Changes in the Cytopathic Effects of Human Immunodeficiency Virus Type 1 Associated with a Single Amino Acid Alteration in the Ectodomain of the gp41 Transmembrane Glycoprotein

JIE CAO,¹ BALDEV VASIR,¹ AND JOSEPH G. SODROSKI^{1,2*}

Division of Human Retrovirology, Dana-Farber Cancer Institute, Department of Pathology, Harvard Medical School,¹ and Department of Cancer Biology, Harvard School of Public Health,² Boston, Massachusetts 02115

Received 5 November 1993/Accepted 1 April 1994

A human immunodeficiency virus type 1 mutant with a single amino acid change (designated 596 W/M) in the ectodomain of the gp41 transmembrane envelope glycoprotein replicated in T-cell lines and in CD4-positive peripheral blood mononuclear cells identically to the wild-type virus. However, the cytopathic effects associated with infection by the mutant virus were altered, with a marked attenuation of syncytium formation and a significant delay in single-cell lysis relative to those of the wild-type virus-infected culture. The 596 W/M mutant is apparently defective in a function that is dispensable for virus entry but that contributes to the efficiency of induction of viral cytopathic effects.

AIDS, which is caused by human immunodeficiency virus type 1 (HIV-1), is characterized by depletion of CD4-positive lymphocytes (2, 10a, 14). HIV-1 infects and kills CD4-positive lymphocytes in tissue culture. Recent estimates of the virus burden in HIV-1-infected individuals are consistent with the possibility that viral cytopathic effects contribute, at least in part, to lymphocyte depletion in vivo (6–8, 19, 31).

HIV-1 exhibits a tropism for CD4-bearing cells that is mediated by a specific interaction between the viral envelope and the CD4 receptor (9, 22, 26, 27). The HIV-1 envelope glycoproteins are synthesized as a 160-kDa precursor (gp160) that is cleaved into the exterior envelope glycoprotein (gp120) and a transmembrane glycoprotein (gp41), which are associated by noncovalent interactions (1, 17, 24, 28, 30, 38). The external gp120 subunit contains the CD4 binding site, whereas the transmembrane gp41 subunit anchors the complex to the surface. Following CD4 binding, the gp120 and gp41 glycoproteins mediate the fusion of viral and host cell membranes to allow virus entry into the target cell (18, 34). Amino acid changes in certain regions of the gp120 and gp41 glycoproteins can attenuate the fusion process without affecting CD4 binding (4, 5, 11, 12, 15, 16, 18, 20, 21, 24, 29, 35, 37). One such region is the hydrophobic gp41 amino terminus, referred to as the fusion peptide (11, 13, 23, 24). Membrane fusion mediated by HIV-1 envelope glycoproteins expressed on the surface of infected cells leads to fusion of these cells with adjacent CD4-positive cells, resulting in the formation of multinucleated syncytia, which exhibit nuclear pyknosis and membrane fragility and eventually die (25, 32). In addition to syncytium formation, single-cell lysis contributes to the cytopathic effects associated with HIV-1 infection (23, 33). Single-cell lysis, which is CD4 dependent, also appears to be determined by the capacity of the envelope glycoproteins to mediate membrane fusion. This assertion is based on the observation that a HIV-1 virus with a mutation affecting the gp41 fusion peptide replicated comparably to the wild-type virus under some conditions even though the ability of the mutant virus to mediate cyto-

pathic effects, both syncytium formation and single-cell lysis, was attenuated (23). Additional studies demonstrated that some antiviral agents block superinfection and consequently reduced or eliminated formation of unintegrated viral DNA but did not inhibit single-cell killing (3). Thus, single-cell killing may be mediated by intracellular complexes of the envelope glycoprotein and CD4. Here we provide evidence that a single amino acid alteration in the ectodomain of the gp41 glycoprotein, apart from the amino-terminal fusion peptide, is responsible for altering the cytopathic properties of HIV-1.

