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Functional Interaction of Constant and Variable Domains of Human Immunodeficiency Virus Type 1 gp120

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A previously reported amino acid substitution within the second conserved domain of the human immunodeficiency virus type 1 (HIV-1) gp120 envelope results in the production of noninfectious particles. Molecular characterization of spontaneous revertant viruses, which arose during long-term cocultures of this *env* mutant, revealed that an amino acid change within another region of gp120 could functionally compensate for the mutation and restore infectivity. In the current study, we have introduced a conservative amino acid substitution at this second-site revertant codon and observed a marked reduction in HIV-1 infectivity. During the passage of this defective virus in cocultures, yet another revertant appeared which contained an amino acid change within a variable region of gp120 which restored infectivity to near wild-type levels. These results, in combination with other point mutations that have been introduced into the HIV-1 envelope, suggest that at least three discrete regions of gp120 may interact during the establishment of a productive viral infection. This critical step occurs subsequent to the adsorption of virions to the cell surface and either prior to or concomitant with the fusion of viral and cellular membranes.

A characteristic feature of human immunodeficiency virus type 1 (HIV-1) is the extensive genetic variability that exists among different isolates. This genomic diversity is most evident within the *env* gene, which contains areas of pronounced amino acid variability (2, 4, 19, 24). Interspersed among these variable domains, however, are conserved regions, several of which have been shown to mediate critical steps during the productive viral infection of human T cells (12, 15).

Information relating *env* gene structure to function continues to accumulate. Several conserved domains within the gp120 envelope protein have been reported to be the principal determinants of the binding of gp120 to the cell surface viral receptor molecule, CD4 (10, 12). Other regions appear to mediate events which occur subsequent to viral absorption (9, 10, 25). Several reports also indicate that conserved domains are involved in the processing of the HIV-1 envelope proteins (10, 15). Recent studies suggest that the variable domains of gp120 may also play important roles during productive viral infection. In particular, a variable region within gp120 appears to elicit type-specific neutralizing antibodies which can block HIV-1-mediated syncytium formation and infection by cell-free virus (7, 13, 16, 17). While the exact mechanism of this interference is unknown, a likely explanation is that this variable gp120 domain is critical to events in the virus replicative cycle which occur subsequent to adsorption to the CD4 receptor (18). The identification and functional characterization of these and other regions within the HIV-1 envelope may facilitate the development of antiviral intervention strategies.

We previously identified a region within the second conserved domain of gp120 that is critical for viral infectivity (25). Amino acid substitutions at three adjacent codons (266 to 268) within this domain rendered the virus noninfectious. An amino acid substitution at a distant codon (128) of gp120, which spontaneously occurred during long-term cocultivations, compensated for one of these mutations (267gln) (see Fig. 1) and resulted in the emergence of revertant infectious virus particles. In the work presented in this paper, we mutated this second-site change in the gp120 of the revertant and observed a marked reduction in viral replicative capacity. Long-term passage in tissue culture generated yet another revertant virus possessing biological properties similar to those of the first revertant virus. Molecular cloning and DNA sequence analysis of the second revertant revealed that an amino acid substitution within a variable domain of gp120 (codon 308) was responsible for the revertant phenotype. These results suggest that the three regions within HIV-1 envelope, defined by the 128, 267, and 308 mutant and revertant codon changes, interact with one another and mediate a critical early step in the virus life cycle.

MATERIALS AND METHODS

Oligonucleotide-directed mutagenesis. Amino acid codon substitutions were introduced into the *env* genes of the mutant and revertant HIV-1 proviral clones p7052, p7055, and p7055R1A (25) (referred to as 266asp, 267gln, and 267gln/128asn in this report) as previously described (25). Briefly, 2.7-kilobase (kb) *Eco*RI-*Bam*HI *env*-containing DNA restriction fragments from the different HIV-1 proviral DNA clones were inserted into the M13 vector mp19, and specific nucleotide changes were introduced by oligonucleotide-directed mutagenesis. Oligodeoxyribonucleotides were synthesized and purified as previously described (25). The mutagenesis protocols of Zoller and Smith (27) and Kunkel (11) (Bio-Rad Laboratories) were both used. The analogous *env*-containing restriction fragment from an infectious molecular clone of HIV-1, pNL4-3 (1), was also used to generate HIV-1 proviral clones with individual revertant codon substitutions. Dideoxy single-stranded M13 and double-stranded plasmid sequencing (Sequenase; U.S. Biochemical Corp.) were used to confirm the presence of the nucleotide substitutions in the mutated 2.7-kb *Eco*RI-*Bam*HI fragments before and after their reintroduction into the

