JOURNAL OF CLINICAL MICROBIOLOGY, May 1987, p. 845–848
0095-1137/87/050845-04$02.00/0

Vol. 25, No. 5

Monoclonal Antibodies to gp110 and gp41 of Human Immunodeficiency Virus

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Received 3 October 1986/Accepted 12 January 1987

Six mouse hybridomas secreting monoclonal antibodies specific for the glycoproteins of human immunodeficiency virus were developed. All six antibodies reacted by radioimmunoprecipitation with the glycoprotein precursor of 150,000 daltons as well as one of the proteolytic processing products of 110,000 daltons (gp110) or 41,000 daltons (gp41). Recombinant polypeptides spanning the *env* coding region were used to locate epitopes on the glycoprotein molecule. The panel of antibodies detected two distinct epitopes of gp41 and one epitope of gp110. We used the antibodies in indirect immunofluorescence assays to evaluate 13 clinical isolates of human immunodeficiency virus from diverse geographic regions, and we found that the gp110 epitope was recognized on all tested isolates, whereas the two gp41 epitopes were detected on 10 of 13 and 4 of 13 isolates.

The etiologic agent of acquired immune deficiency syndrome is a human retrovirus recently renamed human immunodeficiency virus (HIV) and previously called lymphadenopathy-associated virus (LAV), human T-cell lymphotropic virus type III, or acquired immune deficiency syndrome-associated retrovirus (2, 4, 6, 11). These independent isolates share antigenic characteristics, genomic organization, and nucleic acid sequence homology, indicating that a single virus family is responsible for acquired immune deficiency syndrome (14, 18, 22). The antibody response to this virus is indicative of infection by HIV, since virus can be isolated from 50 to 80% of seropositive persons (12, 17). There are reports of viral isolation from persons whose sera lack a detectable antibody titer which emphasize the importance of supplementing the current HIV antibody tests with antigen detection assays (3, 16).

Sequence and restriction enzyme analyses of different clinical isolates of HIV show that there is more variation in the glycoprotein-coding region than the other structural viral proteins (14, 18, 22). Sera from persons infected with the virus, however, most consistently recognize the envelope glycoproteins throughout the disease course (13, 19; unpublished data). These proteins include the glycoprotein precursor of 150,000 to 160,000 daltons (gp150) and the mature viral glycoproteins of 41,000 daltons (gp41) and 110,000 to 120,000 daltons (gp110) (1, 5, 13). It is important to define the immunologically conserved regions of the glycoprotein as potential targets for antigen detection and for vaccine development. It is also important to define variable regions that may be useful for serotyping analysis of strains of HIV.

We have developed a panel of monoclonal antibodies which react to multiple epitopes on the glycoprotein molecule, including gp110 and gp41, as determined by reactivity to recombinant bacterial fusion proteins. A previous report described a monoclonal antibody to an epitope on gp41 (5). We evaluated our antibodies in immunofluorescence assays on clinical isolates of HIV from diverse geographical regions and demonstrated conserved determinants among the isolates.

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MATERIALS AND METHODS

Virus and viral antigens. The viral strain used for immunization and screening in this study was LAV type 1 (LAV-1) grown in a modified CEM cell line (ATCC CCL119) (2). Twelve additional HIV isolates from patients in the United States, Europe, and Africa were grown in peripheral blood lymphocytes (all HIV isolates kindly provided by Luc Montagnier and Jean-Claude Chermann). Recombinant fusion polypeptides spanning the envelope region were prepared by using a modified *Escherichia coli trp* operon (10, 21). Briefly, LAV sequences were first cycled through M13mp18 to add polylinker restriction sites. Final pJH recombinant constructs contained LAV sequences (22) as follows: pEnv-1, 5889-6598; pEnv-2, 6598-7178; pEnv-3, 7178-7698; pEnv-4, 7698-8572; and pEnv-5, 5889-7698. Growth and induction of *E. coli* C600 transformed by the *trp* expression vector and the partial purification of fusion proteins from cell pellets were as previously described (10, 21).

Viral immunogen was prepared by disrupting sucrose gradient-purified LAV with 1% Nonidet P-40 (Sigma Chemical Co., St. Louis, Mo.), pH 7.8, containing 150 mM NaCl and 1% aprotinin. The viral lysate was clarified by centrifugation at 100,000 × *g* for 45 min and then absorbed to lentil lectin-Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N.J.) preequilibrated with the disruption buffer. The column was washed with 20 volumes of buffer.

Production of monoclonal antibodies. Hybrid cell lines producing monoclonal antibodies were prepared by the method of Kohler and Milstein (9) with previously described modifications (7). Spleen cells for the fusion were obtained from a BALB/c mouse that was immunized with the lectin-bound fraction of a soluble lysate from purified HIV virions. Methods for propagation and stabilization of cloned cell lines and for ascites production have been previously described (7).

