AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 10, Number 11, 1994
Mary Ann Liebert, Inc., Publishers

Reactivity of AIDS Patient Sera with a Peptide Derived from HIV Type 1_{NY5} Glycoprotein 120 V3 Loop and Consensus Sequence of Collagens

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PREVIOUSLY WE HAVE REPORTED the structural similarity between a portion of HIV-1 envelope glycoprotein (gp120) and human immunoglobulin heavy chain variable region (IgV_h) of group III gene products.[1-3] The similarity was detected on a continual fragment of consensus sequences involving about 42 residues. This represents the first reported gp120/Ig homology that includes a hypervariable gp120 portion, i.e., the V3 loop also denoted as the principal neutralizing determinant (PND).[4] That observation led us to suggest that gp120 or V3 loop fragments might play a role in triggering autoimmune responses[5] being thus inadequate for vaccine development.[6] The immunogenic determinant of the virus envelope could initiate the formation of antibodies or effector lymphocytes, which, in turn, might react with homologous host protein sequences. Since similarity of diverse V3 loops with the portion of V_h III molecules was confirmed by several criteria,[3,6] and since the V3 loop is one of the most variable gp120 regions, we suggested that the V3 loop may both share linear epitopes with host proteins and interact with the complementary determinants of antigen receptors.[5,6]

Here, we report the sequence homology between a V3 loop region of HIV-1 strain New York 5 ($HIV\text{-}1_{NY5}$) and several human collagens, particularly collagen type VI α1 chain (Fig. 1a), obtained by a computer-assisted search[7] of protein sequence database Swiss-Prot 25.[8] To answer the question of whether this level of sequence similarity is sufficient for immunological cross-reactivity between the selected peptides, an $HIV\text{-}1_{NY5}$ gp120 V3 loop-derived peptide KKGIAIGPGRTLY and peptide CKGCAGDPGRPDD, which encompasses the consensus KG**G*PGR of homologous collagen sequences (Fig. 1b), were designed and synthesized. In the later peptide, each amino acid on a position not defined by the collagen consensus differs from the amino acid on the corresponding position in the typical V3 loop (Fig. 1c) based on 245 reported V3 loop sequences.[9]

The collagen consensus peptide and $HIV\text{-}1_{NY5}$ gp120 V3 loop-derived peptide were coupled to bovine serum albumin (BSA) and used as antigens in solid phase assay to investigate the reactivity of sera obtained from 16 AIDS patients and 15 healthy HIV-negative individuals (Fig. 2). The results revealed that both the V3 loop-derived and the collagen consensus peptide bind only antibodies from sera of AIDS patients. Only one serum of the tested 16 patients sera showed reactivity at the level of the controls. No reactivity was observed using the unrelated peptide or the control sera (Fig. 2). Furthermore, purified antibodies from AIDS patient serum on the V3 loop-derived synthetic peptide-affinity column reacted with both the V3 loop-derived peptide and with the collagen consensus peptide (Fig. 3).

These data suggest that although there is homology between

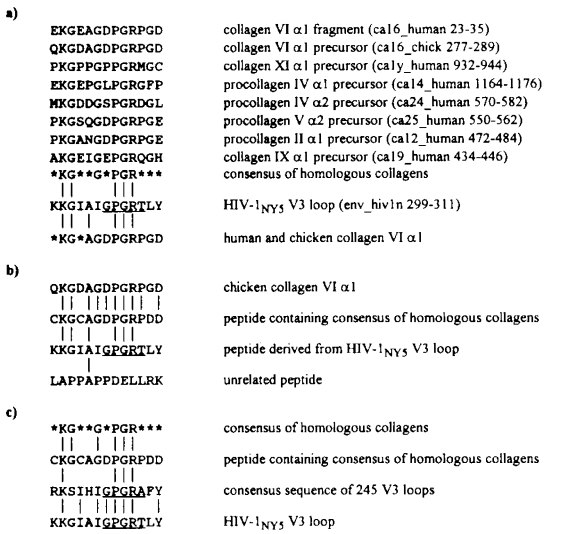

FIG. 1. Sequence similarity between HIV-1 isolate New York 5 gp120 V3 loop fragment KKGIAIGPGRTLY and various human collagens. The "crown" motif at the tip of the V3 loop is underlined. (a) Alignment of the V3 loop fragment with homologous collagen fragments. Among different collagens, the one best aligned with the V3 loop fragment is the α1 chain of collagen VI (6 common residues). Sequence names and residue numbers are from Swiss-Prot 25.[8] (b) Alignment of peptides used in this study with a homologous fragment from chicken collagen type VI α1 chain. (c) The peptide containing the collagen consensus does not have additional common residues (except those defined by the collagen consensus) with the consensus sequence of 245 V3 loops.

