Purification and Characterization of the External Envelope Glycoprotein from Two Human Immunodeficiency Virus Type 1 Variants, HTLV-III_B and HTLV-III_{RF}

S. W. PYLE,^{1*} G. C. DUBOIS,¹ W. G. ROBEY,^{2†} J. W. BESS, JR.,¹ P. J. FISCHINGER,² AND L. O. ARTHUR¹

Program Resources, Inc.,¹ and Office of the Director,² National Cancer Institute-Frederick Cancer Research Facility, Frederick, Maryland 21701

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External envelope glycoprotein from cell membranes and culture media of H9 cells infected with human immunodeficiency virus type 1 (HIV-1) isolate HTLV-III_{RF} was isolated by immunoaffinity chromatography and compared with similar materials isolated from another variant, HTLV-III_B. Envelope glycoprotein from III_B and III_{RF} appears to be identical, whether isolated from infected cell membranes or culture media. The molecular size of the III_{RF} external envelope glycoprotein was 110 kilodaltons, whereas the relative size of III_B gp120 was 123 kilodaltons. Amino-terminal sequence analysis of purified external envelope glycoprotein isolated from infected cell membranes or culture fluids revealed identical single sequences for the first 20 amino acids for each variant. The sequences obtained for III_B gp120 were identical to those reported for the BH10 clone of the III_B isolate, and the sequences determined for III_{RF} gp110 matched the amino acid sequence predicted for the HAT3 clone of the Haitian HIV isolate. The amino-terminal sequences of external envelope glycoproteins isolated from either HIV-1 variant corresponded to the sequence starting at the proposed proteolytic cleavage site for the processing of the signal peptide of gp160. Immunization with external envelope glycoprotein isolated from either of the two HIV-1 variants yielded goat antibodies that primarily precipitated the homologous antigen. Sequential immunization of a single goat with gp120 and then gp110 resulted in the generation of antibodies that precipitated external envelope glycoprotein from both variants.

It is now evident that independent isolates of the human immunodeficiency virus type 1 (HIV-1), although divergent in their genomic sequences, can be considered to be variants of the same retrovirus (19, 28). The genomic diversity of independent isolates of HIV-1 has been observed primarily in the viral *env* gene products (2, 5, 13, 28). The *env* gene codes for a 160,000-dalton glycoprotein, gp160, which is proteolytically cleaved into an external envelope glycoprotein (EEG), gp120, and a smaller transmembrane protein, gp41 (1, 22, 27). Both native and recombinant forms of the EEG have been shown to bind to CD4, which is the initiating event in HIV-1 infection (10, 11). The EEG contains neutralizing epitopes (9) and may cause fusion of CD4⁺ cells, contributing to the pathogenesis of the disease (25). The EEG has been shown to be shed into the media of infected cell cultures (23), and it has been postulated that free glycoprotein might have a role in the pathogenesis of acquired immunodeficiency syndrome (AIDS) (3). While the nucleic acid sequences of several variants have been compared (13, 20, 26), no direct comparisons of the amino acid sequences of the *env* glycoproteins from HIV-1 variants have yet been made.

Variations in the primary structure and the antigenicity of viral envelope proteins would be expected and should be considered in the development of a vaccine for AIDS. In this study, we isolated the EEG from H9 cell membranes and culture media of H9 cells infected with the HIV-1 isolate HTLV-III_{RF} and compared those glycoproteins with similar materials isolated from HTLV-III_B. These two virus isolates were chosen for study because they differ in their nucleotide sequences by 8.5% (28), and the predicted amino acid

sequences for EEGs from their respective molecular clones vary by over 21% (26). Because immunoaffinity chromatography had proven successful in purification of analytical and large-scale quantities of EEG from III_B (18, 21), we utilized this technique to purify EEG from III_{RF}. Amino-terminal amino acid sequences and analyses of the immunological reactivities of antisera generated by immunizing goats with envelope preparations from III_B and III_{RF} revealed differences between the two isolates.

MATERIALS AND METHODS

Cells and virus. Two variants of HIV-1, III_B and III_{RF}, were used in this study (16). Both viruses were propagated by roller culture at 37°C in H9 cells (16), using RPMI 1640 medium containing 5% fetal bovine serum (heat inactivated), 2 mM glutamine, 100 U of penicillin per ml, and 100 U of streptomycin per ml.

