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Immunoglobulin-Like Domain of HIV-1 Envelope Glycoprotein gp120 Encodes Putative Internal Image of Some Common Human Proteins

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ABSTRACT

By examining sequence similarity between the V3-loop of gp120 from various HIV-1 isolates and human proteins, we found that the V3 loop portion KKGIAIGPGR in strain New York 5 (HIV-1_{NY5}) shares 70% identical residues with the collagen-like region (CLR) of human complement component C1q-A. C1q CLR was found to react with autoantibodies from several autoimmune disorders. Thus, we assumed that it would be of interest to find out the C1q reactivity with antibodies from AIDS sera. The results obtained show that the V3 loop-derived synthetic peptide KKGIAIGPGRTLY reacts both with AIDS patients sera and with antibodies purified on the V3 loop peptide-affinity column. The same affinity-purified antibodies bind also to C1q molecules. Since, according to our previous results, HIV-1 V3 loops and immunoglobulin heavy chain variable regions (Ig V_H) share several common features, we suggest that the envelope of HIV-1_{NY5} bears a functional internal image of the C1q-A CLR epitope. Therefore, gp120 could manipulate the immune network and contribute to HIV-induced autoimmunity.

The concept that idiotopes on immunoglobulin variable region (Ig V) can mimic epitopes on other kinds of molecules (1) has been successfully applied to the production of antiidiotype antibodies ($Ab_2\beta$) that mimic the binding domain of viral cell-attachment proteins (2). $Ab_2\beta$ vaccines are now considered a promising route for the induction of antiviral immunity through the involvement of the idiotype network (see ref. 3 for review). A general problem of this approach lies in the fact that viruses very often utilize lymphocyte surface molecules as receptors for infection (4). The normal function of the virus-binding

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membrane structures may be perturbed by $Ab_2\beta$ antibodies, i.e., the same antibody that mimics a viral epitope may also act as an antireceptor autoantibody (5). Immunization with neutralizing antivirus monoclonal antibodies (Ab_1), on the other hand, relies on the involvement of the immunoregulatory network, so the resulting $Ab_2\beta$ level can be quite low, and consequently the amount of Ab_3 synthesized may not be as great as the amount of Ab_1 usually formed in response to a viral antigen.

HIV binds to cell-surface antigen CD4 through its envelope glycoprotein gp120 (6). Despite the polymorphism of HIV-1 gp120, different isolates bind to the same CD4 molecule, suggesting that conserved rather than unique gp120 regions are the likely participants in receptor binding. Antibodies to the CD4 binding site of HIV block CD4 binding (7). These antibodies are group-specific but do not appear to effectively neutralize viral infectivity, suggesting that the primary neutralization epitope(s) and the CD4 binding epitope on gp120 may be separate. The major and highly type-specific neutralizing epitope on gp120 appears to be the so-called V3 loop, which extensively varies in amino acid sequence among different isolates (8,9). The V3-specific neutralizing antibodies occur early in acute infection and prevent the penetration of the virus into the cell. Even though the variability of the V3 loop seems to be the major mechanism for the production of escape mutants (10), the V3 loop is widely considered as one of the main candidates for vaccine design. The molecular basis of this approach is a short consensus motif within the V3 loop (the "crown" motif) shared by HIV-1 isolates from both Europe and the United States (11).

According to our previous results, a gp120 region of about 45 residues, encompassing the V3 loop and 10 residues upstream, shares several features with immunoglobulin heavy chain variable regions (Ig V_H). The common features include (i) similarity of consensus sequences (12,13), (ii) similar distribution of residue variability (13), and (iii) similarity of secondary structure propensity profiles (14). Therefore we have proposed that idiotope structures present on Ig V domains also might be present on gp120 (15). Since the antiidiotype approach is considered as an important vaccine strategy for HIV (3), it would be of interest to investigate possible similarities between the major neutralizing epitope on gp120 and human proteins, especially those of immunological interest.

