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Humoral immune response to the entire human immunodeficiency virus envelope glycoprotein made in insect cells

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ABSTRACT **The human immunodeficiency virus envelope gene was expressed in insect cells by using a *Baculovirus* expression vector. The protein has an apparent molecular mass of 160 kDa, appears on the surface of infected insect cells, and does not appear to be cleaved to glycoproteins gp120 and gp41. Goats immunized with the 160-kDa protein have high titers of antibody that neutralizes virus infection as measured by viral gene expression or cell cytolysis. In addition, immune sera can block fusion of human immunodeficiency virus-infected cells in culture. Both neutralization and fusion-blocking activities are bound to and eluted from immobilized gp120.**

Acquired immunodeficiency syndrome (AIDS) results from infection with the retrovirus most recently referred to as human immunodeficiency virus (HIV) and formerly referred to as human T-cell leukemia/lymphotropic virus (HTLV). Studies on the feasibility of a subunit vaccine to protect against viral infection have mainly focused on the outer envelope proteins of the HIV. These proteins are derived from a precursor glycoprotein, gp160, that is proteolytically cleaved to an external glycoprotein, gp120, and the transmembrane glycoprotein gp41 (1–3). Purified gp120 has been shown to bind the cell receptor, CD4 (4, 5), and to elicit neutralizing antibodies from immunized animals (6). Recombinant proteins that contain portions of gp120 have been produced in mammalian cells (7) and in *Escherichia coli* (8). Synthetic peptides modeling portions of gp120 or gp41 have also raised modest neutralizing titers when used as immunogens (9). The focus of this study was to obtain the entire primary translation product of the envelope gene, gp160, and to evaluate the immunogenic properties of this glycoprotein.

To obtain gp160, we used as the eukaryotic viral expression vector the nuclear polyhedrosis virus of *Autographa californica* (10, 11). Recombinant *Baculovirus* expression vectors are used to infect cultured insect cells and have been shown to produce large amounts of recombinant protein (12). Studies indicate that insect cells properly modify and secrete recombinant proteins such as interferon B (11), human interleukin 2 (13), and human MYC protein (14). Cloning of the fowl plague virus hemagglutinin gene and transcription initiation from the viral polyhedrin promoter led to production of biologically active recombinant protein on the surface of infected insect cells. Chickens immunized with the recombinant protein were protected from challenge with the fowl plague virus (15).

We cloned the entire HIV envelope gene behind the *Baculovirus* polyhedrin promoter in the transfer vector pAc610 (16). Upon cotransfection of recombinant plasmid DNA and viral DNA to *Spodoptera frugiperda* cells, recombinant virus containing the HIV envelope gene was formed and subsequently isolated. Infection of *Spodoptera* cells with the recombinant virus results in expression of a glycosylated protein with an apparent molecular mass of 160 kDa. We report here that immunization of goats with purified recombinant envelope protein generates a strong humoral immune response. Sera to the recombinant 160-kDa (r160) protein blocks fusion of HIV-infected cells in culture.

MATERIALS AND METHODS

Construction of Recombinant Baculovirus. Recombinant *Baculovirus* is obtained by first cloning the isolate HTLV-III_B envelope gene to a plasmid transfer vector, followed by cotransfection of plasmid and *Baculovirus* DNA into the insect cell line *S. frugiperda*. The plasmid transfer vector used in this study was pAc610 (16) obtained from Texas A & M University. This vector contains a multiple cloning site at position −8 in the 5′ untranslated region of the viral polyhedrin promoter.

A DNA fragment, *Mbo* II (position 5801) to *Xho* I (position 8474) containing the entire encoding region of the envelope gene (17) and a synthetic oligonucleotide linker were cloned into the transfer vector by using the *Eco*RI and *Sal* I restriction sites (Fig. 1). The synthetic oligonucleotides (5′-AATTCAACCTATAAATA-3′, 5′-ATTTATAGGTTG-3′) were used to span the junction between the vector *Eco*RI site and the translation initiation codon of the envelope gene (*Mbo* II). This oligonucleotide also makes the −12 to −1 sequence identical to the original polyhedrin promoter sequence (18). This recombinant plasmid, pAcHT3, was used to transfect *S. frugiperda* cells.