Antibody assays. Anti-HIV antibodies were detected by enzyme immunoassay on Genetic Systems LAV enzyme immunoassay plates. Plates were incubated with culture fluids (100 μl per well) at 37°C for 45 min and then washed

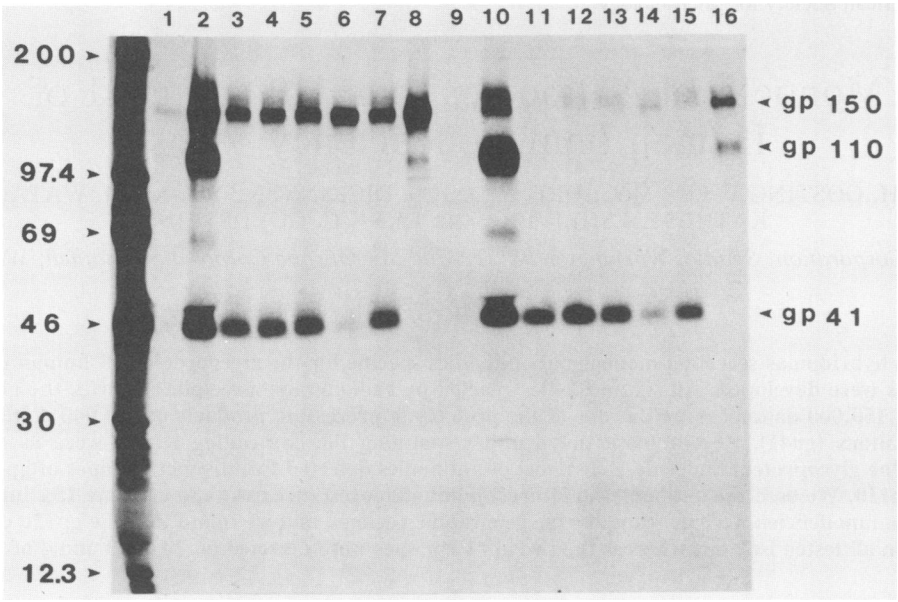

FIG. 1. Immunoprecipitation of [^{3}H]glucosamine-labeled, HIV-infected cell extract (lanes 1 through 8) and purified virus (lanes 9 through 16) by monoclonal antibodies to HIV glycoproteins. Lanes: 1 and 9, reference negative human serum; 2 and 10, reference positive human serum; 3 and 11, HIV-gp41-4; 4 and 12, HIV-gp41-3; 5 and 13, HIV-gp41-5; 6 and 14, HIV-gp41-1; 7 and 15, HIV-gp41-2; and 8 and 16, HIV-gp110-1. ^{14}C-labeled molecular weight standards shown (10^3) include myosin (200,000), phosphorylase B (97,400), bovine serum albumin (69,000), ovalbumin (46,000), carbonic anhydrase (30,000), and cytochrome *c* (12,300).

three times with 0.05% Tween 20 in phosphate-buffered saline. Peroxidase-conjugated goat anti-mouse immunoglobulin G (1:2,000 dilution in 0.05% Tween 20 in phosphate-buffered saline; Zymed Laboratories, Inc., South San Francisco, Calif.) was added (100 μl per well), and the plates were incubated for 45 min at 37°C and washed as described above. Substrate (0.025 M citric acid–0.05 M dibasic sodium phosphate buffer [pH 5.0] containing 14 mg of *o*-phenylenediamine and 10 μl of 30% H_2O_2 per 50 ml) was added, and the plates were incubated for 30 min at room temperature in the dark. The reaction was stopped with 3 N H_2SO_4, and the colorimetric reactions were quantified with an Automated Microplate Reader (model EL310; Bio-Tek Instruments, Inc., Burlington, Vt.).

Indirect immunofluorescence assays were performed on acetone-fixed slides prepared from HIV-infected peripheral blood lymphocytes or CEM cells. Culture supernatant or ascites fluid was applied to cells for 30 min at 37°C, and reactive cells were detected with fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin G F(ab')$_2$ (Zymed).

