Enhanced Recognition and Neutralization of HIV-1 by Antibody-Derived CCR5-Mimetic Peptide Variants

Jessica J. Chiang, Matthew R. Gardner, Brian D. Quinlan, Tatyana Dorfman, Hyeryun Choe, and Michael Farzan

Department of Microbiology and Immunobiology, Harvard Medical School, New England Primate Research Center, Southborough, Massachusetts, USA, and Department of Pediatrics, Harvard Medical School, Children’s Hospital, Boston, Massachusetts, USA

A tyrosine-sulfated CCR5-mimetic peptide, CCR5mim1, inhibits HIV-1 infection more efficiently than sulfopeptides based on the CCR5 amino terminus. Here we characterized sulfopeptide chimeras of CCR5mim1 and the heavy-chain CDR3 of the antibody PG16. Two chimeras bound a range of envelope glycoproteins and neutralized HIV-1 more efficiently than CCR5mim1. An immunoadhesin form of one of these, CCR5mim2-Ig, synergized with CD4-Ig to neutralize HIV-1. These sulfopeptides are among the broadest and most potent CCR5-mimetic peptides described to date.

Human immunodeficiency virus type 1 (HIV-1) entry requires cellular expression of CD4 and a coreceptor, principally CCR5 or CXCR4. Virion association with CD4 triggers conformational changes in the HIV-1 envelope glycoprotein gp120 that promote high-affinity association with the coreceptor. An acidic, tyrosine-sulfated region of the CCR5 amino terminus is critical for gp120 association, and most or all functional HIV-1 and simian immunodeficiency virus (SIV) coreceptors have amino termini bearing multiple sulfotyrosines. These sulfotyrosines interact with conserved pockets in the C4 region and at the base of the V3 loop of HIV-1 gp120. Coreceptor-binding site antibodies with tyrosine-sulfated antigen-combining regions also bind these conserved pockets, including E51, the most potent of the CD4-inducible (CD4i) HIV-1 neutralizing antibodies. Recently, two additional broadly neutralizing antibodies, PG9 and PG16, have been identified. Like E51, these antibodies include sulfotyrosines in their heavy-chains.
chain CDR3. However, unlike CD4i antibodies, their association with the HIV-1 envelope glycoprotein is not enhanced by CD4.

Sulfopeptides based on the sequence of the CCR5 amino terminus specifically bind gp120 and inhibit HIV-1 entry, but only at 50 to 100 μM concentrations (4, 10), precluding their use as therapeutics. A mimetic peptide derived from the heavy-chain CDR3 of E51, p\(p\)PG16v4 (CCR5mim1), associates with gp120 with higher affinity and more efficiently neutralizes HIV-1 entry (6, 14). CCR5mim1 retains the arrangement of CCR5 sulfotyrosines, but it outperforms CCR5-based peptides, likely because it is more flexible and soluble than CCR5-based peptides.

The example of CCR5mim1 suggested that additional antibody-derived peptides may bind gp120 more broadly and efficiently than either CCR5-based peptides or CCR5mim1. Accordingly, we investigated whether pPG16v4, a peptide based on the heavy-chain CDR3 of PG16 (Fig. 1A), could precipitate gp120 using a previously described assay. We observed that pPG16v4-Ig, a fusion of this peptide with the human IgG1 Fc domain, bound metabolically labeled consensus B and C gp120 molecules with efficiency comparable to or better than that of either CCR5mim1-Ig or pPG16v4-Ig (Fig. 2B). We further compared two of these variants, pSwap3-Ig and pSwap7-Ig, with CCR5mim1-Ig and pPG16v4-Ig for their ability to bind a range of cell-surface-expressed HIV-1 envelope glycoproteins. In most cases, these peptides bound envelope-glycoprotein-expressing cells more efficiently than CCR5mim1-Ig, pPG16v4-Ig, or a control Fc fusion with the sulfopeptide derived from the C5a receptor, pC5aR-Ig (Fig. 3A). In some cases, these peptides bound poorly, but their association was markedly enhanced by sCD4 (Fig. 3B). Also, pSwap3-Ig and pSwap7-Ig bound gp120 in the same region as both CCR5 and CCR5mim1, as indicated by
their inability to bind consensus B (ConB) gp120 variants altered in a conserved CCR5-binding region (Fig. 3C) (6, 19). Given its close similarity to CCR5mim1 and its ability to bind envelope glycoprotein with higher affinity, we refer to pSwap7-Ig from this point on as “CCR5mim2-Ig.”