Cotransfection of pAcHT3 and *Baculovirus* DNA was done by the calcium phosphate coprecipitation method exactly as described (16). The resulting viral progeny contain both wild-type and recombinant virus. Recombinant virus was isolated by dilution and propagation in 96-well plates and was identified by DNA hybridization with ^{32}P-labeled HIV envelope sequences as probe. The resulting pure recombinant virus, designated HT3, was used in all experiments.

Production and Purification of Recombinant HIV Envelope Protein. Monolayer cultures of *S. frugiperda* seeded at 1×10^6 cells per ml were infected with HT3 at a multiplicity of 3. Cells were harvested 4 days after infection and resuspended in phosphate-buffered saline at 2×10^7 cells per ml.

Cells were lysed in 20 mM Tris·HCl, pH 7.5/10 mM $Mg(OAc)_2$/1% Triton X-100 and centrifuged for 5 min at 15,000 × *g*. The resulting pellet contained the r160 protein. The precipitate was solubilized in 1% $NaDodSO_4$, and this material was used for immunoblotting, studies with *N*-Glycanase, and preparation of r160 protein for immunization.

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Abbreviations: r160, recombinant 160-kDa protein; AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus; HTLV, human T-cell leukemia virus.

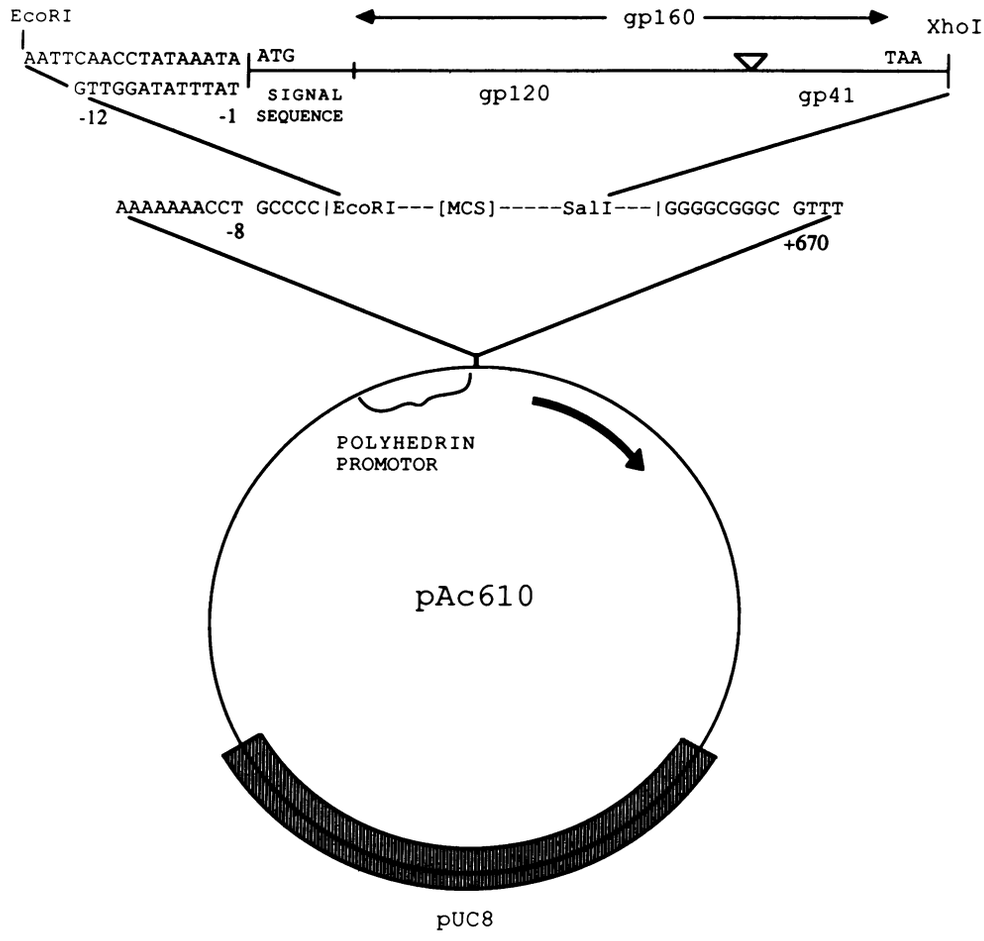

FIG. 1. Transfer vector containing the HIV envelope gene. The vector pAc610 was used as a vehicle for insertion of the HIV envelope gene into the *Baculovirus* genome. Expanded above is the multiple cloning site (MCS) of the vector depicting the two sites used for inserting the HIV envelope gene, *Eco*RI and *Sal* I. The uppermost line shows the envelope gene and the synthetic oligonucleotide used to span the region from *Eco*RI vector linkage to the start codon of the envelope gene. The constructed plasmid is termed pAcHT3.

Recombinant protein for immunization of goats was further purified by electrophoresis. The lysis pellet from about 3×10^8 cells was resuspended in 1% $NaDodSO_4$ and electrophoresed through a preparative $NaDodSO_4$/7.5% polyacrylamide gel. The gel was soaked overnight in 0.25 M KCl to observe the r160 band. The gel slice was mixed with 0.1% $NaDodSO_4$, disrupted by sonication, and used to immunize goats. Each of two goats were immunized with 150 μg of r160 in Freund's complete adjuvant and received boosters at 30-day intervals for 6 months with 100 g of r160 in incomplete Freund's adjuvant.

