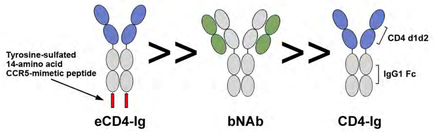
A CCR5-mimetic sulfopeptide converts CD4-Ig to a highly potent inhibitor of HIV-1 entry

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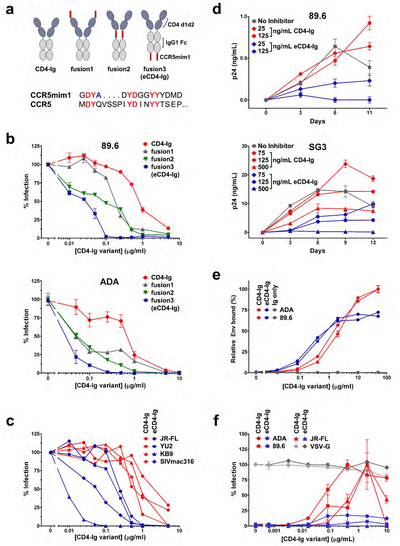


Abstract

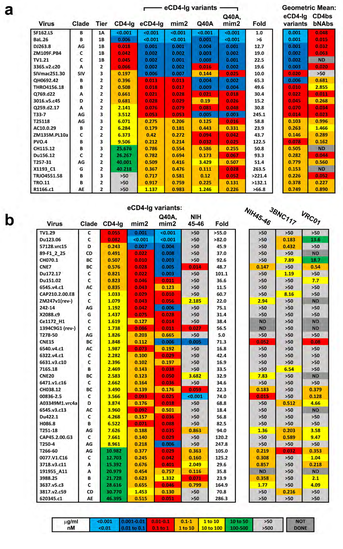
Soluble CD4 and its more bioavailable form, CD4-Ig, neutralize HIV-1 less potently than many broadly neutralizing antibodies (bNAbs) because they bind the HIV-1 envelope glycoprotein with lower affinity, and they promote infection when cellular CD4 is limiting. Here we show that a fusion of CD4-Ig with a small CCR5-mimetic sulfopeptide (eCD4-Ig) overcomes both of these shortcomings: eCD4-Ig bound the envelope glycoprotein with high avidity and its sulfopeptide inhibited infection promoted by CD4-Ig. eCD4-Ig efficiently neutralized a diverse panel of antibody-resistant tier 2 and tier 3 HIV-1 isolates with IC_{50}s ranging from 0.002 to 1.137 μg/ml (0.02-11 nM) − values comparable to or better than those of the best HIV-1 bNAbs.

Because it binds only conserved regions of the HIV-1 envelope glycoprotein, it is also broader than any bNAb. Underscoring this breadth, eCD4-Ig also potently neutralized HIV-2 and antibody-resistant SIV isolates. Further, eCD4-Ig induced markedly more efficient antibody-dependent killing of HIV-1- or SIV-infected cells than did CD4-Ig.

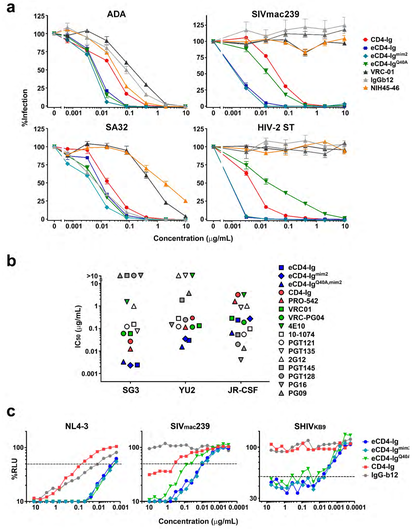
Finally, eCD4-Ig retained its efficacy *in vivo*: it protected humanized mice from a high-dose HIV-1 challenge. In addition, a rhesus macaque analogue could be expressed at 70-160 μg/ml in macaques inoculated with an adeno-associated virus (AAV) vector. eCD4-Ig is thus a highly potent, exceptionally broad and mechanistically distinct inhibitor of HIV-1 entry with the potential to prevent or treat HIV-1 infection.