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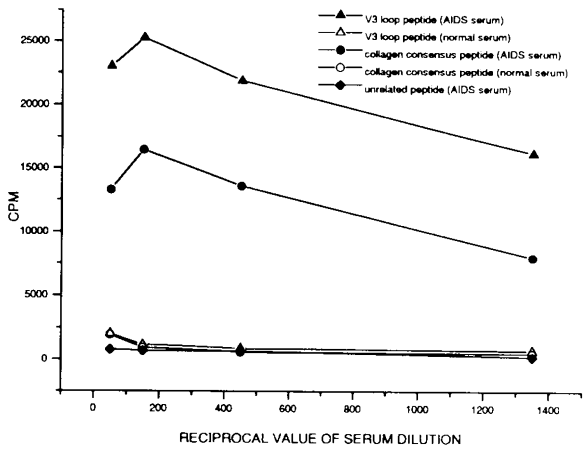

FIG. 2. Binding of human serum antibodies to peptide–BSA conjugate-coated plates. Serial dilutions of sera were obtained from 16 AIDS patients and from 15 normal blood donor controls. Antibody activities were assayed by solid-phase RIA. To duplicate plastic microtiter wells coated with the V3 loop-derived peptide, collagen consensus peptide, and unrelated peptide (0.5 μg/well), serial threefold dilutions of the sera were added. After incubation and washing, bound antibodies were detected with ^{125}I-labeled anti-human κ-light chain. Values obtained on control wells, incubated only with buffer instead of primary antibody or with sera assayed on wells without plated peptide, were subtracted. The results of one representative experiment are presented. Sera giving 2.1 times the mean cpm of negative control were assumed to contain antibodies reactive with the peptides.[10]

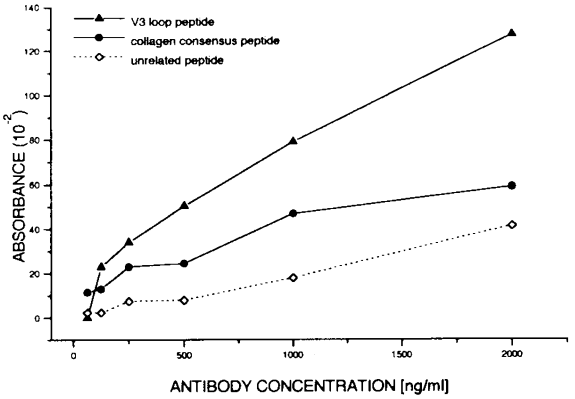

FIG. 3. ELISA reactivity of antibodies purified by absorption to V3 loop-derived peptide-loaded affinity matrix. A V3 loop-derived synthetic peptide-affinity column was prepared by covalently attaching 2 mg of peptide to 1.5 g CNBr-activated Sepharose 4B, using the method suggested by the manufacturer. Microtiter plates were coated with 0.5 μg/well of peptides and then incubated with antibodies purified from AIDS patient serum. Bound antibodies were detected with goat anti-human IgG.

HIV-1_{NY5} gp120 V3 loop and some common human proteins, the organism is not tolerant, i.e., HIV viral determinants are recognized as foreign antigens. The immune response initiated against the HIV-1_{NY5} V3 loop may, however, react with closely homologous self-host proteins such as collagens. Inasmuch as polyclonal anticollagen antibodies (risen against chicken collagen VI α1, containing a region homologous with V3 loop and human collagens, Fig. 1b) bind both to the V3 loop-derived peptide and to the collagen consensus peptide (results not presented), the cross-reactivity between molecules with the degree of sequence similarity here shown could occur. Therefore antibodies or cytotoxic lymphocytes generated against the HIV-1 gp120 V3 loop might interact with some self-determinants, thereby causing cellular autoinjury leading to persistent disease. Furthermore, the results are in accordance with Grant *et al.*[11] who suggested that the presence of antibodies against denatured collagen in HIV-negative persons may augment their susceptibility to the pathogenic effects of HIV infection.

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