drug Administration in an earlier study of the serological evaluation of screening tests for human T-cell leukemia virus type I (HTLV-1) (3). One of the samples was exhausted, and two were known to be positive for antibodies to HIV-1. These were excluded from the analysis, and the remaining 41 serum samples were analyzed in the present study. The sera were originally obtained from Boston Biomedica (BBI), Bridgewater, Mass., and they included sera from multiply transfused hospital patients, one grossly lipemic serum, one serum with anti-HLA-DR antibodies, sera reactive for rhumatoid factor, and sera with reactivities to *Toxoplasma gondii*, HTLV-I, cytomegalovirus, Epstein-Barr virus early antigen, hepatitis B surface antigen, and hepatitis B core antigen. There were sera reactive by the heterophile test and the Rapid Reagin test and sera with antinuclear antibodies.

(iii) Fifteen sera were selected from several blood bank collections for their persistent falsely positive reactions in one or more commercial HIV-1 screening tests. These sera were provided by Organon Teknika Corporation, Durham, N.C.

(iv) The following three groups of serum samples were collected during early seroconversion: BBI panels A and C (each of these panels consists of a longitudinal series of plasma samples collected from a single individual before, during, and after HIV-1 seroconversion [44]) and a panel of four sequential blood samples collected during acute viral meningitis and subsequent HIV-1 seroconversion (24).

(v) One serum sample reacted to p24 only in the Western blot assay, but the donor later became seropositive to additional HIV-1 antigens (sample obtained from Organon Teknika Corporation).

(vi) One thousand serum samples were from healthy blood donors and were collected at American Red Cross centers. These samples were randomly selected and unlinked.

Purification of native HIV-1 antigens. (i) gp160. Envelope glycoprotein gp160 was purified from the extracellular medium of the $6D5_{451}$ cell line, which is chronically infected with the HIV-1_{451} isolate, as described previously (28). Briefly, a series of anti-HIV-1 immunoaffinity chromatographic steps using both human immunoglobulin G (IgG) and mouse monoclonal antibodies was used in the purification procedure. Traces of human and mouse IgGs that coeluted with gp160 were removed by passing the preparation through goat anti-human and goat anti-mouse immunoglobulin columns, respectively. Approximately 300 μg of gp160 was obtained from 1 liter of the conditioned medium.

(ii) gp120. Glycoprotein gp120 was purified from H9 cells infected with HIV-1_{IIIB} as described earlier (36). Briefly, the cells were lysed with a detergent-containing buffer, and the lysate was reacted with an affinity matrix prepared with IgG from the serum of an AIDS patient. The eluate from this human IgG column was passed through a lentil-lectin column, which separated gp120 and p24. Glycoprotein gp120 was eluted from the lectin column and further purified by immunoaffinity and ion-exchange chromatographies.

(iii) p24. The core protein p24 was purified as follows. The flowthrough from the lectin column was dialyzed against 20 mM Tris (pH 8.2) containing 1 M NaCl, after which Triton X-100 was added to obtain a final detergent concentration of 0.2%. The dialyzed material was reacted with an affinity matrix prepared with a monoclonal antibody against p24 (48) and equilibrated with 20 mM Tris-HCl (pH 8.2) containing 1 M NaCl and 0.2% Triton X-100. The bound p24 was eluted with 50 mM diethylamine (pH 11.5). The eluate was extensively dialyzed against 10 mM *N,N'*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) buffer (pH 6.5) containing 1 mM EDTA and loaded on a phosphocellulose column equilibrated with the same buffer. The column was eluted with a linear 0 to 500 mM NaCl gradient, and fractions containing p24 were pooled, concentrated, and dialyzed against phosphate-buffered saline (PBS).

The purities of gp160, gp120, and p24 were assessed by (i) the presence of single Coomassie blue-staining bands on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), (ii) the presence of a single band on Western blots with HIV-1 antibody-positive human serum, and (iii) nonreactivity with goat anti-human and goat anti-mouse IgG by EIA.

EIA using purified viral antigens. Appropriate amounts of purified native viral antigens were dissolved in 50 mM sodium bicarbonate (pH 9.6), and 100 μl of the solution was added to each well of a 96-well polystyrene microplate. The antigens were allowed to absorb overnight at 4°C. The wells were emptied, and nonspecific binding sites were blocked with fourfold-diluted BLOTTO (1.25% nonfat dry milk, 2.5% bovine serum albumin [BSA]) at room temperature for 1 h. The wells were washed with 0.05% Tween 20 in PBS and allowed to react at 37°C for 1.5 h with 100-μl quantities of serum samples diluted fivefold in Dilsim (normal goat serum containing detergents and salts; Organon Teknika Corporation). The wells were then washed five times with 0.05% Tween 20 in PBS and incubated for 1 h at 37°C with peroxidase-labeled goat anti-human IgG at a dilution of 1:1,600 in PBS containing 10% normal goat serum and 0.5% Triton X-100. The wells were washed five times with 0.05% Tween 20 in PBS, and the contents were reacted with 100 μl of a preformulated tetramethylbenzidine peroxidase substrate (TMB microwell peroxidase substrate system; Kirkegaard & Perry, Gaithersburg, Md.). The reactions were stopped by the addition of 100 μl of 1 M phosphoric acid, and the optical densities at 450 nm were measured on a Dynatech reader. Data are shown as a ratio of signal to cutoff value (S/C ratio). Twice the mean of three negative control values is taken as the cutoff. An S/C ratio of ≥ 1 is taken as a positive test. Negative control sera were pools of normal blood donor sera screened for the absence of hepatitis B and HIV-1 antibodies by using licensed enzyme-linked immunosorbent assay (ELISA) tests. For positive controls, high-titer sera were diluted with negative sera to give a test optical density of approximately 0.6. An assay run was considered valid if the mean optical density of the negative controls was below 0.2 and that of the positive control was greater than 0.5.