Site-directed mutagenesis was used to create an *env* gene, derived from the HXBc2 strain of HIV-1, that encodes an envelope glycoprotein (designated 596 W/M) with a substitution of methionine for tryptophan at position 596, within the gp41 glycoprotein. The wild-type and 596 W/M envelope glycoproteins were expressed in COS-1 cells by using the pSVIIIenv plasmid. The pSVIIIenvΔKS plasmid, which contains a frameshift and deletion within the *env* gene, was used as a negative control. The structure of the envelope glycoproteins was analyzed by immunoprecipitation of radiolabeled cell lysates and supernatants with a mixture of sera from AIDS patients. The processing, cell surface expression, and CD4 binding ability of the 596 W/M mutant were comparable to those of the wild-type glycoproteins, as previously reported (5). A slight increase in the amount of the gp120 glycoprotein in the supernatant of the COS-1 cells suggested that the 596 W/M mutant might exhibit a mild decrease in association of the gp120 and gp41 subunits compared with that of the wild-type envelope glycoproteins (Fig. 1A). The steady-state levels of gp41 expression in the transfected-cell lysates of the 596 W/M mutant were reduced compared with those of the wild-type glycoprotein (Fig. 1B), consistent with a mild decrease in subunit association. Previous studies indicate that gp120 dissociation typically results in a rapid turnover of the gp41 glycoprotein (18). The ability of the envelope glycoproteins to induce the formation of syncytia and to complement the replication of an *env*-defective virus was assessed as described previously (16, 36). As shown in Table 1, the 596 W/M mutant was able to complement the early steps in virus replication in SupT1, Jurkat, and C8166 lymphocytes as efficiently as the wild-type HIV-1 envelope glycoproteins. Figure 2 shows that the efficiency of entry into Jurkat lymphocytes was equivalent

* Corresponding author. Mailing address: Division of Human Retrovirology, Dana-Farber Cancer Institute, 44 Binney St., Boston, MA 02115. Phone: (617) 632-3371. Fax: (617) 632-4338. Electronic mail address: Joseph_Sodroski@DFCI.Harvard.edu.

FIG. 1. Expression of envelope glycoproteins in COS-1 cells, showing radioimmunoprecipitation of COS-1 cell lysates (Cell) and supernatants (Sup) with a mixture of sera from HIV-1-infected individuals (A) or with a human monoclonal antibody (98-6) that recognizes gp41 (B) (39). The cells were transfected with pSVIIIenvΔKS (lanes 1), pSVIIIenv (lanes 2), or pSVIIIenv 596 W/M (lanes 3). The gp160, gp120, and gp41 glycoproteins are shown.

for viruses containing the wild-type and 596 W/M envelope glycoproteins over a range of recombinant virus concentrations. By contrast, the syncytium-forming ability of the 596 W/M mutant was significantly lower than that of the wild-type glycoproteins with these three cell lines as targets. As previously reported (4), the C8166 cells formed syncytia with the COS-1 cells expressing the wild-type envelope glycoproteins more efficiently in this assay than did the Jurkat or SupT1 cells. The relative syncytium-forming ability of the 596 W/M mutant was also higher in the C8166 cell line than in the other two cell lines.

Since the gp41 ectodomain has been implicated in oligomerization of the HIV-1 envelope glycoproteins (10, 29a) and since alterations in the oligomeric state of the glycoproteins might influence function, the abilities of the wild-type and 596 W/M glycoproteins to form oligomers were compared. COS-1 cells were transfected with plasmids expressing the wild-type or 596 W/M envelope glycoproteins, radiolabeled with [³⁵S]cysteine, and lysed in buffer (100 mM Tris-HCl [pH 8.0], 100 mM NaCl, 1 mM CaCl₂, 250 mM octylglucoside). Both cell lysates and supernatants were placed on 5 to 20% (wt/vol) sucrose density gradients and centrifuged at 40,000 rpm in an SW41 rotor for 16 h at 5°C. Fractions were collected and precipitated

FIG. 2. Replication complementation ability. The abilities of negative controls (ΔKS) and the wild-type (W.T.) and 596 W/M mutant envelope glycoproteins to complement the entry of an *env*-deficient, chloramphenicol acetyltransferase (CAT)-encoding virus into CD4-positive Jurkat cells were analyzed by titration of the virus used for infection. The amount of virus used was determined by measurement of reverse transcriptase (RT). The amount of CAT activity in the Jurkat target cells is shown on the y axis.

with a mixture of sera derived from HIV-1-infected individuals. The results, shown in Fig. 3, indicate that the 596 W/M gp160 precursor glycoprotein was identified in fractions of the gradient similar to those containing the wild-type gp160 glycoprotein. Apparently the 596 W/M mutant forms oligomers that exhibit stabilities similar to those of the wild-type glycoproteins, under the conditions used in this experiment.