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parental plasmid clones. The mutagenic oligonucleotides and designations used were as follows (5′ to 3′): 128asn, CTCTG TGTTAaTTTAAAGTGC; 128gln, CCACTCTGTGTTcAaT TAAAGTGCACT; 308ile, CAACAATACAAtAAAAAGTA TCC. The lowercase letters identify nucleotide changes that were introduced.

Cell cultures and viral infectivity. The CD4$^+$ lymphocytic leukemia cell line A3.01 (5) and colon carcinoma cell line SW480 (ATCC CCL228) were maintained in supplemented RPMI 1640 and Dulbecco modified Eagle medium as previously described (25). Mutant HIV-1 virions were produced by transfecting uncleaved plasmid DNAs into the SW480 cells, and the infectivity of progeny virions was assessed by establishing cocultures with the A3.01 cells as previously described (25). Cell culture supernatants were collected after transfection and during cocultivation and assayed for the presence of virion-associated reverse transcriptase (RT) activity (6, 25).

Revertant cloning and DNA sequence analysis. Infections of A3.01 cells were established by using cell-free, filtered supernatants (see below) collected from day 21 of the 267gln/128gln coculture experiment (see Fig. 2). Unintegrated proviral DNA was isolated at 10 days postinfection, and molecular clones of the revertant virus were obtained as previously described (8, 25). The 2.7-kb *Eco*RI-*Bam*HI *env*-containing restriction fragment from a revertant recombinant lambda bacteriophage clone was inserted into the 267gln/128gln mutant clone. Progeny virions resulting from transfections with this construction were monitored for infectivity by coculture and RT analyses as described above. Dideoxy DNA sequencing was performed on both strands of the revertant 2.7-kb *Eco*RI-*Bam*HI fragment by using the reconstructed proviral plasmid DNA and synthetic oligonucleotide primers (21, 25).

Infection kinetics and cytopathic effects. The infectivities of the wild-type pNL4-3 and the revertant 267gln/128asn, 267gln/308ile, and 266asp/308ile viruses were monitored by using the A3.01 cell line. SW480 cells were transfected with the revertant plasmid proviral DNAs, and virus-containing supernatants were collected after 24 h. The pNL4-3 virus stock was obtained from supernatants of previously infected A3.01 cells. All supernatants were filtered (0.22-μm-pore-size filters) and assayed for virion-associated RT activity. Comparable amounts of RT activity for each virus were added to 2×10^6 A3.01 cells in 1 ml of tissue culture medium and incubated in 15-ml conical tubes at 37°C for 2 h. Virus-cell suspensions were then transferred to 25-cm^2 culture flasks and maintained in 10 ml of medium. Supernatant samples were collected every 2 days, beginning on day 5, and assayed for RT activity. Cell viability was also monitored at each time point by using trypan blue exclusion, and concentrations were maintained at 10^6/ml. Infections were terminated when extensive cell death was observed.

RESULTS

We have previously shown that the substitution of glutamine for asparagine at codon 267 (a potential N-linked glycosylation site) within the HIV-1 gp120 envelope generates noninfectious virus (25). The mutated gp120 protein, produced from expression vectors, retained its capacity to bind to the CD4 receptor and induce syncytium formation. Collectively, these results suggested that the HIV-1 267gln mutant was blocked at a step in the replicative cycle subsequent to CD4 binding. During long-term cocultures with the 267gln virus, however, infectious revertant virions emerged.