In the present study we report local homology detected between a particular V3 loop and several human proteins. The analysis of 23 different V3 loops found in Swiss-Prot 25 (16), in which all overlapping 11 residue V3 loop segments were aligned with the sequences from the 5th release of the Kabat's collection of immunologically interesting proteins (17), revealed homology (i.e., 70% residue identity) between a 10-residues-long V3 loop segment from isolate New York 5 ($HIV\text{-}1_{NY5}$) and the collagen-like region (CLR) of human complement component C1q-A (Fig. 1a). Their common motif KKG*A**PGR, denoted patt6b, overlaps the conserved "crown" motif GPGRA located at the tip of the V3 loop (Fig. 1b). A further comparison performed on several protein databases revealed that fragments similar to patt6b also can be found in certain other common human proteins. These proteins include different collagens (particularly the $\alpha 1$ chain of collagen type VI), granzyme A (trypsin-like serine-protease specific for cytotoxic T cells and NK cells), complement component C8α, and NGF-β, as summarized on Fig. 1c.

The main objective of this work was to establish whether antibodies from AIDS serum, purified by V3 loop peptide-affinity chromatography, can also recognize human C1q molecules. The reactivity of 12 AIDS patients' sera with the synthetic peptide derived from the V3 loop of $HIV\text{-}1_{NY5}$ (Fig. 1b) was determined by either RIA or ELISA. The majority of the AIDS sera (10 of 12 tested) do contain antibodies recognizing the V3 loop peptide (Fig. 2A). However, neither does the unrelated peptide bind antibodies from AIDS sera, nor does the V3 loop peptide recognize antibodies from 9 healthy blood donors' sera (Fig. 2A). Next, we asked whether antibodies purified from a particular AIDS serum on the V3 loop peptide-affinity column will bind to C1q molecules. Figure 2B shows that affinity-purified antibodies react both with the V3 loop-derived peptide and with C1q.

These findings suggested to us that, as part of its infective strategy, HIV-1 may encode in its envelope functional internal image(s) of self-epitope(s). If so, then gp120 may take part in HIV-1-induced autoimmune pathology in a manner analogous to that of an $Ab_2\beta$ antibody (24). Thus, an individual V3 loop bearing internal image(s) could disturb the hegemony of the idiotypic network by binding to the complementary structures on antigen receptor V domains. Actually, autoantibodies to C1q CLR have been already detected in some autoimmune disorders such as HUVS and SLE (25), meaning that among potentially autoaggressive cells residing in healthy individuals (26) there are also those able to produce and regulate the produc-

a)	KKGIAIGPGR	V3-loop, gp120 of HIV-1_{NY5}	
	KKGEAGRPGR	human C1q-A CLR (res. 10-19)	
	KKG*A**PGR	patt6b	
b)	RKSIHIGPGRAFY	consensus of 245 HIV-1 gp120 V3-loops	
	KKGIAIGPGRTLY	V3-loop derived peptide (gp120 of HIV-1_{NY5})	
	KKG*A**PGR	patt6b	
c)	KKGiAigPGR	V3-loop, gp120 of HIV-1_{NY5}	7
	KKGeAgrPGR	human C1q-A CLR	7
	eKGeAgdPGR	human collagen type VI α1 chain	6
	*KG**g*PGR	consensus of 20 best-matching collagens	5
	*KGaAfyPGh	human and guinea pig NGFβ	5
	qnGgAscPGR	human complement component C8α	5
	KKGgAktkGR	consensus of 5 *E. coli* ssDNA-binding proteins	6
	KKGddvkPGt	human and mouse granzyme A	5