Purification of external envelope glycoproteins. Procedures for purifying EEG from III_B-infected H9 cell membrane extracts and culture fluids have been presented in detail elsewhere (18, 21) and were not modified to purify EEG from III_{RF}.

Briefly, the culture medium from 3- to 4-day-old cultures of HIV-infected H9 cells was removed and clarified of cells, and the virus was purified from the culture fluid by sucrose gradient centrifugation. Membranes from approximately 200 g of infected cells were prepared and extracted with buffers containing 1.0% Triton X-100. Following virus purification, the culture fluids were adjusted to 0.01 M Tris hydrochloride (pH 8.5)–0.1% Triton X-100. The prepared samples were each applied to immunoaffinity resins prepared by coupling immunoglobulins, concentrated from the plasma of selected AIDS patients, to Sepharose CL-4B. Separate resins and apparatus were used to purify glycoproteins from membrane

* Corresponding author.

† Present address: Abbott Laboratories, Abbott Park, IL 60067.

extracts and culture fluids from each of the two virus variants. Adsorbed antigens from cell membrane extracts were eluted from the resins with 4.0 M MgCl_2 and immediately dialyzed against deionized water. Antigens from culture fluids were eluted from immunoaffinity columns with 0.2 M glycine hydrochloride, pH 2.5, immediately adjusted to neutrality with 1 M Tris hydrochloride, pH 8.5, and dialyzed against deionized water. With both preparations, the precipitate that formed during extensive dialysis was removed by centrifugation and the supernatant, containing the external envelope glycoprotein, was lyophilized to dryness.

Routine EEG sample analyses. Quantities of purified EEG were standardized by amino acid analysis measurements. Approximately 1 μg of lyophilized purified protein was hydrolyzed in 200 μl of 5 M HCl containing 0.1% phenol. Reactions were carried out in evacuated glass tubes at 110°C for 20 h. The samples were then dried in vacuo and analyzed on a Durrum model D500 automated amino acid analyzer as reported previously (14). The quantity of protein in a sample was calculated as the sum of the masses of the amino acids detected.

The concentration of endotoxin per unit volume was measured by quantitative chromatogenic *Limulus* amoebocyte lysate assay (QCL-1000; Whittaker M.A. Bioproducts, Walkersville, Md.). Immunologic levels of EEG and core protein in each sample were measured relative to standards by competitive radioimmune assay (RIA), using methods described previously (18). Purified gp120 from III_B-infected H9 cell membranes and purified III_B p24, iodinated with chloramine-T, were used as labeled antigens and as standards in competitive RIAs. Amounts of immunoglobulin contaminating the preparations were estimated by a specific dot-blot immunoassay, using purified human immunoglobulin G as a standard. The sensitivity of the assay was estimated to be 5 ng/ml.

Preparation of deglycosylated EEG. Purified EEG was deglycosylated with endoglycosidase F (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). One volume of EEG solution was adjusted to pH 5.5 with sodium acetate buffer, brought to 0.1% with sodium dodecyl sulfate (SDS), and incubated with 1 volume of endoglycosidase F for 2 h at 37°C. The sample was then diluted in electrophoresis sample preparation buffer for analysis by SDS-polyacrylamide gel electrophoresis (PAGE), using Laemmli buffers (7).

Glycoprotein purification by PAGE. The immunoaffinity eluate containing EEG was further purified to apparent homogeneity by preparative PAGE. Samples containing 100 to 400 μg of protein were subjected to electrophoresis in 10% polyacrylamide gels (7), and the bands were visualized with 0.25 M KCl (4). The region of the gel containing the EEG band was excised, macerated, and forced through a 200-mesh copper screen. The gel particles were suspended in phosphate-buffered saline and used for immunization.