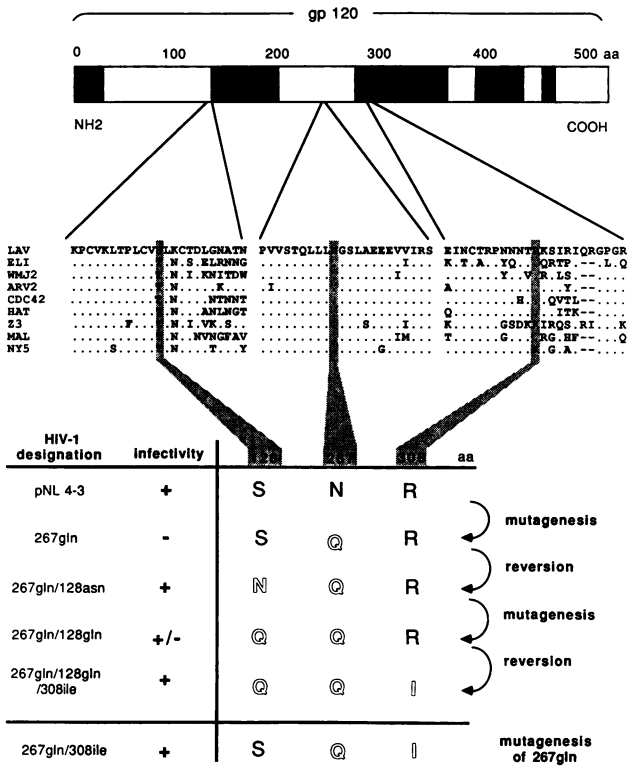

FIG. 1. Location of amino acid substitutions within the HIV-1 envelope gp120 and their effects on viral infectivity. The top of the figure illustrates conserved (open) and variable (shaded) domains within gp120 as previously described (2, 4, 19, 24). The middle section aligns amino acid residues from different HIV-1 clones within the 128, 267, and 308 regions of gp120. The bottom portion indicates amino acid codon substitutions which either were introduced by in vitro mutagenesis or occurred spontaneously during tissue culture. Shaded numbers identify specific codons involved. Solid letters represent codons present in the wild-type infectious (pNL4-3) proviral clone, while open letters indicate amino acid substitutions in the indicated HIV-1 recombinant plasmids. Infectivity of progeny virions from the various HIV-1 proviral clones was determined by transfection, coculture, and RT analyses as described in the text. +/− indicates a low-level infection compared with the other virus infections. The 267gln and 267gln/128asn data have been previously reported (25).

Nucleotide sequencing of revertant molecular proviral clones indicated that the substitution of an asparagine for a serine at codon 128 of gp120 compensated for the retained 267gln mutation and restored infectivity (Fig. 1).

To evaluate the effect of additional substitutions at this second site, a conservative amino acid change was introduced into the 267gln/128asn revertant clone. A glutamine residue was substituted for the asparagine codon at position 128, generating the HIV-1 recombinant plasmid 267gln/128gln. Its biological activity was assessed after transfection into the SW480 codon carcinoma cells and coculturing with the CD4$^+$ A3.01 cells as described in Materials and Methods. The parental 267gln/128asn and wild-type pNL4-3 plasmid clones were similarly examined. Spreading viral infection was monitored by assaying virion-associated RT activity in culture supernatants. Progeny virions from both the wild-type pNL4-3 and the revertant 267gln/128asn clones established productive infections (Fig. 2). The high levels of RT activity detected in the cocultures 9 to 12 days after the addition of the A3.01 cells indicated that peak virus produc-

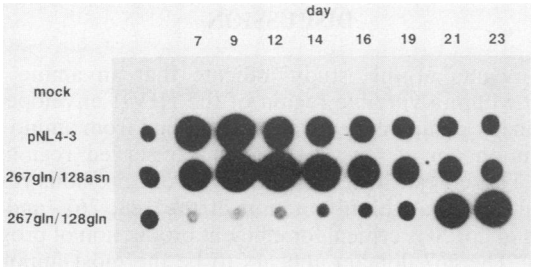

FIG. 2. Infectivity of the 267gln/128gln mutant virus. Virion-associated RT activity was determined in 10-μl culture supernatant samples after transfection of SW480 cells with the wild-type (pNL4-3), revertant (267gln/128asn), and mutant (267gln/128gln) plasmid DNAs (first vertical column) and at the indicated times after coculture with A3.01 cells.

tion occurred during this period. In contrast, only low levels of the 267gln/128gln virus were produced during the first 19 days of coculture, suggesting that the 128gln substitution had significantly reduced, but not completely ablated, viral infectivity. High levels of RT activity were detected on day 21, however, implying the emergence of a revertant virus.

