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A Nonneutralizing Anti-HIV-1 Antibody Turns into a Neutralizing Antibody When Expressed on the Surface of HIV-1-Susceptible Cells: A New Way to Fight HIV

Seung-Jae Lee,* Laura Garza,* Jun Yao,* Abner L. Notkins,† and Paul Zhou‡*

During HIV-1 infection or vaccination, HIV-1 envelope spikes elicit Ab responses. Neutralizing Abs block viral entry by recognizing epitopes on spikes critical for their interaction with receptor, coreceptors or fusion. In contrast, nonneutralizing Abs fail to do so because they recognize epitopes either buried or exposed but not critical for viral entry. Previously, we produced a high-affinity human mAb against the cluster II determinant of gp41. This Ab or its recombinant Fab and single-chain Fv have been repeatedly shown to bind to HIV-1 gp160 or gp41, but fail to block viral entry. We report that, surprisingly, expression of this nonneutralizing anti-HIV-1 gp41 single-chain Fv on the surface of human CD4 T cells markedly inhibits HIV-1 replication and cell-cell fusion. The inhibition targets the HIV-1 envelope at the level of viral entry, regardless of HIV-1 tropism. Although this bona fide nonneutralizing Ab does not neutralize HIV-1 entry when produced as a soluble protein, it acts as a neutralizing Ab when expressed on the cell surface. Expressing Abs on the surface of HIV-1-susceptible cells can be a new way to fight HIV-1. The Journal of Immunology, 2004, 173: 4618–4626.

The HIV-1 envelope spike is a trimeric complex of gp120-gp41 heterodimers. The gp120, a cell surface attachment protein, and the gp41, the membrane spinning protein, are noncovalently linked. They are initially produced as a single glycoprotein precursor gp160, which is cleaved by a cellular protease. During natural HIV-1 infection or vaccination, the envelope spike elicits Ab responses. Neutralizing Abs block viral entry by recognizing epitopes on the envelope spike critical for their interaction with receptor or coreceptors, or for the fusion process. Abs that can neutralize a broad range of primary isolates of HIV-1 have been extremely difficult to generate (1). Despite almost two decades of effort, very few neutralizing Abs have been found (2–9). The lack of broad neutralizing Abs is due to several intrinsic properties of HIV-1 envelope proteins, such as their high mutation rates and resultant extreme sequence diversity (1) and escape mutants (10, 11), heavy glycosylation (1), and the transient and inducible nature of neutralization epitopes (12).

In contrast, nonneutralizing Abs are almost always generated during natural infection or vaccination. Some have very high affinity, but fail to block viral entry, suggesting that nonneutralization epitopes are either buried within the intact envelope spike or exposed but not critical for viral entry. Molecular modeling revealed that the gp120 monomer exists as three faces: neutralizing, nonneutralizing, and silent (1). The neutralizing face corresponds to the surface of the gp120 trimer that interacts with its receptor and coreceptors. This face is exposed at the surface of the intact trimeric envelope spike and conserved. The silent face is heavily glycosylated and does not elicit Ab responses though it is well exposed at the surface of the intact trimeric envelope spike. The nonneutralizing face, which is buried within the intact trimeric envelope spike, elicits strong Ab response. Abs that bind to this face do not bind to the intact envelope spike on virions. This model also implies that the nonneutralizing Abs to gp120 are elicited by either soluble monomeric gp120 shed from the virions or infected cells, or partially shed spike that exposes monomeric gp120 on the surface of the virions or infected cells, or by gp160 precursor proteins found in the debris of dying HIV-1-infected cells. Because gp41 has not been similarly modeled, we do not know the structural basis of neutralizing vs nonneutralizing epitopes in gp41. A few studies have shown that most of the epitopes of gp41 are buried underneath the gp120 in the intact envelope spike (13–17), with the notable exception of epitopes in cluster I determinant (18) and 2F5 (8, 9) and 4E10/Z13 epitopes (6, 7). Interaction of gp120 with CD4 causes conformational changes of the envelope spike, resulting in exposure of some of these hidden epitopes and making them accessible to Ab binding (19). The evolution of nonneutralizing epitopes is usually more conserved than neutralizing epitopes because nonneutralizing Abs do not exert selective pressure on virus survival.