To examine the effect of the change in tryptophan 596 on viral cytopathic effect, the *KpnI*-*Bam*HI *env* fragment containing the appropriate mutation was cloned into the pHXBc2 plasmid. The pHXBc2 plasmid contains an infectious proviral clone of the HXBc2 strain of HIV-1. As a negative control, the deleted *env* gene derived from the pSVIIIenvΔKS plasmid was also cloned into the pHXBc2 plasmid. CD4-positive Jurkat lymphocytes were transfected with 10 μg of the pHXBc2, pHXBc2 596 W/M, and pHXBc2ΔKS plasmids. The supernatants of the transfected cells were used as sources of viruses for infection of Jurkat cells. Equivalent amounts, determined by a

TABLE 1. Syncytium-forming and replication complementation abilities

Envelope glycoprotein	Syncytium formation (%) ^a			Replication complementation (%) ^b		
	SupT1	Jurkat	C8166	SupT1	Jurkat	C8166
ΔKS	0	0	0	0	0	0
Wild type	100	100	100	100	100	100
596 W/M	1	2	16	105	108	100

^a The ability of COS-1 cells expressing the envelope glycoproteins to form syncytia with the three CD4-positive cell lines was determined as previously described (4). Results are expressed as the percentage of the syncytium-forming ability of the wild-type envelope glycoproteins.

^b The ability of the envelope glycoproteins to complement the entry of an *env*-deficient, chloramphenicol acetyltransferase-encoding virus into the three CD4-positive cell lines was determined as previously described (4, 16). The chloramphenicol acetyltransferase values observed for the pSVIIIenvΔKS negative control were subtracted from all chloramphenicol acetyltransferase values, after which the percent activity of the mutant relative to that of the wild-type envelope glycoprotein was calculated.

FIG. 3. Sucrose gradient fractionation of HIV-1 envelope glycoproteins. Transfected, radiolabeled COS-1 cell lysates (A) and supernatants (B) were fractionated on 5 to 20% sucrose gradients. A total of 21 fractions was collected for each gradient and precipitated with a mixture of sera from HIV-1-infected individuals. Fractions 1 to 14 are shown, and bands corresponding to gp160 and gp120 are indicated.

FIG. 4. Replication of wild-type and 596 W/M mutant viruses in Jurkat lymphocytes. The reverse transcriptase (RT) activity in the supernatants of Jurkat lymphocytes following incubation of 1.1×10^7 cells on day zero with 380,000 cpm (reverse transcriptase units) of the wild-type and 596 W/M mutants viruses is shown. Δ KS refers to a replication-defective virus containing a large deletion within the *env* gene.

reverse transcriptase assay, of the wild-type and 596 W/M mutant virus particles were incubated with Jurkat lymphocytes. A similar volume of the supernatant from the pHXBc2 Δ KS-transfected Jurkat cells was incubated with a control Jurkat culture. Following incubation, viable Jurkat cells were counted daily by trypan blue dye exclusion. Equivalent densities of viable cells (0.5×10^6 to 2×10^6 cells per ml) were maintained in all cultures. Viral replication was monitored by measuring reverse transcriptase activity in pelleted virions every 2 days. The replication of the 596 W/M mutant virus was comparable to that of the wild-type virus (Fig. 4). No reverse transcriptase activity was observed in the supernatants of Jurkat cells incubated with supernatants from the pHXBc2 Δ KS-transfected cells.

The cytopathic effects of infection were monitored by counting the total viable cells and by counting the number of syncytia and trypan blue-positive single cells, as previously described (3, 23). As seen in Fig. 5A, the viability of the Jurkat cultures infected with the wild-type HXBc2 virus was comparable to that of the control uninfected culture until day 4 after infection. At this time, the viability of the wild-type virus-infected culture decreased, and it remained significantly less than that of the uninfected culture. The number of viable cells in the 596 W/M virus-infected culture was similar to that in the uninfected culture until day 6 postinfection, after which a decrease in viable cell counts relative to those in the uninfected culture was observed. The total viable cell counts in the 596 W/M virus-infected cultures were significantly greater than those observed in the cultures infected with the wild-type virus.