EIAs using commercial HIV-1 kits. EIAs using virus lysate (Vironostika HIV-1 microelisa system [Organon Teknika Corporation]) and recombinant envelope protein P-Env9 (duPont) were performed according to the manufacturers' instructions.

Western blot immunoassay. Detergent lysates of HIV-1 (produced in our own Food and Drug Administration-licensed virus production facility) were fractionated by SDS-PAGE, and the protein bands were electrophoretically transferred to nitrocellulose sheets according to the procedure of Towbin et al. (46). Nonspecific binding sites were blocked by incubating the nitrocellulose sheets in BLOTTO (5% nonfat dry milk, 10% BSA). Strips containing representative profiles of viral antigens were cut from these sheets and incubated at room temperature for 1 h with test serum diluted 100 times in Dilsim. The strips were washed three times with wash buffer (0.1 M NaCl, 0.25% Tween-20, and 20 mM sodium phosphate [pH 7.2]) and incubated with biotinylated goat antibodies to human IgG in wash buffer for 1 h at room temperature. Strips were washed with wash buffer, reacted with avidin-conjugated horseradish peroxidase diluted 1:1,000 in wash buffer, and again washed with wash buffer before being reacted with

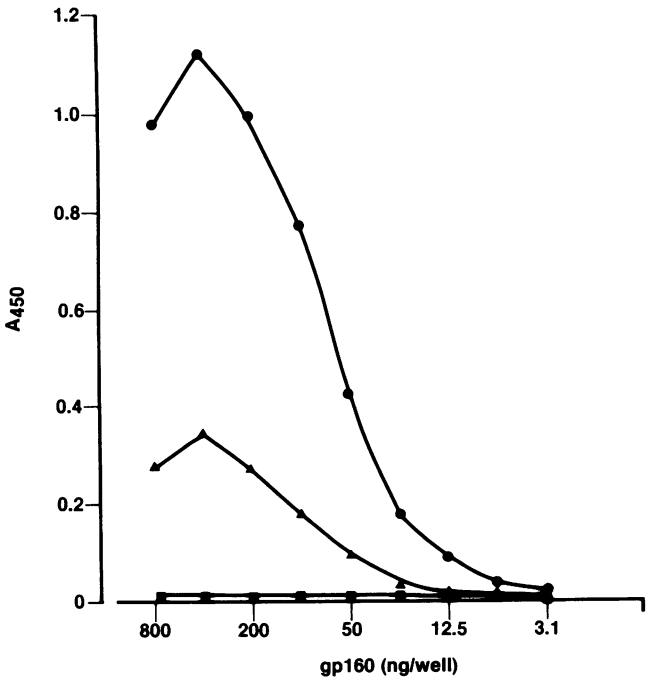

FIG. 1. Optimization of precoating with gp160. The wells of microtiter plates were coated with the indicated concentrations of gp160. Three serum samples that showed medium-positive (●), weakly positive (▲), and false-positive (■) reactions, respectively, in the Vironostika HIV test were studied. The assay was performed at a serum dilution of 1:5 as described in Materials and Methods.

0.003% H_2O_2 containing 600 μg of 3,3′-diaminobenzidine per ml in PBS. The strips were then rinsed with water and dried.

Radioimmunoprecipitation assay. An immunoprecipitation assay using [^{35}S]methionine-labeled proteins was performed as described earlier (47). Briefly, the labeled cells were lysed at 4°C, and the cell lysates were preabsorbed with protein A-Sepharose for 3 h at room temperature. The lysates were clarified by centrifugation, and then 10 μl of human serum and 200 μl of a 10% suspension of protein A-Sepharose were added to 1 ml of the clarified lysate. The samples were then incubated for 18 h at 4°C. The immunocomplexes were pelleted by centrifugation and washed three times with 10 mM sodium phosphate (pH 7.2) containing 0.5% NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, and 0.1% SDS. The washed pellets were suspended in 50 μl of electrophoresis sample buffer, heated for 2 min at 90°C, and centrifuged. The ^{35}S-labeled proteins in the supernatant were separated by electrophoresis on a 12% polyacrylamide gel and visualized by autoradiography.

RESULTS

Optimization of the gp160 ELISA. All available evidence had shown gp41 to be the most desirable HIV-1 antigen for a serological test to identify HIV-1 antibodies (15, 42). We predicted that gp160 would have the same antigenic features as its subunit gp41 for antibody detection but would have the major advantage of being soluble and therefore being amenable to easy manipulation compared with the highly hydrophobic gp41. Our preliminary analysis did confirm that gp160 was a sensitive reagent to detect HIV-1 antibodies in human sera. The system was optimized in an EIA format for antigen and serum concentrations. Because of the purity of the antigen and its freedom from contaminating cellular proteins, the background absorbance was extremely low even at low serum dilutions. All assays were done with 1:5-diluted sera. To