Animal immunization. Goats were immunized with PAGE-purified envelope glycoproteins from III_B and III_{RF} and sequentially with both glycoproteins. The primary immunization consisted of 50 μg of protein immobilized in polyacrylamide gel which was emulsified in complete Freund adjuvant and injected intramuscularly. Secondary immunizations of 50 μg of protein immobilized in polyacrylamide gel and suspended in phosphate-buffered saline were administered by intraperitoneal injection without adjuvant at monthly intervals. All animals received at least five secondary immunizations. Goat 517 was immunized with EEG isolated from III_B-infected cell membranes, while goat 560 was immunized with EEG from culture fluids. The sequen-

tially immunized goat (no. 505) was given six secondary immunizations with EEG from III_B, rested 2 months, and then immunized with the same material from III_{RF}, as above. Animals were bled 10 days after each immunization.

Purification of EEG by reverse-phase HPLC. All EEG samples were purified by reverse-phase high-performance liquid chromatography (HPLC) prior to amino-terminal sequence analysis. This chromatography was necessary to remove low-molecular-weight contaminants which prohibited the identification of the initial three to four residues. A model 244 chromatograph equipped with a model 660 solvent programmer, a model 480 variable-wavelength detector, and a U6K sample injector was used (Waters Associates, Inc., Milford, Mass.). Aquapore RP-300 columns (2.1- by 30-mm cartridge; Applied Biosystems, Inc.) were utilized. Samples of purified EEG (100 μg) were made 5 to 6 M in guanidine hydrochloride containing 0.25% trifluoroacetic acid and applied to a column that had been equilibrated with 0.1% trifluoroacetic acid in water containing 30% organic modifier (acetonitrile-1-propanol, 2:1, with 0.1% trifluoroacetic acid). Application of a 25-min gradient of 30 to 80% organic modifier at a flow rate of 0.5 ml/min resulted in glycoprotein elution in the range of 60 to 70% organic modifier.

Amino-terminal sequence determinations. The HPLC-purified EEG samples (12 to 25 μg) from cell membranes and media of cell cultures infected with III_B and III_{RF} were subjected to amino-terminal sequence analysis, using a model 470A protein sequencer (Applied Biosystems) equipped with a model 120A PTH amino acid analyzer. Approximately 100 pmol of glycoprotein was loaded onto the Polybrene-treated glass fiber disk, and Edman degradation was performed for 30 cycles, using the standard 03RPTH program.

Cleavage at the two Asp-Pro sites present in the sequences of EEG from III_B and III_{RF} was accomplished by subjecting the filter disk to acid hydrolysis following amino-terminal Edman degradation. The filter was first treated with four 20- μl samples of trifluoroacetic anhydride. After 15 min of air drying, 20 μl of 0.2 M *N*-ethylmorpholine was applied to the filter and it was again air dried for 15 min. Trifluoroacetylation was repeated and followed by an additional 15 min of air drying. The filter was reassembled with the sequencer cartridge and washed with S1 (*n*-heptane) and S2 (ethyl acetate) for 2 min each and dried for 2 min with argon. The filter was soaked with 40 μl of 0.015 N HCl, placed in a sealed WISP vial along with a WISP insert vial containing 150 μl of 0.015 N HCl, and heated at 110°C for 30 min. Cleavage at sites other than Asp-Pro was blocked by allowing the dried filter to react with 30 μl of *o*-phthalaldehyde reagent (4.9 mg/ml in 44 mM *N*-ethylmorpholine containing 3% mercaptoethanol) for 50 min at 45°C. The filter was reassembled and washed with S1 and S2 before sequencing continued, using the 03RPTH program but with the acid treatment (trifluoroacetic acid) step omitted in the first cycle (03CBGN).

RESULTS

Purification of HIV-1 EEG. The relative mobilities and purity of EEG preparations from infected cell membrane extracts can be determined by visual assessment of the Coomassie blue-stained gel shown in Fig. 1. Lysed III_B virus, shown in lane 1 for comparison purposes, contains very little gp120, while immunoaffinity-purified gp120 from III_B appears as a single band with a relative molecular size of 123 kilodaltons (kDa) (lane 2). Immunoaffinity-purified EEG