The number and size of the syncytia in the culture infected with the 596 W/M mutant were significantly less than those in the culture infected with the wild-type virus (Fig. 5B and 6). The number of syncytia peaked in both cultures at day 6 after infection.

Single-cell lysis was evident in the wild-type virus-infected culture beginning on day 4 after infection (Fig. 5C). The onset of single-cell lysis was delayed by about 3 days in the 596 W/M virus-infected cultures. Single-cell lysis in the latter cultures

FIG. 5. Cytopathic effects of virus infection in Jurkat lymphocytes. (A) Total viable cell counts determined following infection of Jurkat lymphocytes with the wild-type and 596 W/M mutant viruses. Viable cell counts were determined by trypan blue exclusion. (B) Number of syncytia in the virus-infected Jurkat lymphocyte cultures at different times following infection. (C) Percentage of single cells in the infected Jurkat lymphocyte cultures that were trypan blue-positive at different times following infection.

FIG. 6. Syncytia in Jurkat lymphocyte cultures exposed to the Δ KS control (A), the wild-type HXBc2 virus (B), or the 596 W/M mutant virus (C) on day 6 following infection.

FIG. 7. Viral protein expression in infected Jurkat lymphocytes. Viral proteins were precipitated by the serum from an AIDS patient from Jurkat cells exposed to the Δ KS control, the wild-type HXBc2 virus, or the 596 W/M mutant virus. Results are shown for Jurkat cultures at days 6 (A) and 11 (B) of infection.

was not evident until day 8 postinfection, but after this time the percentage of trypan blue-positive single cells rose sharply, reaching levels even higher than those seen in the wild-type virus-infected culture.

To determine whether differences in the level of viral protein expression could be responsible for the observed differences in cytopathic effects between wild-type and mutant virus-infected cultures, the cultures were labeled with [35 S]cysteine overnight at days 6 and 11 postinfection. Cell lysates, cell supernatants, and pelleted virions were precipitated with a mixture of sera from AIDS patients. Cell surface expression of the viral envelope glycoproteins was also examined by fluorescence-activated cell sorter analysis at day 6 postinfection, using an AIDS patient serum. The level of viral protein expression was at least as great for the 596 W/M virus as for wild-type virus at day 6 of infection (Fig. 7). HIV-1-specific immunofluorescence indicated a similar level of viral protein on the infected Jurkat cell surface at day 6 of infection (data not shown). However, at day 11 of infection, a slight increase in the level of viral *gag* and *env* proteins was observed in 596 W/M-infected cultures in both cell lysates and supernatants relative to that in wild-type virus-infected cultures (Fig. 7 and data not shown).

To examine the replicative and cytopathic properties of the 596 W/M mutant virus in primary cells, CD4-positive lymphocytes were prepared from human peripheral blood by Ficoll-Hypaque gradients (Pharmacia) and removal of CD8-positive cells by magnetic cell separation (MACS) antibody-coated microbeads (Miltenyi Biotec, Inc., Auburn, Calif.). CD4-positive cells were stimulated for 2 days with phytohemagglutinin and cultured in RPMI 1640–10% fetal bovine serum–20 U of interleukin-2 per ml. The cells were then infected with equivalent amounts of reverse transcriptase from Jurkat supernatants producing either the wild-type HXBc2 virus or the 596 W/M mutant. Parallel cultures were incubated with supernatants from Jurkat cells that had been transfected with the pHXBc2 Δ KS control plasmid. The 596 W/M mutant exhibited slightly higher-level and more sustained production of reverse transcriptase in CD4-positive primary lymphocytes than did the wild-type virus. A typical experiment is shown in Fig. 8A.

FIG. 8. Infection of primary CD4-positive peripheral blood lymphocytes. (A) Reverse transcriptase (RT) production from CD4-positive peripheral blood lymphocytes exposed to the Δ KS control or to the wild-type or 596 W/M mutant virus at various times after infection. (B) Percentage of viable cells in the infected CD4-positive peripheral blood lymphocyte cultures that were OKT4-positive on days 9 and 14 after infection.