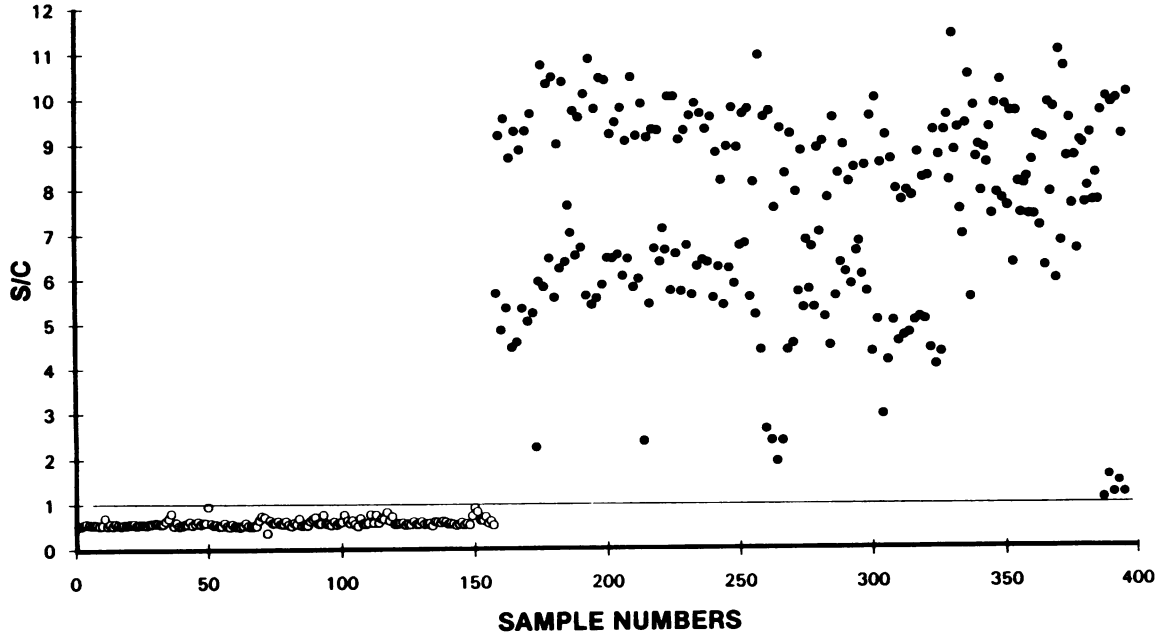

FIG. 2. Reactivity distribution for 395 Western blot-characterized serum samples in native gp160 EIA. One hundred fifty-seven Western blot-negative (○) and 238 Western blot-positive (●) serum samples were tested at a 1:5 dilution with 100 ng of antigen per well on the plate. The S/C ratio at 1.0 indicates twice the absorbance value of the negative controls (see Materials and Methods).

optimize the antigen concentration on the plate, we titrated gp160 from 800 ng per microplate well downward by stepwise twofold dilution. Three serum samples were used to evaluate the effect of the antigen concentration: one that was moderately reactive, one that was weakly reactive, and one that was falsely reactive in a licensed commercial HIV-1 antibody assay, the Vironostika HIV-1 system (Organon Teknika Corporation).

The amount of gp160 required for a maximal response was approximately 400 ng per well (Fig. 1). However, at 50 ng of gp160 per well, even the weakly reactive serum showed considerable reactivity. On the basis of these data, we chose 100 ng of gp160 per well as the antigen concentration for further studies and used the procedure outlined in Materials and Methods. The serum which showed a false-positive reaction in the Vironostika assay was negative with up to 800 ng of gp160 per well.

Assay specificity. The specificity of the native gp160 assay was evaluated by its performance with three panels of sera. These included 157 serum samples that were confirmed negative for HIV-1 antibodies as part of a previous study (15). All 157 samples were nonreactive in the gp160 assay. The S/C ratio for over 90% of the samples was between 0.5 and 0.7 (Fig. 2). A panel of 41 "problem" serum samples representing a variety of serological conditions that might produce false-positive results in the detection of HIV-1 antibodies was also negative in the gp160 assay. Similarly nonreactive was a panel of 15 serum samples which showed false-positive reactions in one or more commercial HIV-1 screening tests.

The performance of the native gp160 assay was further evaluated by using 1,000 randomly selected, unlinked serum samples collected from healthy blood donors by the American Red Cross. The sera were prescreened with a Food and Drug Administration-licensed HIV-1 screening test (Vironostika HIV-1 test). Of the 1,000 serum samples, 2 were repeatedly reactive. The results of the native gp160 assay coincided with the results of the Vironostika HIV-1 test. The two repeatedly reactive serum samples were confirmed positive by Western blot analysis and radioimmunoprecipitation assay (data not shown). The specificity of the gp160 assay was calculated by the following formula: [(total specimens screened − number repeatedly reactive)/(total specimens screened − number confirmed reactive)] × 100 = specificity. Thus, the native gp160 system has a calculated specificity of 100% from results with 1,000 normal donor serum samples tested.

Sensitivity of the gp160 EIA. The sensitivity of the native gp160 assay was initially evaluated by testing 238 serum samples that were confirmed by Western blot to contain HIV-1 antibodies. All 238 samples were positive in the assay. The distribution of S/C ratios for these samples, along with that of S/C ratios for the 157 confirmed negative serum samples described above, is shown in Fig. 2. There is a clear break between the negative and the positive samples. The sensitivity was further evaluated in comparison with the viral lysate-based Vironostika assay by using a dilution panel of sera. The dilution panel was made by serially diluting 14 selected serum samples that showed limited bands on Western blot profiles. These diluted samples were then tested in both assays as described in Materials and Methods. The sensitivities of seven of the serum samples in both tests were about equal (data not shown). The remaining seven samples reacted more strongly in the gp160 assay than in the Vironostika assay (Table 1). Of these latter seven samples, five reacted only to gp41, one reacted to gp41 and p31, and one (an early seroconverter sample) reacted only to p24 in the Western blot assay. The native gp160 assay was then compared with a commercial recombinant envelope EIA by using the same 14-sample dilution panel. Thirteen of these serum samples (Table 2) gave higher absorbance readings with native gp160 than with the recombinant protein at all dilutions tested.