Previously, we established human B cell hybridomas from an HIV-1 patient. The mAb derived from one of these hybridomas displays high affinity binding to the cluster II determinant of the ectodomain of gp41 with a $K_d$ value of $4 \times 10^{-10}$ M (20). This human anti-gp41 Ab or its recombinant Fab or single-chain fragment variable region (scFv) have been repeatedly shown to bind to HIV-1 gp160 or gp41, but fail to block viral entry (20, 21). Thus, it is truly a nonneutralizing Ab.

*Department of Virology and Immunology, Southwestern Foundation for Biomedical Research, San Antonio, TX 78245; and †Experimental Medicine Section, Oral Infection and Immunity Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892

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2 Address correspondence and reprint requests to Dr. Paul Zhou, Department of Virology and Immunology, Southwestern Foundation for Biomedical Research, P.O. Box 760549, San Antonio, TX 78245-0549. E-mail address: pzhou@sfbr.org

3 Abbreviations used in this paper: scFv, single-chain fragment variable; m-scFv, membrane-bound scFv; ER, endoplasmic reticulum; TGN, trans-Golgi network; VSV, vesicular stomatitis virus; rTA, Tet transactivator; TM, transmembrane domain; eGFP, enhanced GFP.
In a series of experiments, we targeted this nonneutralizing anti-
HIV-1 gp41 scFv into the endoplasmic reticulum (ER) and trans-
Golgi network (TGN) of HIV-1-susceptible cell lines and demon-
strated that the anti-HIV-1 gp41 scFv targeted into the ER or TGN
inhibited HIV-1 replication, but the secreted scFv did not (21).
Using a retroviral vector we also transduced anti-HIV-1 gp41
scFv-ER into rhesus macaque primary T cells and demonstrated
that the scFv-ER markedly inhibits pathogenic simian/HIV repli-
cation in vitro (P. Zhou, unpublished observation). More recently,
we transduced this anti-HIV-1 scFv-ER into chronically HIV-1-
infected cells and demonstrated that it significantly blocks the mat-
turation process of HIV-1 envelope proteins. In so doing, it results
in about a 10-fold reduction of gp120 expression on the cell sur-
face and in virions, and a more than two-log reduction of viral
infectivity (J. Yao and P. Zhou, unpublished observation). There-
flecting Ab into a neutralizing Ab by expressing it on the surface
of virus entry, specifically inhibiting HIV-1 replication and cell-cell
fusion. The inhibition is at the level of HIV-1-susceptible CEM-ss cells (22), while not altering the
anti-HIV-1 gp41 scFv to the transmembrane and intracellular signal-
domains of several cytoplasmic membrane-bound receptors. As a
negative control for intracellular signaling, we also constructed a
membrane-bound scFv (m-scFv) in which no signaling domains were
added. We report that expressing this m-scFv on the surface of
HIV-1-susceptible CEM-ss cells (22), while not altering the
surface expression of CD4, CXCR4, or CCR5, markedly inhibits
HIV-1 replication and cell-cell fusion. The inhibition is at the level
of virus entry, specific to HIV-1 envelope and was not affected by
virus tropism. Our results demonstrate that one can turn a nonneu-
tralizing Ab into a neutralizing Ab by expressing it on the surface
of HIV-1-susceptible cells. Expressing Abs on the cell surface can
be a new way to fight HIV-1.

Materials and Methods

Cell lines and viruses

CEM-ss cells (22) were provided by Dr. J. S. Allan (Department of Vi-
rology and Immunology, Southwest Foundation for Biomedical Research,
San Antonio, TX). PT67 packaging cells (23) and 293 FT cells were
purchased from Clontech Laboratories (Palo Alto, CA) and Invitrogen Life
Technologies (San Diego, CA), respectively. Cell line 69T1RevEnv (24) and
HIV-1 viruses 89.6, Ada-M, and Ba-L were obtained through the Na-
tional Institutes of Health AIDS Research and Reference Reagent Program
(Germantown, MD). HIV-1 IIIB was provided by Dr. S. Goldstein at the
National Institute of Allergy and Infectious Diseases, National Institutes of
Health (Bethesda, MD). Cells were maintained in complete DMEM (i.e.,
high glucose DMEM supplemented with 10% FBS, 2 mM l-glutamine, 1
mM sodium pyruvate, penicillin (100 U/ml), and streptomycin (100 μg/
ml); Invitrogen Life Technologies).