The viral cytopathic effect was less pronounced in primary cells than in established CD4-positive T-cell lines (Fig. 8B). By day 14 after infection, a decrease in the percentage of viable cells that stained with the OKT4 antibody was observed in the culture infected with the wild-type virus. Since the OKT4 epitope on CD4 is not masked by gp120 binding, the observed decrease represents a combination of cytopathic effect and some intracellular complexing of CD4 by envelope glycoproteins. The decrease in OKT4-positive cells was less in the 596 W/M mutant virus-infected culture than in the culture infected with the wild-type virus. This observation and the greater production of reverse transcriptase in the culture supernatants are consistent with a decreased cytopathic effect of the 596 W/M virus relative to that of the wild-type virus.

The cytopathogenicity of HIV-1 has been shown to be linked to the late phase of virus infection. The envelope glycoproteins have been implicated in both syncytium formation and single-cell lysis (23, 25, 32). While CD4 binding is important for both processes, postbinding events involved in membrane fusion also appear to be necessary. Here we provide evidence for an envelope glycoprotein function that can influence the efficiency of syncytium formation and single-cell lysis, yet which appears to be dispensable for virus entry. The results underscore the critical role of the envelope glycoproteins in cytopathic effect,

independent of effects on virus replication or protein expression, and support the involvement of events other than receptor binding in syncytium formation and in single-cell lysis.

Both quantitative and qualitative differences in the function of the HIV-1 envelope glycoproteins can account for altered cytopathogenicity with retention of replicative ability. Amino acid changes that decrease the intrinsic fusogenicity of the HIV-1 envelope glycoproteins result in a reduction of viral cytopathic effect that cannot be explained by effects on virus replication rate or protein expression (23). Sensitivity to a reduction in envelope glycoprotein fusogenicity is greatest for the process of syncytium formation, less for single-cell lysis, and even less for virus replication. This pattern of sensitivity can be accounted for by a requirement for a progressively greater number of successful envelope glycoprotein-CD4 interactions for virus entry, single-cell lysis, and syncytium formation, respectively. In this model, the intrinsic fusogenic potential of the envelope glycoproteins in the particular target cell would determine the degree and pattern of cytopathic effect. Thus, for example, replication-competent HIV-1 gp41 mutants with various degrees of fusogenic activity have been shown to lose syncytium-forming ability but to retain the capacity to lyse single cells or have been shown to exhibit reductions in both syncytium-forming and single-cell lysis abilities (9a, 23). Thus, differences in the quantitative requirements for HIV-1 replication and cytopathicity can account for the generation of viruses that, under specific tissue culture conditions, can replicate comparably but differ in cytopathic effects.

The 596 W/M mutant described here differs in phenotype from previously reported mutant viruses, like 517A (23), with lowered intrinsic fusogenic potential. The 596 W/M mutant does not appear to exhibit a defect in intrinsic fusogenicity, since the efficiency of this mutant glycoprotein in mediating virus entry is at least as good as that of the wild-type virus in all target cells examined. The *env* complementation assay is a very sensitive measure of the effects of changes in envelope glycoprotein structure on virus entry. While the *env* complementation assay is clearly able to detect a lower efficiency of virus entry for less-cytopathic viruses with lower fusogenic potentials (16), no defect was discernible for the 596 W/M mutant. For example, while the ability of the fusion-attenuated 517A envelope glycoprotein, associated with a less-cytopathic virus, to complement entry into Jurkat lymphocytes was only 13.7% of that of the wild-type glycoproteins, the comparable activity of the 596 W/M mutant was 108%. Even after continued tissue culture passage, we have never detected a revertant of the 596 W/M mutant virus, further supporting the notion that the change at amino acid 596 is not associated with changes in virus replication rate. By contrast, revertants of the fusion-deficient envelope glycoprotein mutants often arise within 6 weeks of tissue culture passage (5a).