FIG. 1. Coomassie blue-stained SDS-polyacrylamide gel of purified EEG from III_B and III_{RF} . Lane 1, Sucrose-banded III_B virions; lane 2, gp120 immunoaffinity purified from III_B -infected cell membranes; lane 3, endoglycosidase F-treated III_B gp120; lane 4, endoglycosidase F-treated III_{RF} gp110; lane 5, gp110 immunoaffinity purified from III_{RF} -infected cell membranes; lane 6, sucrose-banded III_{RF} virions; lane 7, endoglycosidase F control lane. The relative positions of the molecular size standards (shown at the left in kilodaltons) were, as follows, top to bottom: myosin, galactosidase, phosphorylase B, bovine albumin, ovalbumin, carbonic anhydrase, and soybean trypsin inhibitor. Samples, containing SDS, were subjected to electrophoresis in 10% polyacrylamide and stained with 0.01% Coomassie blue (R-250).

from III_{RF} (lane 5) had a relative molecular size of approximately 110 kDa (gp110), and again, little gp110 appears to be visible in the III_{RF} virus shown for comparison in lane 6. These molecular size relationships were also apparent in gradient polyacrylamide gels (data not shown). To determine whether the EEG migrational differences were due to changes in the protein structure or in the carbohydrate side chains, samples of both were treated with endoglycosidase F. Deglycosylated III_B gp120 migrated as a doublet with estimated molecular size of 58 to 62 kDa (lane 3), while the gp110 from III_{RF} was a single band of approximately 62 to 65 kDa (lane 4). Coomassie blue-stained bands contributed by the endoglycosidase enzyme are shown in lane 7.

In general, the EEG yields from III_{RF} -infected H9 cell membranes and culture fluids were approximately twice those obtained with III_B per unit of starting material (Table 1). Regardless of the virus isolate, approximately 95% of the recoverable EEG was obtained from cell culture fluids and the remaining 5% was from cell membrane extracts. The relative purity of EEG from culture media was lower than the purity of glycoprotein isolated from membranes and has been estimated for some preparations of III_B gp120 at approximately 75% (18). On the whole, comparable values

TABLE 1. Comparison of yields and relative activities of purified EEG from HTLV- III_B and HTLV- III_{RF} ^a

Variant and source	No. of column runs	Cells (g)	Media (liters)	Protein yield (μ g) ^b	Competitive RIA yield (μ g) ^c
III_B					
Cells	10	158.7		252.3	204.5
Media	20		125.1	2,156.1	2,450.2
III_{RF}					
Cells	5	176.6		512.0	199.6
Media	11		123.8	5,410.3	2,156.0

^a Mean values are given.

^b Protein concentration was calculated by using 1 A_{280} unit = 0.14 mg of protein per ml.

^c EEG concentration was measured by competitive RIA, using purified III_B gp120 lot EC-012 as a standard.

were observed for III_B EEG between total protein and gp120 concentration as measured by competitive RIA (Table 1). Similar comparisons were of limited use with gp110 because gp120 from III_B -infected cell membranes was used as the probe and standard and broadly specific AIDS patient serum was used as the antibody source in the RIA. However, using the A_{280} , the mean total protein values for gp110 were approximately twice the concentration of glycoprotein measured by competitive RIA (Table 1). SDS-PAGE comparisons of the preparations showed gp110 preparations to be of similar purity to gp120 (Fig. 1). Levels of p24 and endotoxin contaminants were in the nanogram-per-milliliter range for the measured preparations. Ligand leakage from the immunoaffinity resins was estimated by measuring the amount of human immunoglobulin G and was generally observed to be between 1 and 25 μ g/ml.

Identification of envelope glycoproteins by amino sequence analysis. The envelope glycoproteins isolated from cell membrane extracts and culture media of III_B - and III_{RF} -infected H9 cells were examined by amino-terminal sequence analysis. Immunoaffinity-purified envelope glycoproteins were first purified to homogeneity by reverse-phase HPLC. Sequence analysis revealed identity between the sequences obtained for the gp120 of III_B and those reported for the BH10 clone of the III_B isolate (Fig. 2), and the sequences determined for III_{RF} gp110 (Fig. 2) matched the amino acid sequence predicted for the HAT3 clone of the Haitian isolate (13, 26). The alanine and aspartic acid residues at cycles one and three distinguish the HAT3 clone from the BH10 clone (Fig. 2). The first 20 amino acids for envelope glycoproteins isolated from infected cell membranes and from culture fluids were identical for each virus variant, indicating no difference in materials isolated from both sources.