Other Proteins and Sera. The generation and analysis of sera to polyacrylamide gel-purified HTLV-III_B gp120 envelope (6) and recombinant proteins PB1 (8) and 121 (19) have been described. A monoclonal antibody that binds both gp120 and PB1 was kindly provided by R. Ting, Biotech Research (Rockville, MD). Human serum was from a HIV-seropositive patient characterized as having AIDS-related complex. *N*-Glycanase (peptide *N*-glycosidase, EC 3.5.1.52) was obtained from Genzyme (Boston).

RESULTS

Expression of HIV Envelope in Insect Cells. Purified recombinant *Baculovirus* HT3 containing the HTLV-III_B envelope gene was used to infect monolayers of *S. frugiperda*. Cells were harvested 4 days after infection and examined for expression of HIV envelope polypeptides. Proteins from lysed cells were separated by $NaDodSO_4$/9% polyacrylamide gel and either stained with Coomassie blue or transferred to nitrocellulose and probed with antisera specific for the central portion of the HIV envelope gene (Fig. 2 *A* and *B*). A protein with an apparent molecular mass of 160 kDa was immunoreactive with serum specific for HIV envelope sequences.

The recombinant 160-kDa protein was analyzed for the presence of carbohydrate by digestion with *N*-Glycanase and staining with the carbohydrate-specific stain perchloric acid. Infected cell lysate was digested with *N*-Glycanase as described in Fig. 2. After electrophoresis, proteins were stained with Coomassie blue or transferred to nitrocellulose and probed with envelope-specific antisera. When the extracts were treated with *N*-Glycanase prior to electrophoresis, the immunoreactive 160-kDa band migrated with an apparent molecular mass of 100 kDa. An *N*-Glycanase treatment of extracts containing β-galactosidase expressed from a recombinant *Baculovirus* (16) did not alter the mobility of the β-galactosidase protein (Fig. 2*C*). A duplicate gel was stained with perchloric acid, and the 160-kDa protein but not the *N*-Glycanase-treated 100-kDa protein stained pink, indicating the presence of carbohydrate (data not shown).