If the intrinsic fusogenicity of the 596 W/M envelope glycoprotein, reflected by virus entry, is comparable to that of the wild-type envelope glycoproteins, what processes important for syncytium formation and single-cell lysis might be deficient in this mutant? Such processes are likely to be involved in promoting the efficient fusion of a larger patch of membrane than would apply to virus-cell fusion. For example, the efficiency with which the clustering of envelope glycoprotein tetramers occurs might be affected by the 596 W/M change. This could result from a defect in an active function promoting lateral interaction of envelope glycoprotein spikes or from asymmetries introduced into the complex that decrease the efficiency of lateral packing. One clue to the presence of such asymmetries might be the slight reduction in

the affinity of the gp120-gp41 interaction resulting from the change in tryptophan 596. One would expect that a high concentration of envelope glycoproteins expressed for a sustained period of time might compensate for these kinds of defects. Indeed, the resurgence in single-cell lysis after several days of high-level expression of the 596 W/M envelope glycoproteins may represent such a compensation. One would also expect that processes with a lower required number of envelope glycoprotein-CD4 interactions should be more readily compensated by this mechanism, consistent with the observation that single-cell lysis but not syncytium formation by the 596 W/M mutant eventually approximates that of the wild-type virus.

Further studies of the molecular details of the HIV-1 cytopathic effect may benefit from the availability of the 596 W/M mutant.

We thank Virginia Nixon for manuscript preparation and Amy Emmert for artwork. We thank Susan Zolla-Pazner and the AIDS Research and Reference Reagent Program for the 98-6 monoclonal antibody.

This work was supported by NIH grant AI24755 to Joseph Sodroski, by a Center for AIDS Research grant (AI28691) and a Cancer Center grant (CA06516) to the Dana-Farber Cancer Institute, and by a gift from the G. Harold and Leila Y. Mathers Charitable Foundation.