The opportunity to further characterize purified envelope glycoproteins by amino acid sequence analysis was provided by the existence of two conserved aspartyl-prolyl sites within the molecule. Asp-Pro peptide bonds are very susceptible to low-pH cleavage (8, 15), providing a ready means of cleaving the envelope glycoprotein to expose its internal sequence. According to the sequence determined by Mo-

FIG. 2. Amino acid sequencing yields for amino-terminal analysis of HIV-1 EEG purified from membrane extracts and culture media of H9 cells infected with III_B and III_{RF} . Samples were first purified by reverse-phase HPLC. Approximately 100 pmol of purified protein was loaded onto the Polybrene-treated glass fiber disk, and Edman degradation was performed for 30 cycles. The yield of PTH amino acids (A.A.) at each cycle was corrected for overlap and quantitated by comparison with an appropriate PTH standard of known concentration. Sequences are shown for envelope glycoproteins obtained from (A) III_B -infected H9 cell membranes, (B) III_{RF} -infected H9 cell membranes, (C) III_B -infected H9 cell culture media, and (D) III_{RF} -infected H9 cell culture media.

TABLE 2. Sequencing yields for the Asp-Pro cleavage products of HIV-1 EEG^a

Cycle no.	III _B gp120						III _{RF} gp110					
	DP-1 sequence ^b	pmol		DP-2 sequence ^b	pmol		DP-1 sequence	pmol		DP-2 sequence	pmol	
		Membrane	Media		Membrane	Media		Membrane	Media		Membrane	Media
1	P	33.3	9.5	P	33.3	9.5	P	20.9	24.2	P	20.9	24.2
2	N	17.5	4.1	E	24.3	5.1	N	10.8	14.4	E	14.9	25.9
3	P	24.8	6.6	I	20.6	4.1	P	19.2	12.2	I	18.9	8.9
4	Q	7.0	2.8	V	11.7	4.9	Q	9.1	9.1	V	16.8	7.7
5	E	17.4	1.6	T	7.1	2.9	E	13.2	11.5	L	15.8	9.8
6	V	18.3	2.5	H			V	15.6	6.0	H		
7	V	11.8	2.6	S			L	12.2	8.9	S	11.8	6.7
8	L	12.5	2.8	F			L	11.8	8.4	F	7.4	5.5
9	V	7.8	2.2	N			E	10.6	3.6	N		5.0
10	N			C			N	3.4		C		

^a PTH amino acids were analyzed on an Applied Biosystems model 120A analyzer with 40% of the product of each cycle injected. The amino acids were identified and yields were calculated from chromatographic analyses by comparison with appropriate PTH standards of known concentration.

^b DP-1 and DP-2 represent the predicted cleavage sequence of the envelope gene of the BH10 and HAT3 molecular clones (25).

drow et al. (13), the Asp-Pro sites are located at residues 85 and 373; these sites will be referred to hereafter as DP-1 and DP-2, respectively. Following N-terminal degradation of each sample, the residual protein on the disk was treated with trifluoroacetic anhydride and hydrolyzed with 0.015 N HCl for 30 min at 110°C, and the resultant fragments were sequenced, starting with the proline residues. The sequencing data confirmed that the envelope glycoproteins were from III_B and III_{RF}; again, we obtained identical results regardless of the source of the glycoprotein (Table 2). Substitution of leucine and glutamic acid in the III_{RF} DP-1 for valine residues in the III_B sequence and leucine for threonine in the DP-2 sequence identifies the two variant glycoproteins as homologous with their respective molecular clones.