The location of the glycosylated recombinant protein r160 in HT3-infected insect cells was examined by immunofluorescence of intact cells. HT3-infected cells were harvested 2 days after infection and incubated with a monoclonal antibody specific for the central portion of gp120 (8). Fig. 3 shows that the majority of infected insect cells bound the antibody. Immunofluorescence of formalin-fixed cells with polyclonal

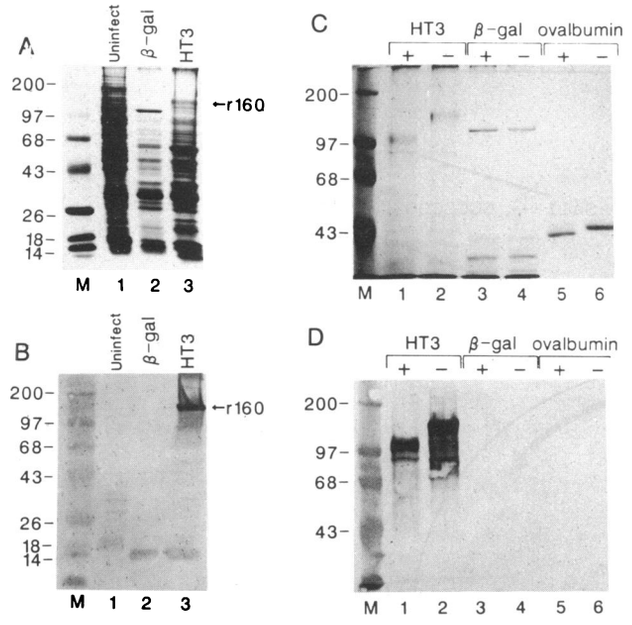

FIG. 2. Characterization of r160 produced in insect cells. Insect cells were infected with recombinant *Baculovirus* as described. Samples of cells (5×10^5 cells) were boiled in 2% $NaDodSO_4$ and electrophoresed through $NaDodSO_4$/9% polyacrylamide gels (20). Duplicate gels were either stained with Coomassie blue or transferred to nitrocellulose and probed with antibody to HIV envelope (8). (*A*) Coomassie blue-stained gel. (*B*) Immunoblot. Lanes: 1, uninfected cells; 2, cells infected with β-galactosidase (β-gal) (16) recombinant *Baculovirus*; 3, cells infected with HT3. Infected insect cells were lysed in 1% Triton X-100, and the pellet was resuspended in 0.5% $NaDodSO_4$. Samples were treated 30 min at 37°C with *N*-Glycanase and electrophoresed on $NaDodSO_4$/9% polyacrylamide gels. Duplicate gels were stained with Coomassie blue (*C*) or were transferred to nitrocellulose (*D*) and probed with antibody to the HIV envelope as above. Lanes: 1, HT3 and *N*-Glycanase; 2, HT3; 3, β-galactosidase and *N*-Glycanase; 4, β-galactosidase; 5, ovalbumin and *N*-Glycanase; 6, ovalbumin.

serum to the central portion of gp120 also localized the r160 protein to the surface of infected insect cells (data not shown).

Immunoblots of HT3-infected insect cells and HIV-infected H9 cells were probed with sera specific for different portions of the envelope gene and human sera positive for HIV antibodies (Fig. 4). The r160 protein was reactive with antisera raised to the deglycosylated 120-kDa envelope protein and antisera raised to an *E. coli* recombinant from the central portion of gp120, PB1 (8). In addition, r160 bound antibodies from serum specific for gp41 (anti-121)—the carboxyl-terminal portion of the HIV envelope gene. When human serum was used as the probe, r160 was immunoreactive and comigrated with gp160 from HIV-infected cells.

The mobility in polyacrylamide gels, increased electrophoretic mobility after *N*-Glycanase treatment, and immunoreactivity of recombinant gp160 suggests that insect cells glycosylate r160 and insert it into the insect cell membrane but do not cleave it into gp120 and gp41.