Retroviral vector, stable packaging cells, and recombinant
viruses

To construct the m-scFv, a 138-bp DNA fragment containing an 8-bp se-
nquence of 3’ end of the anti-HIV-1 gp41 scFv, a 108-bp sequence encoding
11 aa residues of human IgG3 hinge region, 24 aa residues of the trans-
membrane domain (TM) of the subunit 1 of the type 1 IFN receptor (25)
and a stop codon, and a 12-bp sequence of a HindIII site and an addi-
tional 6 bp was generated by a recursive PCR (26). Another DNA fragment
containing a 12-bp sequence of a HindIII site and an additional 6 bp, a
sequence encoding the signal peptide and anti-HIV-1 gp41 scFv, and an
8-bp sequence of 5’ end human IgG3 hinge region was amplified by PCR
using pRC/CMV anti-HIV-1 gp41 scFv-ER as a template (21). These two
fragments were designed so that there are 16 nt overlapping between the 3’
end of scFv and 5’ end of IgG3/TM fragments. The whole fragment en-
coding the m-scFv was then generated by an overlapping PCR as described
before (27). The amplified cDNA was then ligated in a TA vector system
for sequence analysis (Invitrogen Life Technologies). cDNA containing the
correct m-scFv sequence was cloned into the HindIII site of the retroviral
vector pLNCX-TCRenh (28), which is a modified version of pLNCX
(Clontech Laboratories) into which we had inserted a TCR enhancer se-
derence into the BamHI site of pLNCX. The resulting retroviral construct,
designated pLNCX-TCR-m-scFv (see Fig. 1), was linearized at a XmnI site.
Linearized DNA (20 μg) was mixed with 107 PT67 cells in 0.8 ml of
RPMI 1640 (Invitrogen Life Technologies). Electroporation was per-
formed at the capacitance of 960 μF and 200 V/4.4 cm. Cells then were
seeded onto 96-well plates. Stable transfectants were selected by G418
selection (0.8 mg/ml) for 2–3 wk. The resulting stable packaging lines were
designated PT67-m-scFv.

To measure viral titers of supernatants, stable PT67-m-scFv cell lines
were randomly selected and grown onto six-well plates. After the cells
reached confluence, 2-ml fresh complete DMEM was added to each well
and cells were incubated at 32°C overnight. Supernatants were harvested,
filtered, and titrated on HeLa cells as described (28).

To generate recombinant retrovirus stocks, stable PT67-m-scFv and pre-
viously generated PT67 enhanced GFP (eGFP) (28) cell lines were ex-
anded as described by Kotani et al. (29). Supernatants were harvested,
filtered, and either used fresh or stored at −80°C.

Stable transduced CEM-ss cell lines

To transduce CEM-ss cells, a 24-well nonissue culture plate was coated with recombinant fibronectin fragment (CH-296) as previously described (28).
CEM-ss cells (1 × 107) and 2.5 μl of recombinant virus-containing
supernatants were then added onto the CH-296-coated well. The plate was
centrifuged at 32°C for 1 h and incubated at 32°C overnight. The next day
cells were harvested and reseded onto a 96-well plate at ~1000 cells per
well in complete DMEM. G418 at the final concentration of 2 μg/ml was
added in 24 h. Stable CEM-ss cell lines were generated in ~3 wk.

Immunoprecipitation

To study m-scFv expression, eGFP- and m-scFv-transduced CEM-ss cells
were immunoprecipitated with KappaLock-Sepharose as previously de-
scribed (21).