TABLE 1. Sensitivity of native gp160 assay with a panel of diluted sera

Sample	Initial dilution[a]	Result with:	
		Native gp160 (100 ng/well)	Vironostika assay
425	500	+	+
	1,000	+	+
	1,500	+	+/−[b]
	2,000	+	−
528	500	+	+
	1,000	+	+/−
	1,500	+	−
	2,000	+	−
554	10	+	+
	50	+	−
	100	+/−	−
210	1,000	+	+
	1,500	+	+
	2,000	+	−
	2,500	+	−
268	2	+	+
	5	+	−
	10	−	−
264	2	+	+
	5	+	−
	10	+/−	−
ESC	20	+	+
	40	+	+/−
	60	+	−
	120	+	−
	240	−	−

[a] The samples were further diluted 1:5 for the gp160 assay and 1:76 for the Vironostika assay.
[b] +/−, result in the gray zone.

Performance in early seroconversion panels. Three serum panels were tested to evaluate the performance of the native gp160 assay in the detection of HIV-1 antibodies during seroconversion. Panels 1 and 2 were BBI seroconversion panels A and C, respectively (44). Panel 3 consisted of four sequential blood samples collected during an episode of acute viral meningitis and subsequent HIV-1 seroconversion (24). The reactivity of the gp160 assay with each panel was compared with those of EIAs using virus lysate (Vironostika HIV-1) and the recombinant envelope protein P-Env9 (du-Pont). With serum panels 1 and 3, HIV-1 antibodies were detected in exactly the same samples by all three tests (data not shown). With panel 2 (BBI panel C), the gp160 system detected antibodies in the second blood sample of the panel (sample 2C) (Table 3). Both the Vironostika HIV-1 and P-Env9 assays detected the antibody only in later blood samples.

Reactivity of gp160 compared with those of gp120 and p24. Since the serum to be tested contains a mixture of antibodies to different antigens, the sensitivity of the test will depend on the specific antigen or mixture of antigens on the solid phase.

TABLE 2. Comparison between the native gp160 assay and a recombinant envelope peptide-based EIA with a panel of diluted sera

Sample	Initial dilution	S/C ratio[a] in:	
		Native gp160 assay	Recombinant P-Env9 assay (duPont)
432	50	6.6	>3.0
	500	3.2	2.4
	1,000	1.9	1.7
528	50	6.2	>3.0
	500	3.4	2.4
	1,000	2.3	1.8
523	500	6.3	>3.0
	1,000	4.3	2.2
	2,000	3.3	1.5
554	10	4.9	1.9
	100	1.9	1.5
	200	1.2	0.99
150	100	5.7	>3.0
	1,000	2.9	1.8
	2,000	1.9	1.3
210	100	6.3	>3.0
	1,000	3.0	2.1
	2,000	2.0	1.5
215	100	6.3	>3.0
	1,000	3.9	2.0
	2,000	2.9	2.0
425	50	6.3	>3.0
	500	3.3	2.3
	1,000	2.1	1.8
491	50	6.5	>3.0
	500	4.0	2.5
	1,000	2.4	2.0
446	50	6.2	>3.0
	500	3.3	2.6
268	2	4.2	1.9
	40	0.9	0.63
264	2	4.3	2.1
	40	0.9	0.71
259	2	1.2	1.2
	100	0.65	0.63
ESC	80	2.4	1.9
	160	1.8	1.5

[a] Absorbance ÷ twice the mean of three negative control values for native gp160 assay; absorbance ÷ one-half of the mean of three positive control values for recombinant P-Env9 assay.

The reactivities of a number of sera with native gp160, native gp120, and native p24 were studied to make an evaluation of the comparative reactivities to the three proteins. The wells were coated with equal amounts of each purified antigen. A panel consisting of six serum samples reacting strongly in the Vironostika HIV-1 assay was used. Four of the six samples reacted more strongly with gp160 than with gp120 and reacted only poorly with p24. The other two serum samples, SP1009 and GC258, reacted almost equally with all three proteins (Fig. 3A). The reactivities of another panel of six weakly Western blot-positive serum samples are shown in Fig. 3B. These sera were initially diluted to near end point in the Vironostika assay before being tested to determine what specific reactivity remained. All the sera showed high reactivities with gp160 at end point dilutions. The reactivities of gp120 and p24 with these sera were extremely poor. Even though the total number of sera tested in the above-described side-by-side comparison was not large, the results showed that native gp160 was clearly superior as the antigen of choice to detect antibodies to HIV-1 in sera of humans infected with HIV-1.