REFERENCES

- Allan, J., T. H. Lee, M. F. McLane, J. Sodroski, W. A. Haseltine, and M. Essex. 1985. Identification of the major envelope glycoprotein product of HTLV-III. *Science* **228**:1091-1094.
- Barre-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nuygère, S. Chamaret, J. Gruest, C. Dautet, C. Axler-Blin, F. Vézinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier. 1983. Isolation of a T-lymphocyte retrovirus from a patient at risk for acquired immunodeficiency syndrome (AIDS). *Science* **220**:868-871.
- Bergeron, L., and J. Sodroski. 1992. Dissociation of unintegrated viral DNA accumulation from single-cell lysis induced by human immunodeficiency virus type 1. *J. Virol.* **66**:5777-5787.
- Bergeron, L., N. Sullivan, and J. Sodroski. 1992. Target cell-specific determinants of membrane fusion within the human immunodeficiency virus type 1 gp120 third variable region and gp41 amino terminus. *J. Virol.* **66**:2389-2397.
- Cao, J., L. Bergeron, E. Helseth, M. Thali, H. Repke, and J. Sodroski. 1993. Effects of amino acid changes in the extracellular domain of the human immunodeficiency virus type 1 gp41 envelope glycoprotein. *J. Virol.* **67**:2747-2755.
- Cao, J., and J. Sodroski. Unpublished observations.
- Cheng-Mayer, C., D. Seto, D. Tateno, and J. A. Levy. 1988. Biologic features of HIV-1 that correlate with virulence in the host. *Science* **240**:80-82.
- Connor, R. I., H. Mohri, Y. Cao, and D. D. Ho. 1993. Increased viral burden and cytopathicity correlate temporally with CD4⁺ T-lymphocyte decline and clinical progression in human immunodeficiency virus type 1-infected individuals. *J. Virol.* **67**:1772-1777.
- Coombs, R., A. Collier, J.-P. Allain, B. Nikora, and L. Corey. 1989. Plasma viremia in HIV infection. *N. Engl. J. Med.* **321**:1626-1631.
- Dalglish, A. G., P. C. L. Beverly, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss. 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature (London)* **312**:763-767.
- Dedera, D., and L. Ratner. 1991. Demonstration of two distinct cytopathic effects with syncytium formation-defective human immunodeficiency virus type 1 mutants. *J. Virol.* **65**:6129-6136.
- Earl, P., and B. Moss. 1993. Mutational analysis of the assembly domain of the HIV-1 envelope glycoprotein. *AIDS Res. Hum. Retroviruses* **9**:589-594.
- Fauci, A., A. Macher, D. Longo, H. C. Lane, A. Rook, H. Masur, and E. Gelmann. 1984. Acquired immunodeficiency syndrome: epidemiologic, clinical, immunologic, and therapeutic considerations. *Ann. Intern. Med.* **100**:92-106.
- Freed, E., D. Meyers, and R. Risser. 1990. Characterization of the fusion domain of the human immunodeficiency virus type 1 envelope glycoprotein gp41. *Proc. Natl. Acad. Sci. USA* **87**:4650-4654.
- Freed, E., D. Meyers, and R. Risser. 1991. Identification of the principal neutralizing determinant of human immunodeficiency virus type 1 as a fusion domain. *J. Virol.* **65**:190-194.
- Gallagher, W. R. 1987. Detection of a fusion peptide sequence in the transmembrane protein of human immunodeficiency virus. *Cell* **80**:327-328.
- 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.
- Grimaila, R., B. Fuller, P. Rennert, M. B. Nelson, M.-L. Hammar-skjold, B. Potts, M. Murray, S. Putney, and G. Gray. 1992. Mutations in the principal neutralization determinant of human immunodeficiency virus type 1 affect syncytium formation, virus infectivity, growth kinetics, and neutralization. *J. Virol.* **66**:1875-1883.
- Helseth, E., M. Kowalski, D. Gabuzda, U. Olshevsky, W. Haseltine, and J. Sodroski. 1990. Rapid complementation assays measuring replicative potential of human immunodeficiency virus type 1 envelope glycoprotein mutants. *J. Virol.* **64**:2416-2420.
- Helseth, E., U. Olshevsky, C. Furman, and J. Sodroski. 1991. Human immunodeficiency virus type 1 gp120 envelope glycoprotein regions important for association with the gp41 transmembrane glycoprotein. *J. Virol.* **65**:2119-2123.
- Helseth, E., U. Olshevsky, D. Gabuzda, B. Ardman, W. Haseltine, and J. Sodroski. 1990. Changes in the transmembrane region of the human immunodeficiency virus type 1 gp41 envelope glycoprotein affect membrane fusion. *J. Virol.* **64**:6314-6318.
- Ho, D., T. Moudgil, and M. Alam. 1989. Quantitation of HIV-1 in the blood of infected persons. *N. Engl. J. Med.* **321**:1621-1625.
- Ivanoff, L., D. Looney, C. McDanal, J. Morris, F. Wong-Staal, A. Langlois, S. Petteway, and T. Matthews. 1991. Alteration of HIV-1 infectivity and neutralization by a single amino acid replacement in the V3 loop domain. *AIDS Res. Hum. Retroviruses* **7**:595-603.
- Javaherian, K., A. J. Langlois, C. McDanal, K. L. Ross, L. I. Eckler, C. L. Jellis, A. T. Profy, J. R. Rusche, D. P. Bolognesi, S. D. Putney, and T. J. Matthews. 1989. Principal neutralizing domain of the human immunodeficiency virus type 1 envelope protein. *Proc. Natl. Acad. Sci. USA* **86**:6768-6772.
- Klatzmann, D., E. Champagne, S. Chamaret, J. Gruest, D. Guetard, T. Hercend, J. C. Gluckman, and L. Montagnier. 1984. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature (London)* **312**:767-768.
- Kowalski, M., L. Bergeron, T. Dorfman, W. Haseltine, and J. Sodroski. 1991. Attenuation of human immunodeficiency virus type 1 cytopathic effect by a mutation affecting the transmembrane envelope glycoprotein. *J. Virol.* **65**:281-291.
- Kowalski, M., J. Potz, L. Basiripour, T. Dorfman, W. G. Goh, E. Terwilliger, A. Dayton, C. Rosen, W. Haseltine, and J. Sodroski. 1987. Functional regions of the human immunodeficiency virus envelope glycoproteins. *Science* **237**:1351-1355.
- Lifson, J. D., M. B. Feinberg, G. R. Reyes, L. Rabin, B. Banapour, S. Chakrabarti, B. Moss, F. Wong-Staal, K. S. Steimer, and E. B. Engleman. 1986. Induction of CD4-dependent cell fusion by the HTLVIII/LAV envelope glycoprotein. *Nature (London)* **323**:725-728.
- Maddon, P. A., Dalglish, J. S. McDougal, P. Clapham, R. Weiss, and R. Axel. 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* **47**:333-348.
- McDougal, J. S., M. Kennedy, J. Sligh, S. Cort, A. Mowie, and J. Nicholson. 1986. Binding of the HTLV-III/LAV to T4⁺ T cells by a complex of the 100 K viral protein and the T4 molecule. *Science* **231**:382-385.
- McDougal, J. S., J. Nicholson, G. Cross, S. Cort, M. Kennedy, and A. Mawle. 1986. Binding of the human retrovirus HTLV-III/LAV/ARV/HIV to the CD4 (T4) molecule: conformation dependence, epitope mapping, antibody inhibition, and potential for idiotypic mimicry. *J. Immunol.* **137**:2937-2944.