To study the expression of HIV-1 envelope proteins in 69T1RevEnv cells,
cells were cultured with or without the treatment of tetracycline (2 μg/ml) for
6 days. Cells then were labeled with [35S]methionine as described earlier.
HIV-1 envelope proteins gp120 and gp160 were immunoprecipitated with a mAb
against HIV-1 gp120 (cat. no. 43107; Advanced Bioscience Laborato-
ries, Kensington, MD) and followed by protein G-Sepharose (Invitrogen Life
Technologies). The precipitated samples were electrophoresed on 10% SDS-
PAGE gels. The gels were treated with ENHANCE (DuPont-NEN, Boston,
MA) and dried before autoradiography.

FACS analysis

To study cell surface expression of m-scFv, 1 × 106 parental and m-scFv-
transduced CEM-ss cells were incubated with rabbit anti-human x-chain
Ab (Boehringer Mannheim, Indianapolis, IN) for 45 min on ice. Cells then
were washed twice with FACS buffer (PBS containing 1% BSA and 0.02%
Na3) and stained with PE-conjugated goat anti-rabbit IgG Ab (Sigma-
Aldrich, St. Louis, MO) for another 45 min on ice. Cells were washed twice
and fixed with 1% formaldehyde in 0.5 ml of FACS buffer. Cytol-
fluorography was performed on a FACSscan (BD Biosciences, Mountain
View, CA).

To study expression of eGFP, 1 × 106 eGFP-transduced, and m-scFv-
transduced CEM-ss cells were harvested, washed, and fixed before FACS
analysis.

To study surface expression of CD4, 1 × 106 parental, eGFP-, and
m-scFv-transduced CEM-ss cells were incubated with PE-conjugated anti-
human CD4 Ab (clone Leu3a; BD Biosciences) for 45 min on ice. Cells
were then washed and fixed before FACS analysis.

To study surface expression of CXCR4 and CCR5, 1 × 106 parental,
eGFP-, and m-scFv-transduced CEM-ss cells were incubated with mouse
anti-human CXCR4 mAb (clone 12G5; BD Biosciences) and mouse anti-
human CCR5 mAb (clone 3D9; BD Biosciences) for 45 min on ice. Cells
were then incubated with PE-conjugated anti-mouse IgG Ab (Sigma-
Aldrich, St. Louis, MO) for another 45 min on ice. Cells were washed and fixed before FACS
analysis.

HIV-1 infection and p24 assay and syncytial formation

Pooled eGFP- and m-scFv-transduced CEM-ss cell lines (1 × 106) were
incubated overnight with HIV-1 89.6, Ada-M, or Ba-L or 2 h with
HIV-1 strain IIIB (60,000 cpm reverse transcriptase activity) in a final
volume of 0.5 ml. Cells were then washed three times with HBSS and
resuspended in 6 ml of complete DMEM and incubated at 37°C for 21 or
35 days. Every 3 or 4 days, 4.5 ml of cell suspensions were harvested and
replaced with the fresh medium. The supernatants were then collected.
HIV-1 p24 in the supernatants were measured by ELISA (Beckman Coulter, Fullerton, CA) according to the manufacturer’s instruction.

Cell-cell fusion assay

69T1RevEnv cells (24) (2 × 10^5 per well) with or without the tetracycline treatment were seeded in 24-well plate at 37°C overnight. eGFP- or m-scFv-transduced CEM-ss cells (2 × 10^5) were then added onto 69T1RevEnv cells. At various time intervals, cell-cell fusion was monitored under the light microscopy and recorded by a digital camera (Nikon Coolpix 995, Melville, NY).

One-step viral infectivity assay

One-step viral infection assay was performed as previously described (30). Briefly, eGFP- and m-scFv-transduced cells (5 × 10^6) were incubated at 37°C overnight with HIV-1 strains IIIB or Ba-L (600,000 cpm reverse transcriptase activity) for 2 h. Cells were then washed three times with HBSS, treated with 5 U of RNase-free DNase I (Sigma-Aldrich) in 10 mM MgCl_2 at 37°C for 30 min, washed three times with HBSS and placed in complete DMEM. At 1, 3, 5, and 16 h postinfection, 10^6 cells were collected for DNA isolation.