TABLE 3. Reactivities of an early seroconverter panel in the viral lysate, recombinant envelope peptide, and native gp160 systems

Sample	Collection date (mo-day-yr)	Result with:		
		Viral lysate assay (Vironostika)	Recombinant P-Env9 assay (DuPont)	Native gp160 assay
1C	7-16-85	–	–	–
2C	7-23-85	–	–	+
3C	7-25-85	–	+	+
4C	7-30-85	+	+	+

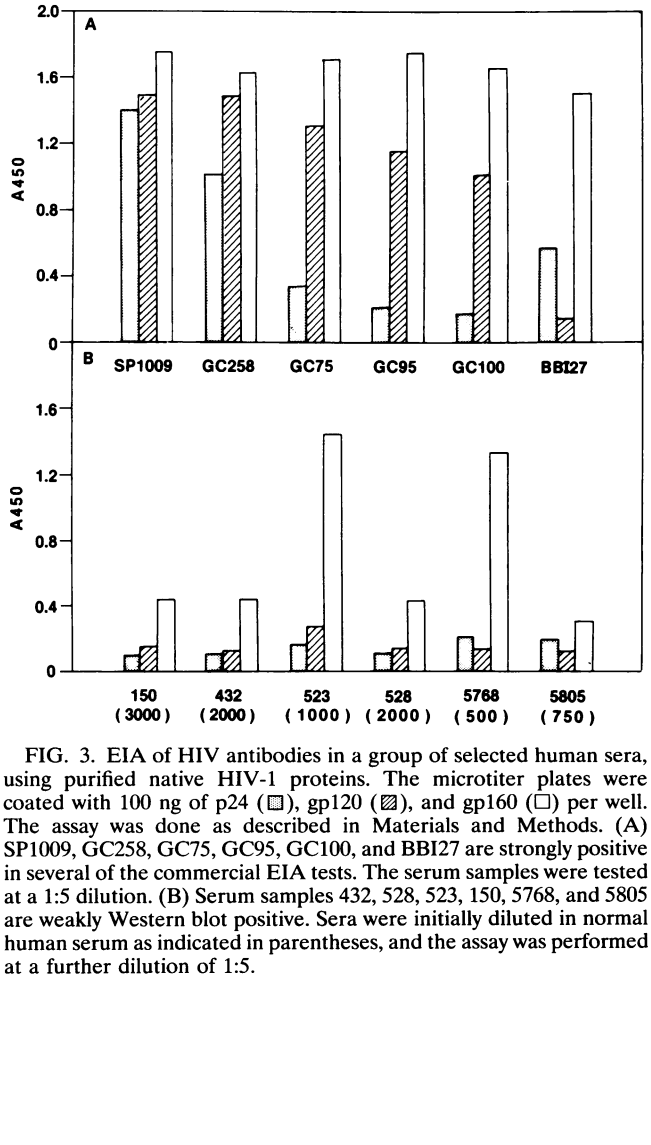

FIG. 3. EIA of HIV antibodies in a group of selected human sera, using purified native HIV-1 proteins. The microtiter plates were coated with 100 ng of p24 (▩), gp120 (▨), and gp160 (□) per well. The assay was done as described in Materials and Methods. (A) SP1009, GC258, GC75, GC95, GC100, and BBI27 are strongly positive in several of the commercial EIA tests. The serum samples were tested at a 1:5 dilution. (B) Serum samples 432, 528, 523, 150, 5768, and 5805 are weakly Western blot positive. Sera were initially diluted in normal human serum as indicated in parentheses, and the assay was performed at a further dilution of 1:5.

DISCUSSION

In this report we describe a highly specific and sensitive EIA for the detection of HIV-1 antibodies by using pure gp160 from a HIV-1-infected cell culture. The antigen is native and homogeneous by several criteria. The background absorption by cellular contaminants was extremely small. In this study the gp160 assay yielded 100% specificity. The assay, therefore, clearly eliminates those false-positive reactions usually encountered with systems using virus lysates. None of the 15 potentially false-positive samples tested showed any reaction in the gp160 assay. Although recombinant DNA technology has clearly increased the specificities of second-generation EIAs, false-positive reactions caused by endogenous bacterial products copurified with the antigen preparations are frequently seen. One of the 15 false-positive serum samples we tested in this study (GC9) was found to be false positive in a recombinant envelope assay (data not shown). Furthermore, because of extremely low nonspecific background absorbance in the gp160 assay, we could use low serum dilutions (up to 1:5) without compromising specificity. In contrast, virus lysate-based assays cannot accommodate high serum concentrations and have to use higher serum dilutions to minimize reactivities to nonspecific proteins. This built-in advantage makes the native gp160 assay two- to fivefold more sensitive than the virus lysate-based assay.

The relative concentrations of antibodies to major HIV-1 proteins appear to vary widely during early seroconversion following HIV-1 infection (1, 38). Although some studies seemed to identify the *gag* protein (p24) as the first antigen recognized by sera from patients in the early stages of HIV infection (8, 17), radioimmunoprecipitation studies identified gp160 as the primary seroconversion antigen, recognized even before p24 (5, 30, 33). EIA using recombinant envelope peptides has been shown to be more sensitive than some commercial assays employing solubilized virus in detecting antibodies early in seroconversion (44). The present study demonstrates that the gp160 assay was even more sensitive in detecting seroconversion than a recombinant envelope protein-based EIA. A significant feature of gp160 is that it is a fully glycosylated viral protein secreted into the culture medium in its native soluble form. These observations would seem to support the view that conformational structures are of great importance in recognition of the earliest antibodies to HIV.