FIG. 1. Local homology between the V3 loop from gp120 of HIV-1 isolate New York 5 (HIV-1_{NY5}) and some common proteins. The "crown" motif at the tip of the V3 loop is underlined. Asterisk (*) indicates one nonconserved residue. The sequence of the V3 loop, found in Swiss-Prot 25 (16), was compared as a whole and in 11-mer fragments with the proteins from the Kabat 5 database (17) using Fastdb (18,19) and Chopper (20). **(a)** Alignment of the V3 loop from HIV-1_{NY5} with the collagen-like region (CLR) of human complement component C1q-A. Their common pattern patt6b is present uniquely in these two sequences, as established by pattern-matching searches performed by Quest (19) against databases Swiss-Prot 25, Kabat 5, and HIV 4 (21). **(b)** Alignment of patt6b, shared by the V3 loop and human C1q-A CLR, with the consensus of 245 V3 loops (11). **(c)** Best similarities to patt6b in database Swiss-Prot 25, identified using programs Fastdb and Fasta (22,23). Residues identical to those in patt6b (i.e., *both* to C1q-A CLR and to the V3 loop) are capitalized and their total number per sequence (maximally 7) is given in the third column.

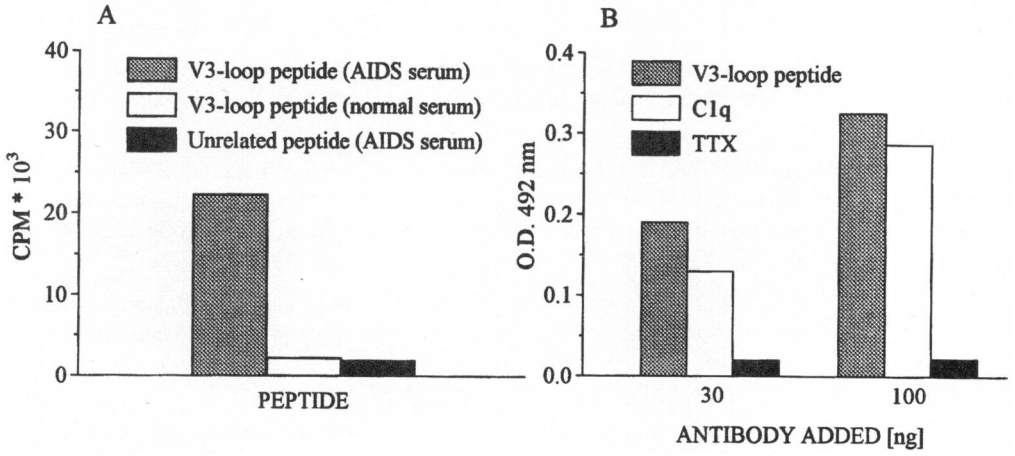

FIG. 2. Binding of antibodies from AIDS patient's sera to HIV-1_{NY5} gp120 V3 loop-derived synthetic peptide KKGIAIGPGRTLY and human complement component C1q. Microtiter plates were coated with BSA-coupled V3 loop peptide (200 ng/well), unrelated peptide LAPPAPPDELLRK (200 ng/well), human C1q, and TTX (500 ng/well), then blocked with 2% BSA. Diluted (1:200) AIDS patient serum or approximately 30 and 100 ng of the purified antibodies was added. Bound antibodies were detected in RIA or ELISA assays. Samples were considered positive if their CPM or OD readings were more than 3 SD above the mean obtained for the control samples. Representative experiments are illustrated.

tion of autoantibodies to C1q CLR. Under certain conditions these cells, being normally under strict control, might become active in producing autoantibodies. We recently proposed a possible mechanism for the interaction of the Ig-like portion of gp120 with V domains of some Ig superfamily members (27) that might help in the explanation of the uncontrolled immune system activation observed in HIV infection (28). In addition to a possible serological link between the central portion of the gp120 V3 loop and C1q-A CLR, gp120 may also interact with the C1q receptor, since C1q CLR represents the binding site for the receptor (29). The interaction of C1q with B cells and subsequent enhancement of Ig production was found to occur via Cq1 CLR (30). Furthermore, tryptase TL_2, a membrane-bound serine esterase present on the surface of human monocytoid and $CD4^+$ lymphoid cells (31), specifically binds to the V3 loop GPGR motif (32). The question of whether the V3 loop can still be considered as a suitable candidate for developing a safe anti-HIV vaccine remains more than actual.

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