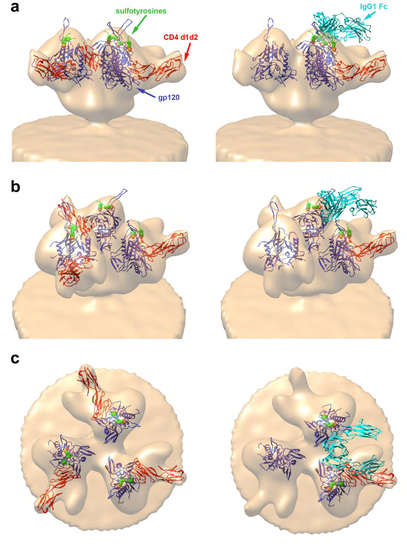
Characterization of CD4-Ig/CCR5-mimetic peptide fusion proteins. **a**, CD4-Ig and fusion constructs with CCR5mim1 peptide are represented. CD4-Ig is comprised of CD4 domains 1 and 2 (blue) fused to a human IgG1 Fc domain (grey). In fusion3 (renamed eCD4-Ig), a sulfopeptide (red) is fused to the carboxy-terminus of CD4-Ig, preceded by a tetraglycine linker. **b**, HIV-1 pseudotyped with the envelope glycoproteins of the 89.6 or ADA isolates was incubated with GHOST-CCR5 cells and the indicated concentrations of CD4-Ig variants. Infection was measured by flow cytometry 2 days later. **c**, Experiments similar to those in panel b using HIV-1 pseudotyped with the indicated envelope glycoproteins. **d**, Infectious HIV-1 was incubated with PBMC in the presence of the indicated concentrations of CD4-Ig or eCD4-Ig. Supernatants were collected on the day indicated and viral p24 levels were measured by ELISA. **e**, 293T transfected to express the indicated envelope glycoprotein were incubated on ice with indicated concentrations of CD4-Ig, eCD4-Ig, or IgG. Binding was analyzed by flow cytometry. **f**, HIV-1 expressing luciferase and pseudotyped with enveloped glycoproteins of the indicated isolates or with VSV-G was incubated with Cf2Th-CCR5 cells in the presence of CD4-Ig or eCD4-Ig. Luciferase activity was measured two days post infection to assess viral infection.



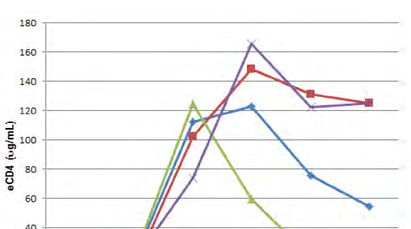
eCD4-Ig variants efficiently neutralize Tier 2 and Tier 3 HIV-1 and SIV isolates.
a, The IC50 values (μg/mL) of CD4-Ig, eCD4-Ig, eCD4-Ig^{mim2}, eCD4-Ig^{Q40A}, and eCD4-Ig^{Q40A,mim2} against 24 viral isolates selected for their neutralization resistance are shown. HIV-1 pseudotyped with the indicated envelope glycoprotein was incubated in triplicate with TZM-bl cells and varying concentrations of the inhibited CD4-Ig variant. Luciferase activity was determined two days post-infection. 'Fold' indicates the ratio of the IC_{50} value of CD4-Ig to the IC_{50} value of the geometric mean of the assayed eCD4-Ig variants. The geometric mean of CD4bs antibodies 3BCN117, NIH45-46, and VRC01 was calculated from values reported in Huang et al. and Sheid et al. **b**, Experiments similar to those in panel a except that the IC_{50} values of CD4-Ig, eCD4-Igmim2, eCD4-IgQ40A,mim2 and NIH45-46 were determined for a panel of 40 viral isolates selected for resistance to the CD4bs bNAbs 3BNC117 and NIH45-46. IC_{50} values of the CD4bs antibodies VRC01, NIH45-46,and 3BNC117 listed in the three rightmost columns were reported in Huang et al. and Scheid et al.