Since the envelope glycoproteins contain epitopes naturally accessible to the immune system, recent studies have been directed to determine whether gp160 could be a safe and efficacious AIDS vaccine (21, 39). The antibodies which recognize the conformational epitope of envelope glycoprotein may be more likely to be functional in vivo than those which bind to denatured proteins or synthetic peptides. Therefore, EIA using native gp160 may be ideal for the detection of the humoral immune response to candidate HIV vaccines incorporating envelope glycoproteins or inactivated whole virus.

The availability of sufficient quantities of purified antigen is a prerequisite for development of serological tests. The necessary amounts of gp160 can be economically purified from the serum-free culture medium of $6D5_{451}$ cells. Unlike the recombinant gp160 produced in an insect tissue culture cell line (13), the native gp160 produced by $6D5_{451}$ cells is soluble in the absence of detergents. Although the native gp160 secreted by the $6D5_{451}$ cells has a 60-amino-acid truncation at the carboxy terminus (27), this small deletion does not affect the immune recognition of the molecule because it is substantially removed from all the known immunodominant epitopes of gp160 (9).

There are additional advantages for native gp160 in an immunodiagnostic test. Since it is a well-defined molecule, the coating density on the solid surface can be controlled in order to provide optimum sensitivity. Undefined proportions of viral proteins on the solid surface may produce variations in assay sensitivity (7, 14, 22). EIAs designed to detect antibodies to envelope proteins have been shown to be more sensitive than the Western blot assay in earlier studies (43, 45). The extreme sensitivity of the gp160 assay renders it suitable for further evaluating the indeterminate or weakly positive samples in Western blot assay, thus eliminating the need for a second test to confirm seropositivity. Since the antigen molecule is in its native configuration, it provides epitopes that may be important in the earliest recognition of antibodies during seroconversion.

REFERENCES

1. **Allain, J. P., Y. Laurian, D. A. Paul, and D. Senn.** 1986. Serological markers in early stages of human immunodeficiency virus infection in haemophiliacs. Lancet **ii:**1233–1236.
2. **Allan, J. S., J. E. Coligan, F. Barin, M. F. McLane, J. G. Sodroski, C. A. Rosen, W. A. Haseltine, T. H. Lee, and M. Essex.** 1985. Major glycoprotein antigens that induce antibodies in AIDS patients are encoded by HTLV-III. Science **228:**1091–1094.
3. **Anderson, D. W., J. S. Epstein, T. H. Lee, M. D. Lairmore, C. Saxinger, V. S. Kalyanaraman, D. Slamon, W. Parks, B. J. Poiesz, L. T. Pierik, H. Lee, R. Montagna, P. A. Roche, A. Williams, and W. Blattner.** 1989. Serological confirmation of human T-lymphotropic virus type I infection in healthy blood and plasma donors. Blood **74:**2585–2591.
4. **Bäcker, U., A. Gathof, J. Howe, E. Strobel, F. Deinhardt, L. Gürtler, P. Grob, H. Joller-Jemelka, P. Kühnl, S. Seidl, R. Fico, J. Shih, and H. Troonen.** 1988. A new second-generation anti-HIV-1 enzyme immunoassay using recombinant envelope and core proteins. AIDS **2:**477–480.
5. **Barin, F., M. F. McLane, J. S. Allan, T. H. Lee, J. E. Groopman, and M. Essex.** 1985. Virus envelope protein of HTLV-III represents major target antigen for antibodies in AIDS patients. Science **228:**1094–1096.
6. **Barré-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier.** 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science **220:**868–871.
7. **Behets, F., A. Disasi, R. W. Ryder, K. Bishagara, P. Piot, M. Kashamuka, M. Kamenga, N. Nzila, M. Laga, G. Vercauteren, V. Batter, C. Brown, and T. Quinn.** 1991. Comparison of five commercial enzyme-linked immunosorbent assays and Western immunoblotting for human immunodeficiency virus antibody detection in serum samples from Central Africa. J. Clin. Microbiol. **29:**2280–2284.
8. **Biggar, R. J., M. Melbye, P. Ebbesen, S. Alexander, J. O. Nielsen, P. Sarin, and V. Faber.** 1985. Variation in human T lymphotropic virus III (HTLV-III) antibodies in homosexual men: decline before onset of illness related to acquired immune deficiency syndrome (AIDS). Br. Med. J. (Clin. Res. Ed.) **291:**997–998.
9. **Bolognesi, D. P.** 1990. Immunobiology of the human immunodeficiency virus envelope and its relationship to vaccine strategies. Mol. Biol. Med. **7:**1–15.
10. **Brun-Vezinet, F., C. Rouzioux, F. Barre-Sinoussi, D. Klatzmann, A. G. Saimot, W. Rozenbaum, D. Christol, J. C. Gluckmann, L. Montagnier, and J. C. Chermann.** 1984. Detection of IgG antibodies to lymphadenopathy-associated virus in patients with AIDS or lymphadenopathy syndrome. Lancet **i:**1253–1256.
11. **Burke, D. S., R. R. Redfield, P. Putman, and S. S. Alexander.** 1987. Variations in Western blot banding patterns of human T-cell lymphotropic virus type III/lymphadenopathy-associated virus. J. Clin. Microbiol. **25:**81–84.
12. **Chang, T. W., I. Kato, S. McKinney, P. Chanda, A. D. Barone, F. Wong-Staal, R. C. Gallo, and N. T. Chang.** 1985. Detection of antibodies to human T-cell lymphotropic virus-III (HTLV-III) with an immunoassay employing a recombinant *Escherichia coli* derived viral antigenic peptide. Bio/Technology **3:**905.