Evaluation of immunologic cross-reactions. Antiserum was produced in goats against envelope glycoproteins purified to apparent homogeneity by PAGE. Goats were immunized with multiple doses of PAGE-purified EEG from III_B or III_{RF} or sequentially with both. Iodinated EEG from III_B and III_{RF}, regardless of source, was precipitated by broadly specific serum from an AIDS patient but not by normal human or goat serum (representative data shown in Fig. 3, lanes 1 to 3). When specific goat antisera were allowed to react with purified EEG from the two HIV-1 variants, isolate-specific reactivity was observed. Goat antiserum to III_B gp120 precipitated the homologous antigen, regardless of the source of the glycoprotein (Fig. 3, lanes 4 and 10), and only minimally precipitated the heterologous antigen, III_{RF}

FIG. 3. Autoradiographs of immunoprecipitates of ¹²⁵I-labeled EEG from III_B (gp120) and III_{RF} (gp110) isolated from infected cell membranes and culture media. Goat 517 was immunized with gp120 from cells, goat 560 was immunized with gp110 from media. Antisera was diluted for immunoprecipitation reactions as shown: lane 1, undiluted normal human serum versus gp120 from media; lane 2, undiluted AIDS patient serum versus gp120 from media; lane 3, undiluted normal goat serum versus gp120 from media; lane 4, 1:25 goat 517 versus gp120 from cells; lane 5, 1:25 goat 560 versus gp120 from cells; lane 6, 1:25 goat 505 versus gp120 from cells; lane 7, 1:25 goat 517 versus gp110 from cells; lane 8, 1:25 goat 560 versus gp110 from cells; lane 9, 1:25 goat 505 versus gp110 from cells; lane 10, 1:5 goat 517 versus gp120 from media; lane 11, 1:5 goat 560 versus gp120 from media; lane 12, 1:5 goat 505 versus gp120 from media; lane 13, 1:5 goat 517 versus gp110 from media; lane 14, 1:5 goat 560 versus gp110 from media; lane 15, 1:5 goat 505 versus gp110 from media.

gp110 (Fig. 3, lanes 7 and 13). The consistent relative intensity of the contaminating 60-kDa bands in each group of three lanes in Fig. 3 can be used as an indication of similar probe concentrations allowing comparisons of the EEG band intensity. Goat antiserum to III_{RF} also primarily precipitated the homologous glycoprotein (Fig. 3, lanes 8 and 14) and partially precipitated III_B gp120 (Fig. 3, lanes 5 and 11). Antiserum from the sequentially immunized goat precipitated both envelope glycoproteins (Fig. 3, lanes 6, 9, 12, and 15). The apparently isolate-specific relationships shown by radioimmune precipitation and PAGE were observed when these same antisera were allowed to react with labeled III_B gp120 in a quantitative radioimmune precipitation assay (data not shown).

No differences were observed in the precipitation patterns of envelope glycoproteins isolated from infected cell membranes or from media, but quantitative differences in antisera reactivity were noted. By using equivalent quantities of iodinated protein, antisera could be diluted to 1:25 for reactions with cell membrane-extracted material, although the best results with medium-extracted glycoproteins were obtained with serum dilutions of 1:5 or less. These differences might indicate minor antigenic variations, differences in structure and epitope availability, or the presence of impurities in the preparations. It should also be noted that these experiments were conducted with a single goat in each group and individual variation in their response cannot be discounted.

DISCUSSION

The purification of significant quantities of biologically active EEG from III_B by immunoaffinity chromatography has been described before (18, 21). The potential utility of native HIV-1 EEG in receptor-binding studies, pathogenicity studies, and vaccine development is enormous. However, it is apparent that the *env* gene which codes for EEG and, particularly, those regions of the glycoprotein with predicted antigenicity is the most variable between different HIV-1 isolates (26). The purification of outer membrane glycoprotein from a divergent HIV-1 isolate, III_{RF}, was therefore undertaken to provide the means for evaluating this polymorphism directly by amino acid sequencing and immunologically by producing antisera in goats.

Envelope glycoprotein from either III_B or III_{RF} isolates appears to be identical whether isolated from infected cell membranes or culture media. Amino-terminal sequence analysis of purified EEG isolated from either source showed an identical single sequence for the first 20 cycles for each variant. The observed sequence matched the amino acid sequences predicted for their respective molecular clones (III_B matched the BH10 clone and III_{RF} matched the HAT3 clone). Other accounts of amino acid sequence analysis of native HIV-1 EEG (1, 21) matched the III_B sequences reported here exactly. Additional sequence information, provided by cleaving the isolated glycoprotein samples at two conserved Asp-Pro sites within the polypeptide chain and by subsequent sequence analysis, further verified the fit with predicted sequences.