Immune Serum to r160 Neutralizes HIV Infection of Cells in Culture. Goats were immunized with purified r160, and the serum was assayed for the ability to neutralize infection of cells by $HTLV\text{-}III_B$. The ability of this antiserum to neutralize HIV infection was assayed by measuring viral gene expression or viral-induced cell death. Viral infection of the H9 clone of the HT cell line (21) was monitored by expression of either the p24 core protein or reverse transcriptase after 7–10 days. The other assay used an interleukin 2-dependent helper T-cell line, ATH8, which is highly susceptible to

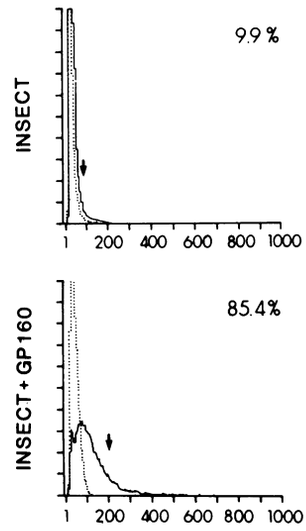

FIG. 3. Cell surface immunofluorescence (—) showing reactivity of anti-gp120 monoclonal antibody on normal insect cells (*Upper*) and insect cells infected with HT3 (*Lower*). Linear green fluorescence (*x* axis) was plotted versus relative cell number (*y* axis). The percentages of cells staining are shown. The arrows represent the mean fluorescence channel. ·····, Autofluorescence. Cells were analyzed on an Ortho Cytofluorograf H50-4.

cytolysis by HIV. Protection from cytolysis by immune sera was measured by visual inspection after 10 days.

Fig. 5 shows the neutralization of viral gene expression with various dilutions of immune sera from AIDS patients and from goats immunized with native gp120 or r160. Both animals immunized with r160 from insect cells show significantly higher titers than sera from gp120-immunized animals. Reduction in viral gene expression from the divergent variant $HTLV\text{-}III_{RF}$ was not observed with sera from either gp120- or r160-immunized animals (data not shown). Table 1 summarizes the neutralization titers obtained by using the three measurements of p24 expression, reverse transcriptase expression, and cytolysis. Although the sensitivities of the assays differ, the relative neutralization titer consistently showed that r160-immunized animals have ≈5-fold higher titers than antisera to gp120. The titer of sera to r160 shown

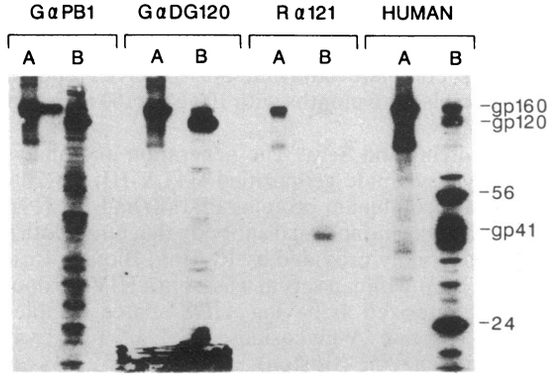

FIG. 4. Immunoreactivity of r160 protein from insect cells. Duplicate samples of insect cells expressing r160 (lane A) and HIV-infected H9 cells (lane B) were electrophoresed (20) and transferred to nitrocellulose. Filters were probed with the sera from the following sources at a 1:50 dilution: goat anti-PB1 (GαPB1); goat anti-deglycosylated 120 (GαDG120); rabbit anti-121 (Rα121); and human AIDS-related-complex patient. After being washed, the filters were incubated with ^{125}I-labeled protein A for 1 hr, washed, and exposed to film overnight.

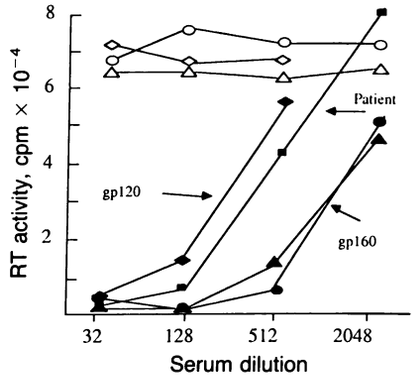

FIG. 5. Neutralization of HTLV-III$_B$ infection with immune goat sera. Preimmune (open symbols) and immune serum (closed symbols) from goats immunized with gp120 or r160 (two goats) were tested for virus neutralization in cell culture. The indicated dilutions of goat serum and patient serum were mixed with virus at 37°C for 30 min, and the mixture was added to H9 cells. After 7–10 days, cultures were measured for reverse transcriptase (RT) activity (6).

in Fig. 5 was determined after immunization and a single-booster immunization. The titer did not change significantly after administration of five additional boosters at monthly intervals.