29. **Page, K., S. Stearns, and D. Littman.** 1992. Analysis of mutations in the V3 domain of gp160 that affect fusion and infectivity. *J. Virol.* **66**:524–533.
- 29a. **Poumbourios, P., D. McPhee, and B. Kemp.** 1993. Antibody epitopes sensitive to the state of HIV-1 gp41 oligomerization map to a putative alpha-helical region. *AIDS Res. Hum. Retroviruses* **8**:2055–2062.
30. **Robey, W. G., B. Safai, S. Oroszlan, L. Arthur, M. Gonda, R. Gallo, and P. J. Fischinger.** 1985. Characterization of envelope and core structural gene products of HTLV-III with sera from AIDS patients. *Science* **228**:593–595.
31. **Schnittman, M., M. Psallidopoulos, H. C. Lane, L. Thompson, M. Baseler, F. Massari, C. Fox, N. Salzman, and A. Fauci.** 1989. The reservoir for HIV-1 in human peripheral blood is a T cell that maintains expression of CD4. *Science* **245**:305–308.
32. **Sodroski, J., W. C. Goh, C. A. Rosen, K. Campbell, and W. Haseltine.** 1986. Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. *Nature (London)* **322**:470–474.
33. **Somasundaran, M., and H. L. Robinson.** 1987. A major mechanism of human immunodeficiency virus-induced cell killing does not involve cell fusion. *J. Virol.* **61**:3114–3119.
34. **Stein, B. S., S. D. Gouda, J. F. Lifson, R. C. Penhallow, K. G. Bensch, and E. G. Engleman.** 1987. pH-independent HIV entry into CD4-positive T cells via virus envelope fusion to the plasma membrane. *Cell* **53**:483–496.
35. **Thali, M., C. Furman, E. Helseth, H. Repke, and J. Sodroski.** 1992. Lack of correlation between soluble CD4-induced shedding of the human immunodeficiency virus type 1 exterior envelope glycoprotein and subsequent membrane fusion events. *J. Virol.* **66**:5516–5524.
36. **Thali, M., U. Olshevsky, C. Furman, D. Gabuzda, J. Li, and J. Sodroski.** 1991. Effects of changes in gp120-CD4 binding affinity on human immunodeficiency virus type 1 envelope glycoprotein function and soluble CD4 sensitivity. *J. Virol.* **65**:5007–5012.
37. **Travis, B., T. Dykers, D. Hewgill, J. Ledbetter, T. Tsu, S.-L. Hu, and J. Lewis.** 1992. Functional roles of the V3 hypervariable region of HIV-1 gp160 in the processing of gp160 and in the formation of syncytia in CD4+ cells. *Virology* **186**:313–317.
38. **Veronese, F. D., A. Vico, T. Copeland, S. Oroszlan, R. Gallo, and M. Sarngadharan.** 1985. Characterization of gp41 as the transmembrane protein encoded by the HTLV-III/LAV envelope gene. *Science* **229**:1402–1405.
39. **Xu, J., M. Gorny, T. Palker, S. Karwowska, and S. Zolla-Pazner.** 1991. Epitope mapping of 10 human monoclonal antibodies to gp41, the transmembrane protein of human immunodeficiency virus type 1. *J. Virol.* **65**:4832–4838.