To isolate total cellular DNA, the cell pellets were resuspended in 100 μl of solution A (10 mM Tris-HCl, pH 8.3, and 100 mM KCl) and lysed in 100 μl of solution B (10 mM Tris-HCl, pH 8.3, 1% Tween 20, 1% Nonidet P-40) containing 25 μg of proteinase K for 30 min at 60°C. The samples were then boiled for 30 min. Viral DNA was amplified by PCR with a pair of primers (5’-GGCTAACTAGGGAACCACTG-3’ and 5’-CTGCTAGATTTCCACACGTAG-3’) as described by Zack et al. (31) and separated on 2% agarose gels, transferred onto nylon membrane, and probed with 5’ end-labeled oligonucleotide probe (5’-CCGTTCTTGTGTAG-3’). The internal control β-actin DNA was measured as previously described (28).

Generation and transduction of recombinant HIV-1 vector

Recombinant HIV-1 vectors were prepared as described by Follenzi et al. (32). Briefly, 293 FT cells were transfected with the transfer vector pRRLsin18.PPT.hPGK.eGFP.Wpre (32; a generous gift from Dr. L. Naldini, University of Turin, Italy), a packaging vector encoding HIV-1 gag/pol proteins (pLP1) and two other plasmid vectors encoding vesicular stomatitis virus (VSV)-G envelope (pLP/VSVG) and HIV-1 Rev protein (pLP2) (Invitrogen Life Technologies). Twenty-four hours later, the supernatant was harvested and ultracentrifuged. The vector pellet was resuspended in a small volume of DMEM. Vector titer was determined by adding serial dilutions to 10^5 293 FT cells in six-well plate (Corning, Corning, NY) and 72 h later measuring eGFP-positive cells by FACS analysis. The amount of HIV-1 gag p24 in concentrated vector stocks was determined by ELISA.

To transfer recombinant HIV-1 vectors into parental and m-scFv-transduced CEM-ss cells, 1 × 10^5 cells were incubated overnight at 37°C with serial dilutions (corresponding to 140, 28, and 2.8 ng of p24) of the above concentrated HIV-1 vector in the presence of 8 μg/ml polybrene (Sigma-Aldrich). Cells then were washed twice with HBSS and cultured in the complete DMEM. eGFP expression was analyzed by FACS in 48 h.

Results

Expression of transgenes in eGFP- and m-scFv-transduced CEM-ss cells

Fig. 1a shows the retroviral vector containing m-scFv (see Materials and Methods for details). The retroviral vector containing...
eGFP was reported before (28). Fig. 1b shows that a 32-kDa protein band corresponding to m-scFv was precipitated by the KappaLock-Sepharose in four representative m-scFv-transduced CEM-ss lines, but not in the parental CEM-ss cells. In addition, m-scFv was detected in cell lysates, but not in culture supernatants, suggesting that the m-scFv was retained in the cells (data not shown). Fig. 1c shows a representative FACS analysis of the surface expression of m-scFv in transduced CEM-ss cells using a rabbit anti-human κ-chain serum as compared with nontransduced CEM-ss cells stained with the same antiserum. Fig. 1d shows a representative FACS analysis of eGFP expression in eGFP-transduced CEM-ss cells as compared with m-scFv-transduced CEM-ss cells. These results demonstrate that m-scFv and eGFP are properly expressed in these stable transduced CEM-ss lines.