Antisera to r160 Inhibits Fusion of HIV-Infected Cells. CD4-positive T lymphocytes can be infected with HIV by either receptor-mediated viral entry or receptor-mediated cell fusion with an infected cell expressing gp120 (24–26). Therefore, we examined whether immune serum to r160 could block fusion of HIV-infected cells and CD4-positive cells. Chronically infected CEM cells (8) were mixed with uninfected cells that express the HIV viral receptor CD4. Results of incubating HTLV-III$_B$-infected cells with various dilutions of antiserum to r160 are shown in Table 2. Fusion inhibition was demonstrated at a 1:30 dilution at the first bleeding for antiserum. Fusion was also blocked by incubation with AIDS patient serum but not with serum to isolated gp120. Limited fusion inhibition could be observed with anti-gp120 antiserum 24 hr after mixing. As with neutralization, fusion inhibition was observed with isolate HTLV-III$_B$ and not with the distinct variants HTLV-III$_{RF}$ and HTLV-III$_{MN}$ (data not shown).

The question can be raised as to the location in r160 of the antigenic epitopes that elicit neutralizing and fusion-inhibiting sera. Immune serum to r160 was fractionated by passage over a column containing immobilized gp120 protein. Both the column effluent and eluate were assayed for fusion inhibition and neutralization (Table 3). The column eluate contained both fusion-blocking and neutralizing activities, whereas the effluent did not. This suggests that antibodies neutralizing viral infection and antibodies blocking cell fusion bind to the gp120 portion of the envelope gene.

Table 1. Neutralizing antibody titers elicited by r160

Immunogen	HIV-neutralizing titers		
	Immunofluorescence of p24	Reverse transcriptase	Cell cytolysis
r160	1:255	1:1500	1:320
r160	1:190	1:1600	1:640
gp120	1:50	1:350	1:120
AIDS sera	NT	1:450	NT

Neutralizing titers by reverse transcriptase were calculated from the assay in Fig. 5 and are the serum dilutions at which HIV infection is 60% of that using preimmune serum from the same animal. Neutralizing assays measuring p24 were done as described (21). Assays measuring cytolysis of ATH8 cells (22) after 10 days were done as described (23). NT, not tested.

Table 2. Activity of serial antisera from two goats immunized with r160

Serum	Bleeding date, wk after immunization	Cell fusion assay, mean no. of giant cells at serum dilution		
		1:10	1:30	1:90
Goat anti-r160 993 antiserum	6	0	0	47
	9	0	5	48
	11	0	55	58
	15	0	50	60
	19	0	8	54
Goat anti-r160 994 antiserum	6	0	7	49
	9	0	1	48
	11	0	19	62
	15	2	49	63
	19	0	7	36
Preimmune goat serum		63	72	64
Human*		0	42	61
Anti-gp120 antiserum†		57	55	60

Chronically infected CEM cells (HTLV-III$_B$), uninfected CEM cells, and the indicated dilution of sera were incubated at 37°C. The number of giant cell foci was determined after 48 hr.
*Serum from patient with AIDS-related complex.
†Serum from goat immunized with purified gp120.

DISCUSSION

We have used a recombinant *Baculovirus* vector to infect cultured insect cells and produce the entire HIV envelope gene product. The recombinant protein is located on the surface of infected insect cells and migrates on NaDodSO$_4$/polyacrylamide gels as a 160-kDa protein. The electrophoretic mobility of r160 after treatment with *N*-Glycanase and the binding of antibodies from animals immunized with portions of either gp120 or gp41 shows r160 contains epitopes from both the amino and carboxyl portions of native envelope protein. These data suggest that HIV envelope protein made in insect cells is glycosylated and inserted into the cell membrane but is not cleaved to gp120 and gp41 as observed in mammalian cells.