13. **Cochran, M. A., B. L. Ericson, J. D. Knell, and G. E. Smith.** 1987. Use of baculovirus recombinants as a general method for the production of subunit vaccines, p. 384–388. *In* R. M. Chanock, R. A. Lerner, F. Brown, and H. Ginsberg (ed.), Vaccines 87. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Deinhardt, F., J. Eberle, and L. Gürtler.** 1987. Sensitivity and specificity of eight commercial and one recombinant anti-HIV ELISA tests. Lancet **i:**40.
15. **DeVico, A. L., F. D. Veronese, S. L. Lee, R. C. Gallo, and M. G. Sarngadharan.** 1988. High prevalence of serum antibodies to reverse transcriptase in HIV-1-infected individuals. AIDS Res. Hum. Retroviruses **4:**17–22.
16. **Döpel, S. H., T. Porstmann, P. Henklein, and R. von Baehr.** 1989. Fine mapping of an immunodominant region of the transmembrane protein of the human immunodeficiency virus (HIV-1). J. Virol. Methods **25:**167–177.
17. **Esteban, J. I., J. W. Shih, C. C. Tai, A. J. Bodner, J. W. Kay, and H. J. Alter.** 1985. Importance of western blot analysis in predicting infectivity of anti-HTLV-III/LAV positive blood. Lancet **ii:**1083–1086.
18. **Gallo, R. C., S. Z. Salahuddin, M. Popovic, G. M. Shearer, M. Kaplan, B. F. Haynes, T. J. Palker, R. Redfield, J. Oleske, B. Safai, G. White, P. Foster, and P. D. Markham.** 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science **224:**500–503.
19. **Gnann, J. W., Jr., J. B. McCormick, S. Mitchell, J. A. Nelson, and M. B. Oldstone.** 1987. Synthetic peptide immunoassay distinguishes HIV type 1 and HIV type 2 infections. Science **237:**1346–1349.
20. **Gnann, J. W., Jr., J. A. Nelson, and M. B. Oldstone.** 1987. Fine mapping of an immunodominant domain in the transmembrane glycoprotein of human immunodeficiency virus. J. Virol. **61:**2639–2641.
21. **Gorse, G. J., S. E. Frey, F. K. Newman, and R. B. Belshe.** 1992. Detection of binding antibodies to native and recombinant human immunodeficiency virus type 1 envelope glycoproteins following recombinant gp160 immunization measured by flow cytometry and enzyme immunoassays. J. Clin. Microbiol. **30:**2606–2612.
22. **Gürtler, L. G., J. Eberle, B. Lorbeer, and F. Deinhardt.** 1987. Sensitivity and specificity of commercial ELISA kits for screening anti-LAV/HTLV$_{III}$. J. Virol. Methods **15:**11–23.
23. **Hardy, C. T., T. A. Damrow, D. B. Villareal, and G. E. Kenny.** 1992. Evaluation of viral-lysate enzyme-linked immunosorbent assay kits for detecting human immunodeficiency virus (type 1) infections using human sera standardized by quantitative western blotting. J. Virol. Methods **37:**259–273.
24. **Ho, D. D., M. G. Sarngadharan, L. Resnick, F. D. Veronese, T. R. Rota, and M. S. Hirsch.** 1985. Primary human T-lymphotropic virus type III infection. Ann. Intern. Med. **103:**880–883.
25. **Hoxie, J. A., T. P. Fitzharris, P. R. Youngbar, D. M. Matthews, J. L. Rackowski, and S. F. Radka.** 1987. Nonrandom association of cellular antigens with HTLV-III virions. Hum. Immunol. **18:**39–52.
26. **Kalyanaraman, V. S., R. Pal, R. C. Gallo, and M. G. Sarngadharan.** 1988. A unique human immunodeficiency virus culture secreting soluble gp160. AIDS Res. Hum. Retroviruses **4:**319–329.
27. **Kalyanaraman, V. S., V. Rodriguez, S. Josephs, R. C. Gallo, and M. G. Sarngadharan.** 1990. Differential viral gene expression and its effect on the biological properties of the cell clones of an HIV-1-infected cell line. Virology **177:**380–383.
28. **Kalyanaraman, V. S., V. Rodriguez, F. Veronese, R. Rahman, P. Lusso, A. L. DeVico, T. Copeland, S. Oroszlan, R. C. Gallo, and M. G. Sarngadharan.** 1990. Characterization of the secreted, native gp120 and gp160 of the human immunodeficiency virus type 1. AIDS Res. Hum. Retroviruses **6:**371–380.
29. **Kenealy, W., D. Reed, R. Cybulski, D. Tribe, P. Taylor, C. Stevens, T. Matthews, and S. Petteway.** 1987. Analysis of human serum antibodies to human immunodeficiency virus (HIV) using recombinant ENV and GAG antigens. AIDS Res. Hum. Retroviruses **3:**95–105.
30. **Kitchen, L., G. Malone, S. Orgad, F. Barin, R. Zaizov, B. Ramot, E. Gazit, J. Kreiss, M. Leal, I. Wichmann, U. Martinowitz, and M. Essex.** 1986. Viral envelope protein of HTLV-III is the major target antigen for antibodies in hemophiliac patients. J. Infect. Dis. **153:**788–790.
31. **Kühnl, P., S. Seidl, and G. Holzberger.** 1985. HLA DR4 antibodies cause positive HTLV-III antibody ELISA results. Lancet **i:**1222–1223.