**Inhibition of HIV-1 replication and syncytia in CEM-ss cells expressing m-scFv**

We next examined the effect of the m-scFv on the surface expression of CD4, CXCR4, and CCR5. We found no significant changes in CD4, CXCR4, and CCR5 expression among m-scFv- or eGFP-transduced or parental CEM-ss cells (Fig. 2). Thus, these results demonstrated that the surface expression of m-scFv does not alter HIV-1 receptor and coreceptor expression. Similar results were obtained with three other eGFP-transduced and three other m-scFv-transduced lines.
We next examined the effect of the m-scFv on HIV-1 replication. Four eGFP-transduced or four m-scFv-transduced CEM-ss lines were pooled and infected with four HIV-1 strains 89.6, Ada-M, Ba-L, and IIIB. Pooled cell lines were used for the infection to minimize potential variation among individual cell lines. The 89.6 strain, a dual tropic virus, uses either CD4/CCR5 or CD4/CXCR4 to infect cells (33). The Ada-M and Ba-L strains, both R5 viruses, use CD4 and CCR5 to infect cells (34, 35). The IIIB, an X4 virus, uses CD4 and CXCR4 to infect cells (36). As shown in Fig. 3, HIV-1 replication was inhibited in all four strains. For cells infected with 89.6, inhibition was apparent at day 7 and continued throughout the experiment of 35 days. At the peak of viral production up to a 4-log reduction was observed (Fig. 3a). For cells infected with Ada-M, inhibition was apparent at day 4 but moderate. Supernatant collected on day 7 showed 98% reduction. Supernatants collected on day 11, 14, 18, and 21 only showed 20–52% reduction (Fig. 3d). The experiment was repeated two additional times with similar results. At this time, it is not clear why m-scFv inhibits HIV-1 strains 89.6, Ada-M, and Ba-L much better than IIIB. It could be due to the difference in antigenicity and replication rate among these virus strains, but not the amount of viruses used for the infection because they were similar. Furthermore, when 5-fold fewer IIIB and 5-fold more Ba-L were included in subsequent experiments, no significant changes in the level of inhibition to either HIV-1 strain were observed (data not shown).

Syncytia induced by HIV-1 IIIB 8 days after infection in pooled eGFP-transduced CEM-ss cell lines (e) vs pooled m-scFv-transduced CEM-ss cell lines (f). Original magnification, ×320.

FIGURE 3. p24 Activity in pooled eGFP- and m-scFv-transduced CEM-ss cell lines infected with HIV-1 strains 89.6 (a), Ada-M (b), Ba-L (c), and IIIB (d). Syncytia induced by HIV-1 IIIB 8 days after infection in pooled eGFP-transduced CEM-ss cell lines vs pooled m-scFv-transduced CEM-ss cell lines (f). Original magnification, ×320.
In m-scFv-transduced cells, syncytia were smaller size, fewer, and had delayed kinetics. Eight days postinfection, syncytia of considerably larger size were observed in eGFP-transduced (Fig. 3e), but not in m-scFv-transduced cells (Fig. 3f). On average, ~4 x 10^4 syncytia/ml were detected in eGFP-transduced cells, but none of the syncytia in m-scFv-transduced cells (S.-J. Lee and P. Zhou, unpublished observation and data not shown).

**Inhibition of HIV-1 envelope-induced cell-cell fusion by m-scFv**

To directly test the effect of m-scFv on HIV-1 envelope-mediated cell-cell fusion, we cocultured the eGFP- or m-scFv-transduced CEM-ss cells with 69T1RevEnv cells as previously described by Yu et al. (24). The latter contains a gene encoding HIV-1 envelope protein under an inducible promoter. This envelope gene was derived from molecular clone pLAI3 (24). In the absence of tetracycline, Tet transactivator (tTA) binds to and transactivates the inducible promoter resulting in HIV-1 envelope protein expression; in the presence of tetracycline, binding of tetracycline to tTA causes conformational change of tTA, which blocks tTA binding to the inducible promoter and prevents HIV-1 envelope protein expression (Fig. 4a). Coculturing the eGFP- or m-scFv-transduced cells with tetracycline-treated 69T1RevEnv cells results in no cell-cell fusion (Fig. 4, b and d). In contrast, coculturing the eGFP-transduced cells with tetracycline-untreated 69T1RevEnv cells results in massive cell-cell fusion (Fig. 4c). The fusion begins after 5–6 h and peaks at 24 h. Coculturing the m-scFv-transduced cells


Discussion

In a series of studies including this one, we demonstrated that a bona fide nonneutralizing human anti-HIV-1 gp41 Ab, when produced as a soluble protein, does not neutralize HIV-1 entry. However, when expressed on the surface of HIV-1-susceptible cells, it blocks HIV-1 replication and cell-cell fusion. The inhibition appears to be at the level of virus entry, HIV-1 envelope specific, and independent of virus tropism. On the cell surface this Ab acts as a neutralizing Ab.