Immune sera to r160 were assayed for HIV virus neutralization by viral gene expression and T-cell cytolysis. In comparison with gp120, deglycosylated 120 protein, or serum from an AIDS-related complex patient, the serum from r160-immunized goats showed 5-fold higher neutralization titers. Perhaps the presentation of antigen as the entire 160-kDa protein or the specific glycosylation patterns of the insect cell makes the r160-neutralizing epitopes more antigenic. Further immunization studies are necessary to discern whether this increased neutralizing titer is intrinsic to the immunogen, r160, or a result of immunization protocol. Antiserum to r160 shows high neutralizing titers only against the homologous HIV variant. Neutralization of the HIV

Table 3. Fractionation of r160 serum

Sera	Neutralization titer	Fusion inhibition, no. of foci
r160	680	0
Effluent	0	63
Eluate	350	0

Purified native gp120 was immobilized to CNBr-activated Sepharose, and 0.5 ml of r160 serum was fractionated as described (23). Fusion inhibition was measured at a 1:10 dilution of serum.

variants HTLV-III$_{MN}$ and HTLV-III$_{RF}$ was detectable only with the cell cytolysis neutralization assay (data not shown). This weak cross-neutralization was not observed with antisera to gp120 or deglycosylated 120 proteins. Sera of AIDS patients generally showed neutralization of multiple virus variants (27). Whether a patient's serum is directed to conserved viral epitopes distinct from the variant specific neutralizing epitope or results from exposure to multiple viral variants is unclear.

Cells can be infected by cell–cell fusion, thus avoiding any inhibition by neutralization of free virus. Fusion of HIV-infected cells and susceptible T cells in culture is blocked by antisera to r160, suggesting that immune sera can block viral propagation by either route of virus entry. As with neutralization of virus infection, blockage of viral-induced cell fusion by anti-r160 is variant specific. By analogy with other virus infections that demonstrate variations in surface proteins, a strong neutralizing antiserum will protect against closely related variants albeit at reduced titers. It is not yet possible to group variants of HIV by patterns of cross-neutralization. Further experimentation is necessary to discern if a broader humoral immune response can be obtained with multiple variants of r160 proteins.

Fractionation of immune sera to r160 by adsorption to immobilized gp120 protein suggests the location of antigenic epitopes within the envelope gene. Analysis of fractionated serum for neutralization and cell-fusion blockade showed that both antiserum activities bound to gp120. This suggests that epitopes eliciting these activities are located within the gp120 portion of the envelope protein. Sera that can block cell fusion always show neutralization of virus infection, although the reciprocal is not true. The *in vitro* assays of fusion blockade and neutralization of virus infection measure two different activities whose relatedness is still unclear.

In addition to a humoral response to r160 proteins, the immunized animals have lymphocytes primed to proliferate in response to HIV antigens (data to be presented elsewhere). The cellular response does not appear restricted to a single viral variant. A broad cell-mediated immune response together with high virus-neutralizing titer make r160 proteins attractive candidates for a vaccine to AIDS. The critical determination necessary is whether the humoral and cellular immune response attained with r160 is protective to viral challenge.