Next we analyzed pC5aR-Ig, CCR5mim1-Ig, pSwap3-Ig, and CCR5mim2-Ig using a previously described entry inhibition assay (6, 10). Both pSwap3-Ig and CCR5mim2-Ig neutralized the dualtropic, clade B isolate 89.6 more efficiently than CCR5mim1-Ig, with 50% inhibitory concentrations (IC50s) of approximately 10 nM (Fig. 4A). They better neutralized consensus B and C isolates, but with IC50s of approximately 4 μM (Fig. 4B). Using a previously described TZM-bl cell neutralization assay (16), we next investigated the ability of CCR5mim2-Ig to enhance CD4-Ig-mediated neutralization of HIV-1. When total protein was kept constant at the IC50 of CD4-Ig alone, a 9:1 ratio of CD4-Ig to CCR5mim2-Ig (4.5:1 on a molar basis) neutralized all pseudoviruses assayed more efficiently than CD4-Ig alone (Fig. 4C). This effect was evident at a 9:1 ratio for a range of total protein concentrations (Fig. 4D).

The synergy between CCR5mim2-Ig and CD4-Ig raises the possibility that a CCR5-mimetic peptide such as CCR5mim2 might increase the therapeutic utility of CD4-Ig. Despite its necessary breadth, CD4-Ig has been disappointing therapeutically because it has lower affinity for gp120 than effective neutralizing antibodies and because at low concentrations CD4-Ig enhances rather than inhibits HIV-1 entry (18, 20). This enhancement may occur because soluble forms of CD4 promote virion association with the coreceptor (21, 23). CCR5mim2-Ig can prevent coreceptor association with the virus and limit this enhancement. CCR5 mimetics like CCR5mim2-Ig have a second property that may also contribute to its potency as a partner for CD4-Ig. Unlike CD4-Ig, both arms of the dimeric CCR5 mimetic immunoadhesin can bind to gp120 monomers of an envelope glycoprotein trimer (14), perhaps more effectively preventing virus association with the cell. Finally, it is well established and consistent with our data here that sCD4 and CD4-Ig markedly enhance gp120 association with CCR5-mimetic peptides (4, 6, 10). We have also shown that in some cases, CCR5-mimetic peptides can decrease the off rate of CD4-mimetic peptides (14) and perhaps CD4-Ig from the envelope glycoprotein.

Combinations of CD4- and CCR5-mimetic peptides have one key advantage over most neutralizing antibodies: they associate with necessarily conserved regions of gp120 (15, 24). In contrast,
antibody epitopes are larger than the CD4- and CCR5-binding sites and so must include variable residues that permit escape. Recent advances in gene therapy, such as self-complementary adeno-associated virus (scAAV), have enabled persistent expression of high levels of immunoadhesins but impose a size limit that precludes expression of full-length antibodies (13, 17). In contrast, both CD4-Ig and CCR5mim2-Ig can be easily expressed by scAAV vectors. Further study of their use in this context and further improvement of CCR5-mimetic peptides are therefore warranted. Finally, CCR5mim1, CCR5mim2, and pSwap3 may be useful in exploring the variation in the sulfotyrosine-binding pockets of gp120 or investigating the conformational transitions of the HIV-1 envelope glycoprotein.

REFERENCES