Comparison of eCD4-Ig variants and HIV-1 neutralizing antibodies.
a, HIV-1 pseudotyped with the envelope glycoproteins of the indicated HIV-1, HIV-2 or SIV isolates were incubated in triplicate with TZM-bl cells and the indicated concentrations of CD4-Ig, eCD4-Ig variants, or the CD4bs antibodies IgGb12, VRC-01 or NIH45-46. Error bars represent standard errors. **b**, Experiments similar to those in panel a except that a wider array of entry inhibitors was characterized with HIV-1 pseudotyped with the SG3, YU2, or JR-CSF isolates, and IC50 values are plotted. eCD4-Ig variants are represented with blue, CD4-Ig and the tetrameric CD4-Ig construct PRO-542 with red, and HIV-1 neutralizing antibodies with green, grey, and white. **c**, Antibody-dependent cell-mediated cytoxicity (ADCC) was measured using CEM.NKR-CCR5 target cells incubated with infectious HIV-1 NL4-3, SHIVKB9 or SIVmac239 for four days. Cells were then incubated with KHYG-1 NK effector cells21 for 8 hours in the presence of the indicated concentrations of CD4-Ig, eCD4-Ig variants, or the HIV-1 neutralizing antibody IgGb12, and ADCC activity was measured as loss of luciferase activity from the target cells.



A model of eCD4-Ig "claw" bound to the HIV-1 envelope glycoprotein trimer.
a, On the left, the structure (2QAD) of gp120 (YU2 isolate) bound to the tyrosine-sulfated CD4i antibody 412d and CD4 domains 1 and 2, was fitted into a cryoelectron micrograph of HIV-1 envelope glycoprotein (Bal isolate) bound to CD4. gp120 and CD4 are shown in blue and red, respectively. 412d sulfotyrosines are represented as green (carbon), red (oxygen), and yellow (sulfur) spheres. On the right, the Fc domain of human IgG1 (cyan) is oriented to avoid steric interaction with the envelope glycoprotein while minimizing distances between the carboxy terminus of domain 2 of CD4 and the amino-terminus of one Fc domain monomer, and between the carboxy-termini of the two Fc domain monomers and sulfotyrosine 100 of the 412d heavy chain (Tys 100). This sulfotyrosine occupies a pocket in gp120 thought to bind CCR5 sulfotyrosine 104. This pocket is also critical for binding CCR5mim1 and CCR5mim25-6. Consistent with previous studies, the resulting distances are short enough to permit each sulfopeptide to bind a gp120 monomer, whereas only one CD4 can bind . For clarity only the bound CD4 is shown. **b**, The same structures shown in panel a, rotated 30 degrees about the horizontal axis. **c**, The same structures shown in panel a rotated 90 degrees about the horizontal axis.



eCD4-Ig delivered with an AAV vector can be expressed at high-titers in rhesus macaques
Four Indian-origin rhesus macaques were inoculated intramuscularly with 2 x 10^{13} single-stranded adeno-associated virus (ssAAV) vector particles expressing a rhesus eCD4-Ig transgene together with 0.5 x 10^{12} ssAAV vector particles expressing rhesus tyrosine protein sulfotransferase 2. eCD4-Ig titers were measured by gp120 ELISA at the indicated time points. Experiments are currently underway to determine if expression of eCD4-Ig can protect against infection with SHIV-AD8.

Conclusions

1. eCD4-Ig is at least as potent as the best broadly neutralizing HIV-1 antibodies (bNAb)
2. It is broader than any bNAb, for example neutralizing HIV-2 and SIV
3. It binds Env in a claw-like configuration, with two sulfopeptides and one CD4 moiety binding the Env trimer
4. It can be expressed in rhesus macaques to levels that should be protective and which may suppress an ongoing infection