To determine whether another revertant had emerged, cell-free virus from day 21 of the coculture (Fig. 2) was used to directly infect the A3.01 cells as described in Materials and Methods. The kinetics of RT activity detected during this infection resembled those of the parental 267gln/128asn virus (data not shown) and suggested that another reversion in the *env* gene had occurred. We therefore isolated unintegrated proviral DNA from the infected A3.01 cells and obtained several lambda phage clones of the putative revertant proviral DNA, as described in Materials and Methods. When the 2.7-kb *Eco*RI-*Bam*HI *env*-containing restriction fragment from one of the lambda phage clones was introduced into the defective 267gln/128gln proviral plasmid clone, full infectivity was restored. The infection kinetics of the resultant progeny virions were indistinguishable from those of the original parental 267gln/128asn virus (data not shown).

To identify the molecular basis for this change, the complete nucleotide sequence of the revertant 2.7-kb *Eco*RI-*Bam*HI fragment was determined. This analysis revealed that the 267gln and 128gln codons were both retained in the revertant virus; however, four additional nucleotide substitutions, not present in the defective 267gln/128gln provirus, were detected. The first was a second-position change within codon 84 of the HIV-1 *R* gene (26) resulting in an isoleucine-for-threonine substitution. The remaining three nucleotide changes were all located within the *env* gene. Two consisted of third-position base changes (G to A and C to T within codons 367 and 705, respectively) and did not alter the codon specificities for lysine and phenylalanine. The final change was located at amino acid position 308, situated in a variable domain of gp120. Within this codon, a second-position change of G to T resulted in the substitution of an isoleucine for an arginine residue. Thus, the 308ile substitution had restored the infectivity of the defective 267gln/128gln virus (Fig. 1).

The results of these revertant virus analyses did not indicate whether the 308ile substitution had compensated for the 128gln mutation or the defect associated with the original 267gln mutation, both of which were retained in the second revertant. To examine this question further, the 308ile codon change was introduced into the original noninfectious 267gln HIV-1 proviral clone by oligonucleotide-directed mutagenesis; the infectivity of the resultant 267gln/308ile virions was evaluated by transfection and coculture. The 267gln/308ile virus particles were able to establish a spreading viral infection (Fig. 1) and exhibited infection kinetics that were similar to those of the wild-type virus (data not shown). This result indicated that the 308ile substitution, like the previously described 128asn change (25), could functionally correct the 267gln mutation.

TABLE 1. Infectivity of wild-type HIV-1 containing revertant envelope codon substitutions

HIV-1 designation[a]	Amino acid at envelope codon[b]:		
	128	267	308
pNL4-3 (wild type)	S	N	R
128asn	**N**	N	R
308ile	S	N	**I**
128asn/308ile	**N**	N	**I**

[a] On the basis of data from transfection, coculture, and RT analyses with the indicated HIV-1 plasmid clones, every virus was fully infectious.

[b] Numbers refer to amino acid codons within gp120 (Fig. 1); boldface letters indicate codon substitutions which were introduced into the pNL4-3 proviral clone as described in the text.

Since the 128asn and the 308ile substitutions could each restore envelope function in the noninfectious 267gln mutant, we wondered whether either revertant change could alter the infectivity of the original, fully infectious pNL4-3 provirus. The introduction of the 128asn and 308ile substitutions, alone or in combination, did not affect viral infectivity (Table 1). Thus, the revertant amino acid changes were functionally significant only in the presence of the 267gln mutation.

We next asked whether the 308ile substitution could compensate for other mutations within the 267 region of gp120 that also impair HIV-1 infectivity. One such HIV-1 clone, designated 266asp, has an aspartic acid codon substituted for a leucine at position 266 in gp120 (25) (see the amino acid alignment in Fig. 2). Low but measurable levels of the 266asp virus were produced (compared with the wild-type pNL4-3) after transfection and coculture (Fig. 3). When the 308ile substitution was introduced into the 266asp clone by oligonucleotide-directed mutagenesis, higher levels of progeny virions were generated (Fig. 3), demonstrating again a functional interaction between the 267 and 308 regions of gp120.

