Fine Mapping of an Immunodominant Domain in the Transmembrane Glycoprotein of Human Immunodeficiency Virus†

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Sera from virtually all individuals infected with human immunodeficiency virus contain antibodies against the viral envelope glycoproteins. By using a series of synthetic peptide antigens, we identified an immunodominant domain at amino acid position 598–609 of gp41. The minimal essential epitope is a 7-amino-acid sequence (amino acids 603–609) containing two cysteine residues. Both cysteine residues are required for the antigenic conformation of the sequence, possibly due to creation of a cyclic structure via disulfide bond formation.

During the last 5 years, the acquired immunodeficiency syndrome (AIDS) has evolved from a disease only recognized in a restricted host population to a global epidemic (7, 16). Advances in diagnostic testing and vaccine development will require a clearer understanding of the humoral and cellular immune responses to infection by human immunodeficiency virus (HIV), the etiologic agent of AIDS (2, 8). One approach to identifying and mapping antigenic determinants on HIV is to synthesize peptides representing potential antigenic domains of HIV proteins and to analyze the binding of these peptides to antibodies from HIV-infected patients (10, 25; J. W. Gnann, P. L. Schwimmbeck, J. A. Nelson, A. B. Truax, and M. B. A. Oldstone, J. Infect. Dis., in press). These peptides can also be injected into rabbits to elicit antibodies that can be tested for biologic activity (e.g., neutralization, inhibition of cell fusion).

The general rules for selecting sequences for synthesis are that the peptides should, first, be 10 to 20 amino acids in length and, second, have a surface location as predicted by hydrophilicity and secondary structure analysis (9, 11, 21, 23). Using these criteria, we synthesized over 20 peptides representing various proteins encoded by the HIV *gag*, *pol*, and *env* genes. Although all the peptides were highly immunogenic when injected into rabbits, only *env* peptides reacted with sera from HIV-infected patients (Gnann et al., in press). We have identified a 12-amino-acid peptide (Leu-Gly-Leu-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys; amino acids 598–609) derived from gp41, the transmembrane glycoprotein of HIV, that is recognized by 100% of the sera from HIV-infected patients that we have tested, but by none of the sera from healthy controls. In this report, we further characterize this immunodominant domain by fine mapping the binding epitope through the use of a series of deleted peptides, and we provide evidence that disulfide bond formation plays a key role in the antigenic conformation of the epitope.

Peptides were synthesized by solid-phase methods (12), using an automated peptide synthesizer, and analyzed for purity by high-pressure liquid chromatography. Sera were obtained from HIV-infected patients followed in an outpatient clinic and from uninfected individuals. All of the patients were HIV seropositive when tested by commercially available enzyme-linked immunosorbent assays that

utilize whole-virus preparations as antigens. Specimens were coded, divided into aliquots, and stored at −20°C until tested. Peptide antigens were tested against sera in a standard enzyme-linked, immunosorbent assay (Gnann et al., in press), and optical densities were analyzed on an automated scanner at 492 nm. The cutoff for positivity was defined as the mean optical density at 492 nm plus 3 standard deviations of a panel of 22 negative control sera.

Using the synthetic 12-amino-acid peptide from gp41 as an antigen, we tested sera from 84 HIV-seropositive patients and 80 HIV-seronegative controls (Table 1). The gp41 peptide reacted with 84 of 84 HIV-positive sera and with 0 of 80 HIV-negative sera. In results not shown here, J. McCormick (Centers for Disease Control, Atlanta, Ga.) found this peptide to be reactive with sera from 78 of 79 AIDS patients from the United States and with 0 of 10 negative control sera (McCormick, personal communication). Hence, >99% of HIV-infected patients, but none of the uninfected controls, had antibodies against this gp41 peptide.

To establish which residues comprise the binding epitope essential for reactivity, we constructed a nested set of synthetic peptides. The peptides were synthesized with sequential single-amino-acid deletions from the amino terminus of the original 12-amino-acid peptide. Deletion of three amino acids resulted in a minor reduction in recognition by HIV-positive sera (Table 2, part A). Removal of the next two amino acid residues produced eight- and seven-residue oligopeptides that reacted with 64 and 48%, respectively, of sera from HIV-positive individuals. Removal of the amino-terminal cysteine from the 7-mer yielded a hexapeptide that was not recognized by any of the sera tested. None of the truncated peptides reacted with sera from HIV-negative controls. Next, we synthesized peptides with deletions from the carboxy terminus of the reactive 12-amino-acid peptide (Table 2, part A). None of these peptides was reactive with HIV-positive sera. Thus, the smallest peptide retaining a degree of reactivity is a 7-amino-acid sequence containing a cysteine residue at each terminus. The peptides that contained one or the other of the cysteine residues (but not both) were not reactive.