Biochemical procedures. Immunoprecipitation assays were performed on extracts from CEM cells infected with LAV-1 and radiolabeled with [^{3}H]glucosamine (40 μCi/ml) for 24 h in 1/10 glucose–RPMI 1640 supplemented with 2.0% fetal calf serum, glutamine, sodium pyruvate, and antibiotics. Virus was pelleted (1 h at 100,000 × *g*) from the cell-free culture supernatant, and detergent extracts were prepared in P-RIPA buffer (phosphate-buffered saline containing 1% Triton X-100, 1.0% deoxycholic acid, 0.1% sodium dodecyl sulfate, and 1.0% aprotinin), clarified by centrifugation, and stored at −70°C. For the assay, 100 μl of extract was incubated with 4 μl of mouse ascites for 1 h on ice. Four microliters of rabbit anti-mouse immunoglobulin G (Zymed) was added and allowed to incubate for 30 min. Immunoprecipitin (100 μl; Bethesda Research Laboratories, Inc., Gaithersburg, Md.), suspended in P-RIPA containing 1.0% ovalbumin, was added to each tube, and incubation was continued for an additional 30 min. The bound immune complexes were washed and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described previously (15), modified with a 7.0 to 15.0% gradient gel and two washes in lithium chloride buffer (0.1 M Tris [pH 8.0], 0.5 M LiCl, 1.0% 2-mercaptoethanol). After electrophoresis, the gels were fixed, soaked in En^{3}Hance (New England Nuclear Corp., Boston, Mass.), dried, and exposed to XR-5 film.

Immunoblot assays were performed with purified virus or recombinant fusion polypeptides added to sample buffer (2% sodium dodecyl sulfate, 5% 2-mercaptoethanol, 0.002% bromophenol blue, and 10% glycerol in 0.06 M Tris hydrochloride [pH 6.8], final concentrations), heated at 100°C for 5 min, and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 7.0 to 15.0% polyacrylamide gradient gels. Antigens in the gel were transferred to nitrocellulose membranes by electrophoresis for 4 h at 25 V in 25 mM sodium phosphate buffer (pH 7.0). The antibodies were evaluated by immunoblot methods as previously described (8) except that goat anti-mouse immunoglobulin G-horseradish peroxidase was used.

RESULTS AND DISCUSSION

Hybridizations were performed between NS-1 myeloma cells and lymphocytes from a BALB/c mouse immunized with a lectin-bound solubilized fraction of HIV. Culture fluids from hybrid cells were tested for anti-HIV antibodies by an enzyme immunoassay utilizing replicate plating techniques with disrupted HIV and uninfected CEM cell preparations. From one fusion, six hybrids were produced which secreted monoclonal antibodies reacting specifically with HIV. The hybrid cell lines were subsequently cloned by limiting dilution until phenotypically stable. The immunoglobulin subclass of the six antibodies was immunoglobulin G1 as determined by agar gel immunodiffusion with specific typing sera.

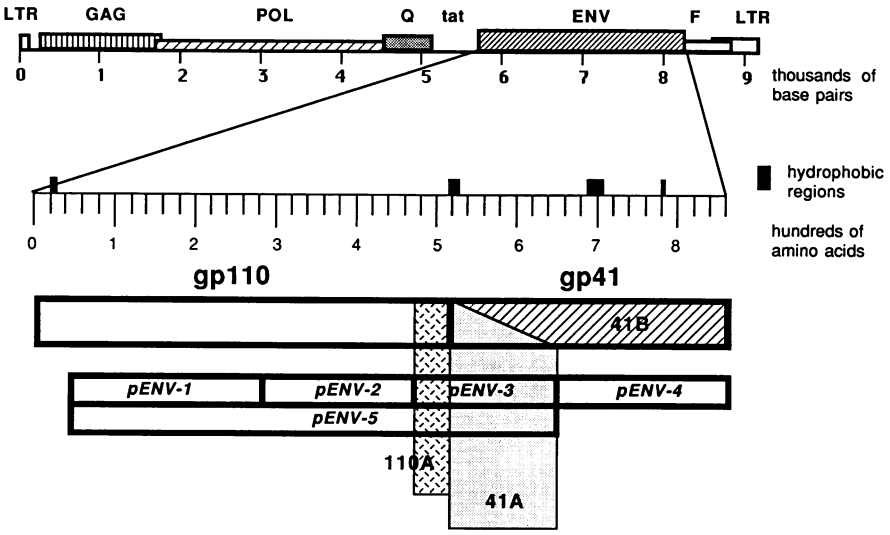

FIG. 2. Graphic representation of the HIV genome (14, 18, 20, 22) with an underlying expanded scale of the *env* region. The bacterial *env* recombinant fusion proteins (pEnv-1 through -5) are shown in relative position to the mature *env* products, gp110 and gp41. Monoclonal antibody-reactive regions are labeled 110A, 41A, and 41B.