These results show that the 128asn and 308ile codon substitutions can independently compensate for functional defects in HIV-1 envelope arising from mutations with the

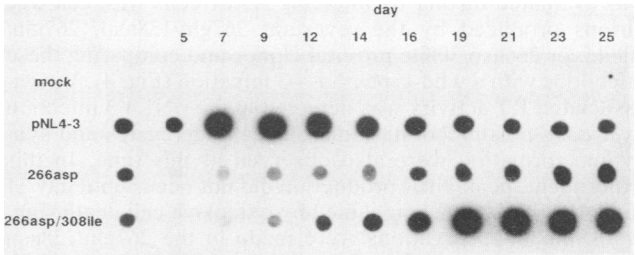

FIG. 3. Functional compensation of the 308ile amino acid change for the defect associated with the 266asp mutation in gp120. Virion-associated RT activity was detected in culture supernatants after transfection of SW480 cells with the pNL4-3, 266asp, and 266asp/308ile plasmid DNAs (first vertical column) and at the indicated times after coculture with A3.01 cells.

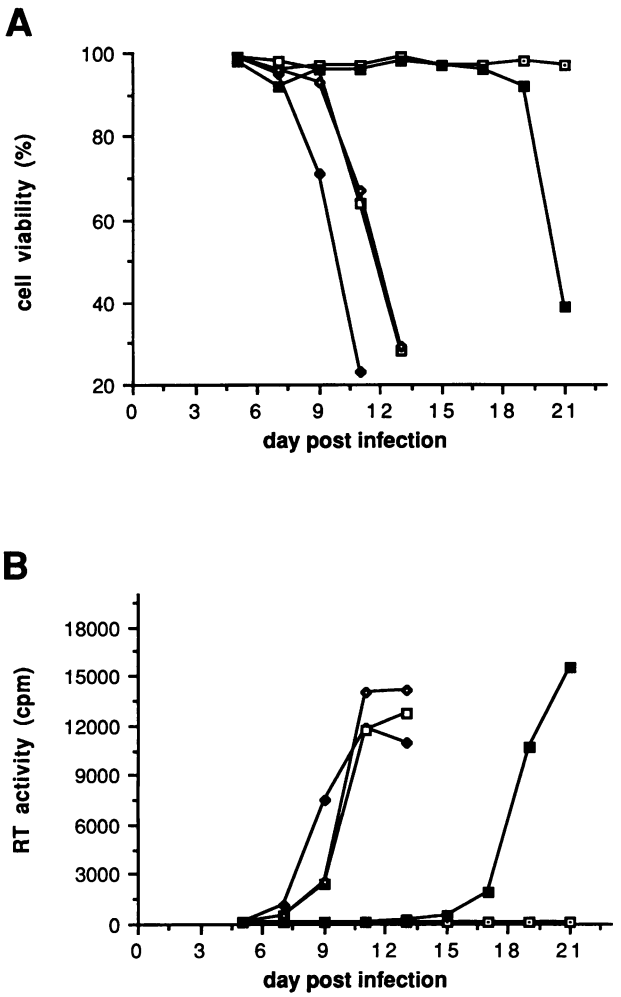

FIG. 4. Infectivity of HIV-1 revertant viruses. Parallel infections of A3.01 cells were established by using cell-free virions produced by the 267gln/128asn, 267gln/308ile, and 266asp/308ile revertant and the pNL4-3 wild-type proviral clones as described in Materials and Methods. Cell viability and virion-associated RT activities in culture supernatants were evaluated for each infection at the indicated time intervals. Symbols: ⊡, mock; ◆, pNL4-3; ◆, 267gln/128asn; □, 267gln/308ile; ■, 266asp/308ile.

267 region; however, the delayed infection kinetics displayed by the 266asp/308ile virus suggested that infectivity may not always be restored to wild-type levels. This issue was evaluated further by infecting A3.01 cells with cell-free virions produced by the revertant 267gln/128asn, 267gln/308ile, or 266asp/308ile proviral clones and comparing these infections with a wild-type pNL4-3 infection (Fig. 4). Virion-associated RT activity was detected in the pNL4-3 infection by 7 days postinfection; numerous cell aggregates and syncytium formation were also observed at this time. In this experiment, peak virus production did not occur until day 11 (Fig. 4B) and was accompanied by extensive cell death (Fig. 4A). Similar observations were made in the 267gln/128asn and 267gln/308ile infections, although associated cell death was delayed 2 days relative to the pNL4-3 infection. The infection kinetics of the 266asp/308ile virus was markedly delayed, with detectable levels of RT activity not present until day 17. Despite this delay, the spreading 266asp/308ile virus infection eventually led to extensive cell death.