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1. Allan, J. S., Coliga, J. E., Barin, F., McLane, M. F., Sodrowski, J. G., Rosen, C. A., Haseltine, W. A., Lee, T. H. & Essex, M. (1985) *Science* **228**, 1091–1093.
2. Veronese, F. D., DeVico, A. L., Copeland, T. D., Oroszlan, S., Gallo, R. C. & Sarngadharan, M. G. (1985) *Science* **229**, 1402–1405.
3. Robey, W. G., Safai, B., Oroszlan, S., Arthur, L. O., Gonda, M. A., Gallo, R. C. & Fischinger, P. J. (1985) *Science* **228**, 593–595.
4. Dalgleish, A. G., Beverly, P. C. L., Clapham, P. R., Crawford, D. H., Greaves, M. F. & Weiss, R. A. (1984) *Nature (London)* **312**, 763–768.
5. McDougal, J. S., Mawle, A., Cort, S., Nicholson, J., Cross, D., Scheppler, J., Hicks, D. & Sligh, J. (1985) *J. Immunol.* **135**, 3151–3157.
6. Robey, W. G., Arthur, L. O., Matthews, T. J., Langlois, A., Copeland, T. D., Lerche, N. W., Oroszlan, S., Bolognesi, D. P., Gilden, R. V. & Fischinger, P. J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7023–7027.
7. Lasky, L. A., Groopman, J. E., Fennie, C. W., Benz, P. M., Capon, D. J., Dowbenko, D. J., Nakamura, G. R., Nunes, W. M., Renz, M. E. & Berman, P. W. (1986) *Science* **233**, 209–212.
8. Putney, S. D., Matthews, T. J., Robey, W. G., Lynn, D. L., Robert-Guroff, M., Mueller, W. T., Langlois, A. J., Ghrayeb, J., Petteway, S. R., Weinhold, K. J., Fischinger, P. J., Wong-Staal, F., Gallo, R. D. & Bolognesi, D. P. (1986) *Science* **234**, 1392–1395.
9. Chanh, T. C., Dreesman, G. R., Kanda, P., Linette, G. P., Sparrow, J. T., Ho, D. D. & Kennedy, R. C. (1986) *EMBO J.* **5**, 3065–3071.
10. Smith, G. E., Fraser, M. J. & Summers, M. D. (1983) *J. Virol.* **46**, 584–593.
11. Smith, G. E., Summers, M. D. & Fraser, M. J. (1983) *Mol. Cell. Biol.* **3**, 2156–2165.
12. Pennock, G. D., Shoemaker, C. & Miller, L. K. (1984) *Mol. Cell. Biol.* **4**, 399–406.
13. Smith, G. E., Ju, G., Ericson, B. L., Moschera, J., Lahm, H., Chizzonite, R. & Summers, M. D. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8404–8408.
14. Miyamoto, C., Smith, G. E., Farrell-Towt, J., Chizzonite, R., Summers, M. D. & Ju, G. (1985) *Mol. Cell. Biol.* **5**, 2860–2865.
15. Kuroda, K., Hauser, C., Rott, R., Klenk, H. & Doerfler, W. (1986) *EMBO J.* **5**, 1359–1365.
16. Summers, M. D. & Smith, G. E. (1986) *Tex. Agric. Exp. Stn. Bull.*, no. 1555.
17. Ratner, L., Haseltine, W., Patarca, R., Livak, K. J., Starcich, B., Josephs, S. F., Doran, E. R., Rafalski, J. A., Whitehorn, E. A., Baumeister, K., Ivanoff, L., Petteway, S. R., Pearson, M. L., Lautenberger, J. A., Papas, T. S., Ghrayeb, J., Chang, N. T., Gallo, R. C. & Wong-Staal, F. (1985) *Nature (London)* **313**, 277–284.
18. Iddekinge, B., van Hooft, J. L., Smith, G. E. & Summers, M. D. (1983) *Virology* **131**, 561–565.
19. Chang, T. W., Kato, I., McKinney, S., Chanda, P., Barone, A. D., Wong-Staal, F., Gallo, R. C. & Chang, N. T. (1985) *Biotechnology* **3**, 905–909.
20. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
21. Robert-Guroff, M., Brown, M. & Gallo, R. C. (1985) *Nature (London)* **316**, 72–74.
22. Mitsuya, H. & Broder, S. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1911–1915.
23. Krohn, K. (1987) *Vaccines* **87**, in press.
24. McDougal, J. S., Kennedy, M. S., Sligh, J. M., Cort, S. P., Mawle, A. & Nicholson, J. K. A. (1986) *Science* **231**, 382–385.
25. Sodroski, J., Goh, W. C., Rosen, C., Campbell, K. & Haseltine, W. A. (1986) *Nature (London)* **322**, 470–474.
26. Lifson, J. D., Feinberg, M. B., Reyes, G. R., Rabin, L., Banapour, B., Chakrabarti, S., Moss, B., Wong-Staal, F., Steimer, K. S. & Engleman, E. G. (1986) *Nature (London)* **323**, 725–728.
27. Matthews, T. J., Langlois, A. J., Robey, W. G., Chang, N. T., Gallo, R. C., Fischinger, P. J. & Bolognesi, D. P. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9709–9713.