The antibodies were analyzed for reactivity to specific glycoproteins by immunoprecipitation analysis with [^{3}H]glucosamine-labeled, HIV-infected cell and purified viral extracts. A representative immunoprecipitation with the six monoclonal antibodies is shown in Fig. 1. Five antibodies (HIV-gp41-1 through -5) reacted with gp41 in both infected cell and purified viral extracts. One antibody (HIV-gp110-1) reacted with gp110 in both preparations. All the antibodies strongly reacted with the precursor gp150 in the infected cell extract, whereas this reaction was weak in the purified viral extract. None of the antibodies recognized both gp41 and gp110. This observation is consistent with previous reports of the glycoprotein processing of the precursor gp150 into two products, gp110 and gp41.

To further define the epitopes to which these antibodies reacted, immunoblot analysis was performed on purified HIV and on recombinant bacterial fusion polypeptides spanning the entire *env* coding region. The monoclonal antibodies

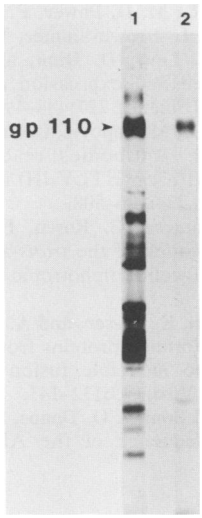

FIG. 3. Immunoblot of LAV-1 with reference positive human serum (lane 1) and monoclonal antibody HIV-gp110-1 (lane 2).

reacted with three distinct epitopes on the glycoprotein molecule (Fig. 2). The gp110-specific antibody reacted with gp110 by immunoprecipitation and immunoblot (Fig. 3) and also reacted by immunoblot with a bacterial fusion protein (pEnv-3). This recombinant protein includes only the carboxyl terminus of gp110 and spans the putative proteolytic cleavage site of gp150 into gp110 and gp41. Therefore the epitope (110A) to which this antibody reacts can be defined as the carboxyl terminus of gp110. This region has been shown to be relatively conserved among isolates by nucleic acid sequence analysis (14, 18, 22).

The gp41-reactive antibodies recognized two epitopes on the molecule. One antibody, HIV-gp41-1, did not react well by immunoprecipitation but reacted strongly by immunoblot to gp41 as well as the bacterial fusion protein pEnv-3. These observations indicated that antibody HIV-gp41-1 reacted with an epitope (41A) which is better exposed after the solubilization or sodium dodecyl sulfate-polyacrylamide gel electrophoresis procedures. The HIV-gp41-1 antibody also recognized a reactive site on the recombinant fusion protein, which might not share native conformation with gp41, further suggesting that 41A is a linear epitope. The other four antibodies reacted with gp41 strongly by immunoprecipitation, but did not react by immunoblot with gp41 or any of the fusion proteins. These gp41 antibodies were specific for a conformational epitope which was altered during solubilization or gel separation. Competition radioimmunoassay results showed that HIV-gp41-2 through -5 inhibited the binding of one another (but not HIV-gp41-1) to HIV (data not shown). The antibodies therefore recognize a single additional epitope (41B) on the gp41 molecule.

Sequence and restriction enzyme analysis of the genomes of several HIV isolates show that the greatest diversity is in the glycoprotein and particularly in the gp110 region (14, 18, 22). We have tested by indirect immunofluorescence assay the panel of monoclonal antibodies to the glycoprotein on 13 different isolates of HIV from diverse geographic regions to assess which epitopes may be conserved and which ones may be variable. The anti-gp110 monoclonal antibody reacted with all 13 isolates tested, including the isolates from Europe and Africa. This epitope appears to be conserved

among isolates of HIV. Furthermore, there is evidence that epitope 110A is exposed on the surface of intact virus since preincubation of virus with HIV-gp110-1 inhibits the binding of virus to susceptible cells (12a). The anti-gp41 monoclonal antibody which reacted with a linear epitope (41A) reacted with 10 of the isolates. The other anti-gp41 antibodies had variable reactions in immunofluorescence assays on multiple isolates of HIV. In the immunofluorescence assays, positive staining was most often associated with the presence of large syncytial cells. Positive cells exhibited a delicate apple-green fluorescence associated with the membrane and frequently the cytoplasm as well. Staining was either diffuse or punctate and was often most intense in a patch in the perinuclear region. Failure of some of the antibodies to stain the isolates could be due to viral expression associated with the stage of infection or to epitope polymorphism.

In summary, the envelope glycoproteins are of interest because they are consistently recognized by patient sera and because the glycoprotein-coding region is more variable than that of the viral structural proteins. Of the three distinct epitopes on the glycoprotein molecule recognized by our panel of monoclonal antibodies, at least one epitope is highly conserved among the clinical isolates of HIV which were tested. This epitope is located on the carboxyl terminus of gp110, and its reactive monoclonal antibody may be particularly useful for antigen detection assays.

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