DISCUSSION

The results of this study indicate that an amino acid change within a variable region of the HIV-1 envelope can functionally compensate for defects arising from amino acid substitutions in at least two other conserved regions of gp120. These data, in conjunction with our previous results (25), suggest that the interaction of the 128, 267, and 308 regions of gp120 is critical for efficient production of progeny virions. The 267 domain appears to be the most important element for this interaction, since amino acid substitutions at codons 128 and 308 were functionally significant only in the presence of mutations within the 267 region.

The 267 region has been previously reported to be critical for the processing of the gp160 precursor to the gp120 and gp41 virion envelope proteins (10). We have observed that the 266asp mutation leads to reduced gp120 production in pulse-chase experiments in HeLa cells transfected with the 266asp or 266asp/308ile proviral clone (unpublished results). Since the 308ile substitution has no effect on processing gp160, yet increases the replicative capacity of the 266asp virus, an envelope function(s) unrelated to processing must be responsible for the augmented infectivity observed. Inefficient processing, however, might contribute to the delayed infection kinetics characteristic of the 266asp/308ile virus.

It is not presently known why viral infectivity is altered by the amino acid substitutions at codons 128, 267, and 308. Results from an earlier study have suggested that the 128 and 308 regions of gp120 may be involved in its association with gp41 (10). We have previously speculated that the 267 domain of gp120 might mediate events occurring subsequent to the binding of virions to the CD4 receptor, since the presence of the 267gln substitution in gp120 produced in expression vectors did not impair binding to CD4 (25). This observation has been supported by another report which demonstrated that antibodies specific for the 267 region blocked HIV-1 infection without interfering with CD4 binding (9). The putative interaction between codon 308 and the 267 domain identified in our current study further suggests that a postbinding event is involved, since the results of several recent studies imply a similar role for the 308 region. Codon 308 is located within a stretch of amino acids which elicits type-specific neutralizing antibodies to HIV-1 (7, 13, 16, 17). These antibodies inhibit infection by cell-free virus as well as fusion of HIV-1-infected cells (13, 16, 17) but do not affect CD4 binding (18). Type-specific virus neutralization even after the adsorption of virions to $CD4^+$ T cells has been reported (P. Nara, personal communication). Thus, type-specific neutralization and the amino acid substitutions we have identified in the gp120 revertants may target the same postbinding event, since both involve the 308 domain of gp120. Our results also imply that the 128 and 267 regions play an important role in this event and suggest that the interaction of at least these three domains of the HIV-1 envelope may be required for productive infection of T cells.

At present, we can only speculate on which postbinding step(s) would involve the 128, 267, and 308 domains of gp120. HIV-1 envelope-mediated fusion of the viral and cellular membranes could require the interaction of the 128, 267, and 308 regions, as described for other viruses (3); however, it is not clear how the 128, 267, and 308 regions of gp120 might participate in the fusion of viral and cellular membranes, since the putative fusogenic domain of HIV-1 is located at the N terminus of the gp41 envelope protein (10). The similarity of HIV-1 entry to that of both the para- and orthomyxoviruses may provide some clues. In Sendai and

influenza virus infections, a postbinding activation step must precede the fusion of the viral and cellular membranes. Extracellular fusion by Sendai virus is dependent on the proteolytic cleavage of the FO precursor envelope protein at the cell surface (3), while a pH-dependent conformational change allows the HA2 envelope cleavage product of the influenza virus hemagglutinin to fuse with endosomal membranes during viral entry (22). HIV-1 entry has been reported to occur by extracellular fusion that is pH independent (14, 20); however, proteolytic processing of the HIV-1 gp120 and gp41 envelope components, which is also essential for infectivity (15), occurs intracellularly (23) prior to CD4 binding. Since the fusogenic domain of gp41 is most likely not exposed on the surface of HIV-1, a conformational change which alters the location of this region after adsorption to human T cells might be required to promote fusion of viral and cellular membranes. Such a conformational change could be triggered by the interaction of gp120 with CD4 and depend on the structural interaction of the 128, 267, and 308 regions of the viral envelope.