These data suggest that the cysteine residues at positions 603 and 609 play a critical role in the antigenic conformation of the peptide, perhaps by formation of a cyclic structure via disulfide bonding. We attempted to reduce the peptide with dithiothreitol and to alkylate the sulfhydryl groups with α-iodoacetamide (3); these experiments were unsuccessful due to the poor solubility of the peptide at pH <10. Instead,

TABLE 1. Detection of antibodies to gp41 in human sera with a synthetic peptide antigen

Test group	No. positive/ no. tested
HIV-infected patients	
AIDS (CDC group IV-C, IV-D)[a]	24/24
ARC[b] (CDC group IV-A) and generalized lymphadenopathy (CDC group III)	32/32
Seropositive, asymptomatic (CDC group II)	28/28
Total[c]	84/84
Uninfected controls	
Homosexual men	0/23
Healthy volunteers	0/47
Random blood donors	0/10
Total[d]	0/80

[a] Classification system proposed by Centers for Disease Control (5).
[b] ARC, AIDS-related complex.
[c] Sensitivity, 100%.
[d] Specificity, 100%.

we synthesized another 12-amino-acid peptide containing a conservative amino acid substitution (serine for cysteine at amino acid position 609) that would prevent the formation of intrapeptide disulfide bonds. The substituted peptide with a serine at position 609 reacted with 2 of 22 (9%) HIV-positive sera. In contrast, the original peptide, containing a cysteine at that position, reacted with 100% of the HIV-positive test sera (Table 2, part B).

Thus, our experiments indicate that (i) amino acid sequence 598–609 of the transmembrane glycoprotein of HIV contains an immunodominant epitope; (ii) the essential epitope for immune recognition is a 7-amino-acid sequence containing two cysteine residues (amino acids 603–609; Cys-Ser-Gly-Lys-Leu-Ile-Cys); and (iii) the presence of both cysteine residues is essential for the antigenic conformation of the epitope, possibly via formation of disulfide bonds.

Other investigators have devised computer programs to predict antigenic determinants on HIV (13, 15, 18). Interestingly, none of these computer algorithms predicted the antigenic site we describe in this report. Several of the computer-predicted sites correspond to amino acid sequences we have previously synthesized that, in fact, proved to be poorly antigenic (Gnann et al., in press), indicating the imprecise predictive ability of the current computer pro-

TABLE 2. Fine mapping of the essential structure of an immunodominant determinant of gp41

	Amino acid position[a]	Sequence	No. positive/ no. tested[b]	% Positive
A.	598–609	L G L W G C S G K L I C	22/22	100
	599–609	G L W G C S G K L I C	21/22	95
	600–609	L W G C S G K L I C	20/22	91
	601–609	W G C S G K L I C	19/22	86
	602–609	G C S G K L I C	14/22	64
	603–609	C S G K L I C	21/44	48
	604–609	S G K L I C	0/22	0
	600–607	L W G C S G K L	0/22	0
	598–603	L G L W G C	0/22	0
B.	598–609	L G L W G C S G K L I C	22/22	100
	598–609'	L G L W G C S G K L I S	2/22	9

[a] Amino acid positions based on the sequence published by Wain-Hobson et al. (24).

[b] Number of HIV-positive sera tested. All peptides were nonreactive with normal control sera.

grams. Although not strongly hydrophilic, amino acid sequence 598–609 does contain two amino acids in a β-turn region (Ser-Gly; amino acids 604–605), a predictor of antigenicity (13, 19). Most importantly, this region of gp41 is very highly conserved among HIV strains (1, 14, 17, 20, 22, 24), an important consideration in selecting a sequence for use as a diagnostic reagent or synthetic vaccine.

Modrow and colleagues recently published a theoretical model of the tertiary structure of the HIV envelope polyprotein gp160 (13). In their model, the reactive gp41 sequence described here (amino acids 598–609) is located in a loop external to the viral membrane. This proposed location on the outside surface of the virion would correlate with the antibody response we observed against the synthetic gp41 peptide. Investigators using recombinant, bacterially synthesized large polypeptides roughly corresponding to the hypothetical external loop as antigens in immunoassays have also reported strong antibody responses (4, 6). We have preliminary data (not shown) that rabbit antibody generated against the 12-amino-acid gp41 sequence can neutralize HIV in vitro, further suggesting that the sequence is located in an accessible position on the external envelope of the virion.

Finally, from our studies it seems likely that custom-made peptides will play an increasingly important role in the diagnosis and characterization of a wide variety of infectious diseases.

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