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Virology 345 (2006) 44 – 55
VIROLOGY
www.elsevier.com/locate/yviro

Analysis of HIV-1 subtype B third variable region peptide motifs for induction of neutralizing antibodies against HIV-1 primary isolates

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Received 28 April 2005; returned to author for revision 14 July 2005; accepted 22 August 2005
Available online 20 October 2005

Abstract

The HIV-1 gp120 V3 loop is a potent inducer of neutralizing antibodies for T cell line adapted-HIV-1, but less so for primary isolates. We hypothesized that peptides representative of the diversity of natural HIV-1 V3 loop variants might capture elements of conserved higher order structures and so stimulate broadly reactive neutralizing antibodies. We designed a panel of 29 subtype B V3 sequences postulated to reflect the range of V3 diversity. These peptides were used to immunize guinea pigs. The most effective peptide (62.19) clustered around the subtype B consensus sequence and induced antibodies that reproducibly neutralized 31% of the subtype B HIV-1 primary isolates evaluated, but exhibited limited cross-neutralization of non-subtype B HIV-1 strains. Taken together, these data demonstrated that the limited neutralization profile of antibodies induced by optimal subtype B V3 motifs likely represents the maximum breadth of neutralization of subtype B HIV-1 primary isolates attainable by anti-V3 peptide antibodies.

Keywords: HIV-1 vaccine; HIV-1 envelope; V3 loop; Neutralization; Antibody; Peptide; Immunogen; Guinea pig

Introduction

Design of HIV-1 immunogens capable of inducing high-titer antibodies that neutralize a broad spectrum of HIV-1 primary isolates is a major priority for HIV-1 vaccine development. When the gp120 third variable region (V3) loop was identified as a potent neutralizing determinant for T cell line adapted (TCLA) strains of HIV-1, it was thought that gp120 or a V3 subunit would be an important component of an HIV-1 vaccine (Javaherian et al., 1989; LaRosa et al., 1990; Palker et al., 1988, 1989; Rusche et al., 1988). However, observations that anti-V3 antibodies poorly neutralize HIV-1 primary isolates in PBMC (Hanson, 1994; Matthews, 1994) dampened enthusiasm for the gp120 V3 loop as a vaccine target.

Recent studies, however, suggested that certain antibodies reactive with the V3 loop can indeed neutralize HIV-1 primary isolates, and have rekindled interest in developing V3 immunogens as targets for antibodies that can broadly neutralize HIV primary isolates. Hioe et al. (1997) have shown that polyclonal and human monoclonal antibodies (mabs) against V3 can neutralize select HIV-1 primary isolates. Liao et al. (2000) used C4–V3 peptides with the V3 loops of $gp120_{89.6}$ and $gp120_{89.6P}$ to induce antibodies in guinea pigs and monkeys that neutralized $SHIV_{89.6}$ in vitro. Krachmarov et al. (2001) affinity purified anti-V3 antibodies from HIV-1+ human serum and demonstrated effective anti-V3 neutralization of HIV-1 primary isolates. Finally, Letvin et al. (2001) showed that C4–$V3_{89.6P}$-immunized rhesus monkeys were protected from $SHIV_{89.6P}$-induced CD4+ decline. Taken

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doi:10.1016/j.virol.2005.08.042

together, these studies suggested that, in certain circumstances, anti-V3 antibodies can prevent primary isolate SHIV-induced disease, and could be a relevant target for neutralization of primary HIV-1 isolates.

The V3 loop has important functions in HIV-1 Env-mediated fusion. Amino acid charge in the V3 loop can determine HIV-1 tropism and co-receptor usage (Chesebro et al., 1988; de Jong et al., 1992; Fouchier et al., 1992; Milich et al., 1993; Shioda et al., 1992). Moreover, the V3 region is located within gp120 adjacent to the co-receptor binding site, and participates in co-receptor interactions with gp120 (Hu et al., 2000; Ping et al., 1999; Shioda et al., 1992; Trkola et al., 1996; Wang et al., 1999; Wu et al., 1996). Thus, in spite of the remarkable variability within V3, it is likely that conserved higher order structures of the V3 region exist that are critical for co-receptor binding and HIV-1 infectivity (Catasti et al., 1996). In this regard, Sharon et al. have studied the structure of V3 peptides bound to a neutralizing human mab, 447-52D, and found structural similarities between the V3 loop and the chemokines that are natural ligands for HIV-1 co-receptors (Sharon et al., 2003) although it was not shown that the chemokine loop is involved in chemokine receptor binding.