The delineation of regions within the HIV-1 envelope which are critical to viral infectivity may help to identify potential targets for antiviral intervention. The fact that antibodies directed against the 308 region can block viral infection raises the possibility that polyvalent vaccines might protect exposed individuals (16); however, the construction of such candidate vaccines may also have to be based on the contribution of the 128 and 267 domains as well. Amino acid changes at any one of these regions may allow the virus to escape neutralizing antibodies which specifically target the 308 region. The types of compensatory amino acid changes that we have observed may represent a mechanism by which HIV-1 can escape immunologic recognition while maintaining functional integrity during a productive viral infection.

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ADDENDUM IN PROOF

During preparation of the manuscript, Felser et al. (Virology **170**:566–670) reported that the 267gln mutation in gp120 leads to a two- to fourfold reduction in HIV-1-mediated cell fusion.

LITERATURE CITED

1. **Adachi, A., H. E. Gendelman, S. Koenig, T. Folks, R. Willey, A. Rabson, and M. A. Martin.** 1986. Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. J. Virol. **59**:284–291.
2. **Alizon, M., S. Wain-Hobson, L. Montagnier, and P. Sonigo.** 1986. Genetic variability of the AIDS virus: nucleotide sequence analysis of two isolates from African patients. Cell **46**:63–74.
3. **Choppin, P. W., and A. Scheid.** 1980. The role of viral glycoproteins in adsorption, penetration, and pathogenicity of viruses. Rev. Infect. Dis. **2**:40–61.
4. **Desai, S. M., V. S. Kalyanaraman, J. M. Casey, A. Srinivasan, P. R. Anderson, and S. G. Devare.** 1986. Molecular cloning and primary nucleotide sequence analysis of a distinct human immunodeficiency virus isolate reveal significant divergence in its genomic sequences. Proc. Natl. Acad. Sci. USA **83**:8380–8384.
5. **Folks, T., S. Benn, A. Rabson, T. Theodore, M. D. Hoggan, M. Martin, M. Lightfoote, and K. Sell.** 1985. Characterization of a continuous T-cell line susceptible to the cytopathic effects of the acquired immunodeficiency syndrome (AIDS)-associated retrovirus. Proc. Natl. Acad. Sci. USA **82**:4539–4543.
6. **Goff, S., P. Traktman, and D. Baltimore.** 1981. Isolation and properties of Moloney murine leukemia virus mutants: use of a rapid assay for release of virion reverse transcriptase. J. Virol. **38**:239–248.
7. **Goudsmit, J., C. Debouck, R. H. Meloen, L. Smit, M. Bakker, D. M. Asher, A. V. Wolff, C. J. Gibbs, and D. C. Gajdusek.** 1988. Human immunodeficiency virus type 1 neutralization epitope with conserved architecture elicits early type-specific antibodies in experimentally infected chimpanzees. Proc. Natl. Acad. Sci. USA **85**:4478–4482.
8. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:364–369.
9. **Ho, D. D., J. C. Kaplan, I. E. Rackauskas, and M. E. Gurney.** 1988. Second conserved domain of gp120 is important for HIV infectivity and antibody neutralization. Science **239**:1021–1023.
10. **Kowalski, M., J. Potz, L. Basiripour, T. Dorfman, W. C. Goh, E. Terwilliger, A. Dayton, C. Rosen, W. Haseltine, and J. Sodroski.** 1988. Functional regions of the envelope glycoprotein of human immunodeficiency virus type 1. Science **237**:1351–1355.
11. **Kunkel, T. A.** 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82**:488–492.
12. **Lasky, L. A., G. Nakamura, J. H. Smith, C. Fennie, L. Shimosaki, E. Patzer, P. Berman, T. Gregory, and D. J. Capon.** 1987. Delineation of a region of the human immunodeficiency virus type 1 (HIV-1) gp120 glycoprotein critical for interaction with the CD4 receptor. Cell **50**:975–985.
13. **Matsushita, S., M. Robert-Guroff, J. Rusche, A. Koito, T. Hattori, H. Hoshino, K. Javaherian, K. Takatsuki, and S. Putney.** 1988. Characterization of a human immunodeficiency virus neutralizing monoclonal antibody and mapping of the neutralizing epitope. J. Virol. **62**:2107–2114.
14. **McClure, M. O., M. Marsh, and R. A. Weiss.** 1988. Human immunodeficiency virus infection of CD4-bearing cells occurs by a pH-independent mechanism. EMBO J. **7**:513–518.
15. **McCune, J. M., L. B. Rabin, M. B. Feinberg, M. Lieberman, J. C. Kosek, G. R. Reyes, and I. L. Weissman.** 1988. Endoproteolytic cleavage of gp160 is required for the activation of human immunodeficiency virus. Cell **53**:55–67.
16. **Palker, T. J., M. E. Clark, A. J. Langlois, T. J. Matthews, K. J. Weinhold, R. R. Randall, D. P. Bolognesi, and B. F. Haynes.** 1988. Type specific neutralization of the human immunodeficiency virus with antibodies to *env*-encoded synthetic peptides. Proc. Natl. Acad. Sci. USA **85**:1932–1936.
17. **Rusche, J. R., K. Javalerian, C. McDanal, J. Petro, D. L. Lynn, R. Grimalia, A. Langlois, R. C. Gallo, L. O. Arthur, P. J. Fischinger, D. P. Bolognesi, S. D. Putney, and T. J. Matthews.** 1988. Antibodies that inhibit fusion of human immunodeficiency virus-infected cells bind a 24-amino acid sequence of the viral envelope, gp120. Proc. Natl. Acad. Sci. USA **85**:3198–3202.
18. **Skinner, M. A., A. J. Langlois, C. B. McDanal, J. S. McDougal, D. P. Bolognesi, and T. J. Matthews.** 1988. Neutralizing antibodies to an immunodominant envelope sequence do not prevent gp120 binding to CD4. J. Virol. **62**:4195–4200.
19. **Starcich, B. R., B. H. Hahn, G. M. Shaw, P. D. McNeely, S. Modrow, H. Wolf, E. S. Parks, W. P. Parks, S. F. Josephs, R. C. Gallo, and F. Wong-Staal.** 1986. Identification and characterization of conserved and variable regions in the envelope gene of HTLV-III/LAV, the retrovirus of AIDS. Cell **45**: 637–648.
20. **Stein, B. S., S. D. Gowda, J. D. 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 **49**:659–668.
21. **Strauss, E. C., J. A. Kobori, G. Siu, and L. E. Hood.** 1986. Specific-primer-directed DNA sequencing. Anal. Biochem. **154**: 353–360.
22. **Wiley, D. C., and J. J. Skehel.** 1987. The structure and function of the hemagglutinin membrane glycoprotein of influenza virus.