How could this happen? The epitope that our Ab recognizes locates in the cluster II determinant (aa residues 644–663) of the ectodomain of gp41 (37). This determinant resides within the second heptad repeat and is immediately N-terminal to two broad neutralization epitopes 2F5 (aa residues 662–667) (8, 9) and 4E10/Z13 (aa residues 669–774) (6, 7). HIV-1 gp41-mediated fusion is triggered by interaction between the second and the first heptad repeats, which converts a prehairpin gp41 trimer into a fusogenic three-hairpin bundle (17). A peptide T20 derived from this second heptad repeat acts as a fusion inhibitor and is the latest anti-HIV-1 drug approved by the Food and Drug Administration (38). We envision two possibilities that are not mutually exclusive as to how our m-scFv operates. The first possibility is that the cluster II determinant is totally buried within the envelope spikes of virions, so scFv does not neutralize viral entry when produced as a soluble protein. However, the interaction of gp120 to its receptor and coreceptor causes drastic conformational changes of gp41 from a prehairpin to a fusogenic three-hairpin bundle (17). During this process, the cluster II determinant and other hidden epitopes in gp41 are exposed. The nearby m-scFv interacts with the inducible cluster II determinant and blocks the subsequent fusion process. It has been reported that the gp41 epitopes spanning aa residues 521–663 are buried under gp120 trimers (19), with the exception of cluster I determinant (18). However, following interaction of gp120 with soluble CD4, these hidden gp41 epitopes, including the cluster II determinant, become accessible to Ab binding (19). The second possibility is that the cluster II determinant is indeed buried within the intact envelope spikes, but exposed when gp120 proteins are shed from the spikes. Because a virion can contain a mixture of intact envelope spikes and so-called dead spikes from which gp120 has been shed (39), it can be bound by m-scFv.
our scFv. When the scFv is produced as a soluble protein, even if it can bind to these dead spikes, it cannot prevent the remaining intact envelope spikes from interacting with their cognate receptor and coreceptors. As a result, it does not neutralize viral entry. However, when the scFv is expressed on the cell surface, the m-scFv directly binds to the dead spikes and traps virions so that they cannot move freely. In so doing, it prevents the intact envelope spikes from interacting with their cognate receptor and coreceptors. The m-scFv may even mediate endocytosis of viruses into the endosome/lysosome, resulting in viral degradation. Currently, we are performing experiments to sort out these underlying mechanisms.

Regardless of the mechanism, our results clearly show that expressing m-scFv against HIV-1 gp41 on the surface of HIV-1-susceptible cells could be a new way to fight HIV-1. For therapeutic purposes, one may perform an ex vivo transfer of m-scFv gene into hematopoietic progenitor cells from an HIV-1-infected patient and infuse the cells into the patient. When these cells differentiate into macrophages or CD4 T cells, the expression of m-scFv may render them resistant to HIV-1 infection. For preventive purposes, one may perform an ex vivo transfer of m-scFv gene into hematopoietic progenitor cells and perform an in vivo transfer directly into rectal and vaginal epithelial stem cells in high-risk individuals. After being infused, the hematopoietic progenitor cells will differentiate into different hematopoietic lineages cells, including intraepithelial lymphocytes, mucosal macrophages, and mucosal dendritic cells. The epithelial stem cells will differentiate into various types of epithelia. Intraepithelial lymphocytes, mucosal macrophages, mucosal dendritic cells, and epithelia are cell types implicated in mucosal transmission of HIV-1 (40). The expression of m-scFv on the surface of these cells may render them resistant to HIV-1 infection. As a result, it prevents HIV-1 transmission through mucosal routes.

Finally, we demonstrated that although m-scFv and scFv-ER both markedly inhibit HIV-1 replication, the mechanism of HIV-1 inhibition by these two molecules is quite different. m-scFv, but not scFv-ER, inhibits HIV-1 envelope-mediated cell-cell fusion and viral entry (S.-J. Lee et al., unpublished observation and data not shown). No effect of scFv-ER on HIV-1 envelope-mediated cell-cell fusion and viral entry is consistent with our previous report that the mechanism of inhibition of scFv-ER is the binding of scFv to HIV-1 gp160 inside ER, which significantly delays the maturation of gp160 to gp120 and gp42 (21). Experiments are now under way to express both m-scFv and scFv-ER in individual cells to test whether they can synergistically inhibit HIV-1.

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