In an effort to design V3 immunogens that would induce neutralizing antibodies of maximum breadth for HIV-1 primary isolates, we have clustered subtype B V3 sequences available in the HIV database into like-groups based on a sequence diversity scoring system (Korber et al., 1994) using an amino acid substitution matrix that incorporated structure (Henikoff and Henikoff, 1993). We have tested selected V3 peptides for their ability to bind human neutralizing anti-V3 mabs, and for their ability to induce neutralizing antibodies against HIV-1 primary isolates in guinea pigs. We found our selection process to clearly define HIV-1 gp120 V3 motifs with better capacities for induction of neutralizing antibodies than others. However, the selected V3 motifs induced neutralizing antibodies against a minor subset of HIV-1 primary isolates, thus defining the limits of V3 peptide subunits as components of an experimental HIV-1 vaccine.

Results

Design of HIV-1 subtype B V3 peptides for immunogenicity studies

In the 2001 Los Alamos HIV Sequence Database (www.LANL.gov), there were 6870 subtype B V3 sequences from the United States of which there were 1514 unique forms. From these 1514 sequences, we chose 30 peptides as described in Methods, of which 29 could be successfully synthesized and purified. The initial selection of representative sequences was based on primary amino acid (aa) alignment, using maximum linkage clustering (Korber et al., 1994). The related clusters were then scored for similarity based on the presence of those aa that are predicted not to markedly affect higher order structures (e.g. Lys → Arg) versus those aa changes that would markedly affect structure (e.g. Phe → Arg) (Henikoff and Henikoff, 1993). Finally, a representative of each of 30 clusters was selected by inspection of viral sequences using two criteria. First, within the context of each cluster, we attempted to select a sequence that was repeated many times in the complete data set of 6870 sequences, as we reasoned that frequent repetition of a sequence was indicative of a loop that retained a favorable conformation for the virus, and that a loop that was naturally repeated many times was more likely to elicit antibodies that could cross-react with multiple isolates. Second, we selected a sequence that was central to the cluster, not an outlier, to minimize the distance to all other sequences in an attempt to provide the best coverage of the cluster (Fig. 1).

The peptides chosen for synthesis are shown in Table 1. Each synthesized peptide had the C4 region synthesized N-terminal to the V3 region for maximum immunogenicity (Haynes et al., 1995; Palker et al., 1989). The consensus sequence (a concatenation of the most common amino acid in every position in the alignment) based on the unique sequence alignment of 1514 peptides of the subtype B V3 region is reflected in sequence 1.481. Peptide 1.481 was present 481 times in the complete set of 6870 sequences. As the most commonly observed peptide, it was ranked first and assigned "1" at the beginning of its identifier. When a sequence/putative motif was present only once, as was the case with sequence 1448.1, it was chosen because it was deemed most representative of a cluster of related peptides, and meeting the second criteria for representative peptide selection in a cluster where no sequence was found repeated in the full set of peptides.

Immunization of guinea pigs with C4–V3 subtype B peptides

A single guinea pig was immunized with each subtype B C4–V3 peptide to generate 29 pre- and post-bleed paired sera that were screened for peptide immunogenicity using a single round pseudotype virion neutralization assay (Tables 1 and 2). A spectrum of breadth of 50% neutralizing antibody responses of 20 HIV-1 isolates (19 B subtype, one AG subtype) was observed; 10/29 C4–V3 peptide sera were unable to neutralize any of the isolates tested, while one peptide sera (62.19) was able to neutralize 8 isolates (Tables 1 and 2). It is interesting to note that peptide 62.19 does not completely correspond to the consensus; the sequence near the tip of the loop is identical, but 62.19 has two substitutions near the C-terminal end.

Because human anti-V3 Mabs 447-52D and 39F neutralize HIV-1 primary isolates (Gorny et al., 2002; Gorny et al., 2004) (James Robinson, personal communication), we tested the ability of these two human Mabs to bind the subtype B C4–V3 peptides in ELISA assays. Peptides that induced antibodies that neutralized ≥3 HIV isolates had significantly higher OD at 405 nm binding to Mabs 39F ($P < 0.0002$) and 447-52D ($P < 0.0001$), than did the 10 C4–V3 peptides that were unable to neutralize any HIV-1 primary isolates (Table 1) (Fig. 2). Thus, from these data of peptide binding to Mab 447-52D, we