Annu. Rev. Biochem. **56**:365–394.

23. **Willey, R. L., J. S. Bonifacino, B. J. Potts, M. A. Martin, and R. D. Klausner.** 1988. Biosynthesis, cleavage, and degradation of the human immunodeficiency virus 1 envelope glycoprotein gp160. Proc. Natl. Acad. Sci. USA **85**:9580–9584.
24. **Willey, R. L., R. A. Rutledge, S. Dias, T. Folkes, T. Theodore, C. E. Buckler, and M. A. Martin.** 1986. Identification of conserved and divergent domains within the envelope gene of the acquired immunodeficiency syndrome retrovirus. Proc. Natl. Acad. Sci. USA **83**:5038–5042.
25. **Willey, R. L., D. H. Smith, L. A. Lasky, T. S. Theodore, P. E. Earl, B. Moss, D. J. Capon, and M. A. Martin.** 1988. In vitro mutagenesis identifies a region within the envelope gene of the human immunodeficiency virus that is critical for infectivity. J. Virol. **62**:139–147.
26. **Wong-Staal, F., P. Chaudra, and J. Ghrayeb.** 1987. AIDS. Res. Hum. Retrovirol. **3**:33–39.
27. **Zoller, M. J., and M. Smith.** 1984. Oligonucleotide-directed mutagenesis: a simple method using two oligonucleotide primers and a single-stranded DNA template. DNA **3**:479–488.