The amino-terminal sequence of the EEG from III_B derived from membrane or culture fluid fractions also corresponds to the sequence generated at the proposed proteolytic cleavage site for the processing of the signal peptide of gp160 (13, 26). The amino acid sequence analysis of EEG isolated from III_{RF}-infected cell membranes or culture fluids shows that the signal peptide of this isolate is cleaved by proteolysis in an identical fashion to the III_B-derived EEG.

Immunization with EEG isolated from either of the two HIV-1 variants induced the formation of precipitating antibodies in goats that were primarily isolate specific. Antisera made against III_B gp120 partially precipitated gp110 from III_{RF}, suggesting that gp110 has some unique determinants. Sequential immunization of a single goat with gp120 and then gp110 resulted in the generation of antibodies that precipitated EEG from both variants equally well. The isolate specificity of the goat-neutralizing response to III_B gp120 has been documented (6, 12, 17), but that isolate-specific response was restricted to neutralization. Goat antiserum to III_B gp120 bound EEG from III_B and III_{RF} equally well in solid-phase immunoassays (12). Antiserum from the sequentially immunized goat has been shown to neutralize both III_B and III_{RF} viruses but not other virus isolates (P. L. Nara, W. G. Robey, S. W. Pyle, W. C. Hatch, N. M. Dunlop, J. W. Bess, Jr., J. C. Kelliher, L. O. Arthur, P. J. Fischinger, J. Virol., in press). Our results indicate that the goat-precipitating antibody response mimics the neutralizing response in isolate specificity and that sequential immunization results in primarily a double isolate-specific precipitating response.

One outcome of these experiments that is difficult to explain is the apparent increase in mobility of III_{RF} gp110 relative to III_B gp120 in polyacrylamide gels. Aberrant electrophoretic mobilities are common with glycosylated proteins, because the carbohydrate moieties usually prohibit SDS-protein binding, reducing the net negative charge to the molecule and lowering polypeptide mobility (24). The data generated with endoglycosidase F, which removes complex and high-mannose N-linked sugar groups, appear conflicting. While glycosylated III_{RF} gp110 has a smaller apparent molecular size than III_B gp120, the situation is reversed with deglycosylated proteins (62 kDa for III_B and 65 kDa for III_{RF}). Whether the variation in the predicted amino acid sequences of III_B and III_{RF} is sufficient to account for the relative mobility differences of the deglycosylated proteins is unknown. There are 28 predicted potential N-linked glycosylation sites in the gp110 of III_{RF} and only 24 in the gp120 of III_B (13, 26). The greater number of predicted N-linked sites might indicate increased total glycosylations of gp110 compared with gp120. By contrast, the more rapid migration of gp110 in SDS-PAGE signifies a higher net negative charge, possibly indicating decreased total glycosylation of gp110 relative to gp120. Additional carbohydrate studies will be necessary if these observations are to be explained.

The indistinguishability of the molecular weights, patterns of immunoreactivity, and partial sequences of membrane- and culture fluid-derived glycoproteins purified from the same variant strongly suggests that proteins from these two sources are identical and that the release of EEG from the membrane does not detectably alter its structure. Approximately 95% of the protein containing EEG was recovered from culture media, and the remaining 5% was isolated from membrane-infected cells of both virus variants. The amount of purified EEG (gp110) recovered from III_{RF} was approximately double that of gp120 from III_B, whether isolated from infected H9 cell membranes or from culture fluids containing infected cells. Variations in assay results with EEGs isolated from infected H9 cell membranes or culture fluids were attributed to differences in overall purity and not to the use of MgCl₂ or glycine hydrochloride as elution buffer. These data imply that culture fluids of HIV-1-infected cells should be considered as a source of native EEG. The isolate-specific precipitating response of serum from goats vaccinated with native HIV-1 EEG and the double-isolate response of a goat vaccinated sequentially with EEG from

both variants could have implications for subunit vaccine development. Since any vaccine against HIV-1 should be capable of eliciting a broadly reactive immune response, immunization with multiple types of EEG might be required to generate a protective immune response against all HIV-1 virus variants.

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