32. **Lasky, L. A., J. E. Groopman, C. W. Fennie, P. M. Benz, D. J. Capon, D. J. Dowbenko, G. R. Nakamura, W. M. Nunes, M. E. Renz, and P. W. Berman.** 1986. Neutralization of the AIDS retrovirus by antibodies to a recombinant envelope glycoprotein. Science **233:**209–212.
33. **Manca, N., F. D. Veronese, D. D. Ho, R. C. Gallo, and M. G. Sarngadharan.** 1987. Sequential changes in antibody levels to the env and gag antigens in human immunodeficiency virus infected subjects. Eur. J. Epidemiol. **3:**96–102.
34. **Moore, J. P., L. A. Wallace, E. A. Follett, and J. A. McKeating.** 1989. An enzyme-linked immunosorbent assay for antibodies to the envelope glycoproteins of divergent strains of HIV-1. AIDS **3:**155–163.
35. **Norrby, E., G. Biberfeld, F. Chiodi, A. von Gegerfeldt, A. Naucler, E. Parks, and R. Lerner.** 1987. Discrimination between antibodies to HIV and to related retroviruses using site-directed serology. Nature (London) **329:**248–250.
36. **Pal, R., B. C. Nair, G. M. Hoke, M. G. Sarngadharan, and M. Edidin.** 1991. Lateral diffusion of CD4 on the surface of a human neoplastic T-cell line probed with a fluorescent derivative of the envelope glycoprotein (gp120) of human immunodeficiency virus type 1 (HIV-1). J. Cell Physiol. **147:**326–332.
37. **Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo.** 1984. Detection, isolation and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science **224:**497–500.
38. **Ranki, A., S. L. Valle, M. Krohn, J. Antonen, J. P. Allain, M. Leuther, G. Franchini, and K. Krohn.** 1987. Long latency precedes overt seroconversion in sexually transmitted human-immunodeficiency-virus infection. Lancet **ii:**589–593.
39. **Redfield, R. R., D. L. Birx, N. Ketter, E. Tramont, V. Polonis, C. Davis, J. F. Brundage, G. Smith, S. Johnson, A. Fowler, T. Wierzba, A. Shafferman, F. Volvovitz, C. Oster, D. S. Burke, and the Military Medical Consortium for Applied Retroviral Research.** 1991. A phase I evaluation of the safety and immunogenicity of vaccination with recombinant gp160 in patients with early human immunodeficiency virus infection. N. Engl. J. Med. **324:**1677–1684.
40. **Saah, A. J., H. Farzadegan, R. Fox, P. Nishanian, C. R. Rinaldo, Jr., J. P. Phair, J. L. Fahey, T. H. Lee, and B. F. Polk.** 1987. Detection of early antibodies in human immunodeficiency virus infection by enzyme-linked immunosorbent assay, Western blot, and radioimmunoprecipitation. J. Clin. Microbiol. **25:**1605–1610.
41. **Samuel, K. P., A. Seth, M. Zweig, S. D. Showalter, and T. S. Papas.** 1988. Bacterial expression and characterization of nine polypeptides encoded by segments of the envelope gene of human immunodeficiency virus. Gene **64:**121–134.
42. **Sarngadharan, M. G., M. Popovic, L. Bruch, J. Schüpbach, and R. C. Gallo.** 1984. Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. Science **224:**506–508.
43. **Schulz, T. F., J. M. Aschauer, P. Hengster, C. Larcher, H. Wachter, B. Fleckenstein, and M. P. Dierich.** 1986. Envelope gene-derived recombinant peptide in the serodiagnosis of human immunodeficiency virus infection. Lancet **ii:**111–112.
44. **Schumacher, R. T., P. E. Garrett, G. E. Tegtmeier, and D. Thomas.** 1988. Comparative detection of anti-HIV in early HIV seroconversion. J. Clin. Immunoassay **11:**130–134.
45. **Thorn, R. M., G. A. Beltz, C. H. Hung, B. F. Fallis, S. Winkle, K. L. Cheng, and D. J. Marciani.** 1987. Enzyme immunoassay using a novel recombinant polypeptide to detect human immunodeficiency virus env antibody. J. Clin. Microbiol. **25:**1207–1212.
46. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
47. **Veronese, F. D., T. D. Copeland, S. Oroszlan, R. C. Gallo, and**

M. G. Sarngadharan. 1988. Biochemical and immunological analysis of human immunodeficiency virus *gag* gene products p17 and p24. J. Virol. **62:**795–801.

48. **Veronese, F. D., M. G. Sarngadharan, R. Rahman, P. D. Markham, M. Popovic, A. J. Bodner, and R. C. Gallo.** 1985. Monoclonal antibodies specific for p24, the major core protein of human T-cell leukemia virus type III. Proc. Natl. Acad. Sci. USA **82:**5199–5202.
49. **Wang, J. J., S. Steel, R. Wisniewolski, and C. Y. Wang.** 1986. Detection of antibodies to human T-lymphotropic virus type III by using a synthetic peptide of 21 amino acid residues corresponding to a highly antigenic segment of gp41 envelope protein. Proc. Natl. Acad. Sci. USA **83:**6159–6163.
50. **Zweig, M., S. D. Showalter, S. V. Bladen, R. V. Gilden, L. O. Arthur, W. G. Robey, P. L. Nara, and P. J. Fischinger.** 1988. Partial purification of native HIV transmembrane protein gp41: generation of polyclonal and monoclonal antibodies. AIDS Res. Hum. Retroviruses **4:**51–62.