Gene Therapy Using a Secreted Single Chain Variable Fragment Targeting CCR5 to Inhibit HIV Infection

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Abstract

Interaction of R5 HIV with cellular CCR5 is mandatory for entry into host cells. The humanized monoclonal antibody PRO 140 masks cell surface CCR5 and efficiently blocks R5 HIV infection. While weekly administrations of PRO 140 have been shown to drastically reduce the viral load in patients, frequent injections of purified antibodies are neither practical nor cost-effective. Engineering gene-modified cells or tissues to secrete therapeutic proteins at sites of viral replication may advantageously replace continued drug administration. We have designed a gene encoding a secreted single chain variable fragment of PRO 140, sscFvPRO140, and have used a lentiviral vector for its delivery and expression. The sscFvPRO140 was effectively secreted from gene-modified cells, remained stable in culture supernatant at 37°C, and specifically bound to cells expressing CCR5. Single-round infection assays were performed to assess inhibition of HIV infection. sscFvPRO140 reduced R5 HIV entry in unmodified target cells, although at a lower potency compared to what has been reported for the parent monoclonal antibody. Entry of X4 HIV, which utilizes CXCR4 as a co-receptor, remained unaltered. Furthermore, viral entry was substantially reduced when gene-modified HIV target cells expressing sscFvPRO140 were infected. Molecules such as ribozymes or Zinc finger nucleases, which act intracellularly, only confer protection to gene-modified target cells. In contrast, the proposed gene therapy strategy based on secreted anti-HIV proteins has the potential to protect the gene-modified as well as unmodified HIV target cell populations.

Keywords: Fusion inhibitor; Gene therapy; HIV; Lentiviral vector; PRO 140; Secreted antiviral proteins; Single-chain variable fragment; Soluble CD4

Introduction

Human immunodeficiency virus type-1 (HIV) entry into target cells requires the presence of CD4 and a co-receptor. R5 HIV, which is predominantly involved in the transmission of the virus, utilizes the chemokine (C-C motif) receptor 5 (CCR5) as a co-receptor. Individuals who are born homozygous with a 32 base pair deletion in the CCR5 gene (CCR5Δ32/Δ32) are generally in good health and highly resistant to R5 HIV [1]. In the famous case of a patient who was treated in Berlin, transplantation of hematopoietic stem cells from a CCR5Δ32/Δ32 donor resulted in a permanent decrease of virus in the absence of antiretroviral therapy [2,3]. Several intracellular gene therapy strategies have been employed to render the target cells resistant to R5 HIV infection. These strategies include excision of the CCR5 gene via Zinc finger nucleases [4], cleavage of CCR5 mRNA by multimeric ribozymes [5], inhibition of CCR5 mRNA translation by small interfering or antisense RNAs [6,7], and prevention of surface CCR5 protein expression by an intrabody or an intrakine [8,9]. These molecules target CCR5 DNA, RNA or protein within the gene-modified target cells. In all cases, the gene-modified target cells were shown to be protected against R5 HIV infection. However, as it is not possible to genetically modify the entire hematopoietic stem/progenitor cell population within a patient, the therapeutic benefit would be limited because unmodified stem/progenitor cells will constantly give rise to susceptible progeny cells that will allow virus replication.

Engineering cells that continuously secrete anti-HIV proteins could extend the protection against infection to the unmodified HIV target cell population, resulting in a systemic antiviral effect. In this study we have assessed the feasibility of developing a novel gene therapy strategy based on a secreted anti-CCR5 antibody that could prevent HIV infection by masking surface CCR5 (Figure 1a). The humanized monoclonal antibody (mAb) PRO 140 is among the most potent CCR5 inhibitors reported to date. The mAb PRO 140 binds to surface CCR5 without inducing CCR5 signaling [10] and is effective against primary HIV isolates, including those resistant to small-molecule CCR5 inhibitors [11]. Remarkably, weekly injections of mAb PRO 140 were shown to reduce R5 HIV load by 100 fold and increase CD4+ T cell counts [12,13]. We designed a secreted single chain variable fragment (sscFv) based on the mAb PRO 140 and investigated whether the sscFvPRO140 can protect gene-modified and unmodified target cells against R5 HIV infection.

Materials and Methods

Cell lines, plasmids, and peptides

Human embryonic kidney 293T cells [14] were a kind gift from Dr. Jason Moffat (University of Toronto, Toronto, Canada). U373-MAGI-CCR5Δ32 and U373-MAGI-CXCR4ΔEM cells [15] were obtained from Dr. Michael Emerman (AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH). 293T cells were cultured in complete DMEM (cDMEM) containing 10% fetal bovine serum (Thermo Fischer Scientific, Waltham, Massachusetts) and 1% Antibiotic-Antimycotic (Life Technologies, Carlsbad, California). U373-MAGI-CCR5Δ32 and
U373-MAGI-CXCR4<sub>end</sub> cells were cultured in cDMEM supplemented with 0.2 mg/ml G418 (Sigma-Aldrich, St. Louis, Missouri), 0.1 mg/ml hygromycin B (Life Technologies), and 1.0 μg/ml puromycin (Bioshop, Burlington, Canada). pLVX-IREs-ZsGreen1 (hereafter referred to as pLVX) was purchased from Clontech (Mountain View, California). The genes encoding sscFv<sub>PRO140</sub> and sCD4 were codon-optimized for expression in human cells and synthesized by Genscript (Piscataway, New Jersey). These genes were cloned into the multiple cloning site of pLVX to generate pLVX-sscFv<sub>PRO140</sub> and pLVX-sCD4. pLJM2, psPAX2, and pMD2G were generously donated by Dr. Moffat. The pJRFL-env and pHXB2-env plasmids were a kind gift from Dr. Donald Branch (University of Toronto). The fusion inhibitor T-20 (hereafter referred to as F<sub>I-20</sub>) was obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH.

Production of lentiviral vector particles

Lentiviral vector particles were generated by calcium phosphate transfection of 293T cells as described previously [16]. Briefly, 293T cells were grown to 90% confluence in a 10 cm dish and co-transfected with 10.5 μg of a lentiviral transfer plasmid (pLVX, pLVX-sscFv<sub>PRO140</sub> or pLVX-sCD4), 7.0 μg of the packaging plasmid psPAX2, and 3.5 μg of the envelope plasmid pMD2G encoding the vesicular stomatitis virus glycoprotein G. 16 hours after addition of the plasmids, the culture supernatants were exchanged with 8 ml of fresh medium. The culture supernatants containing the vector particles were harvested every 12 hours for a total of 4 times. Subsequently, the pooled culture supernatants were clarified by filters with a 0.45 μm pore size and concentrated by polyethylene glycol 8000 precipitation, as described previously [17]. 293T cells were transduced with serial dilutions of the vector particle stocks and the number of transducing units (TU) was determined by counting the ZsGreen<sup>+</sup> cells [16].

Transductions

To generate gene-modified producer cells, 293T cells were transduced with LVX, LVX-sscFv<sub>PRO140</sub> or LVX-sCD4 vector particles. Briefly, lentiviral vector particles (1.6×10<sup>6</sup> TU) were mixed with 2×10<sup>5</sup> 293T cells (multiplicity of infection of 8) in 500 μl cDMEM containing 8 μg/ml polybrene (Sigma-Aldrich) and the cells were seeded into 12 well plates. The medium was changed the next day, and the cells were incubated for an additional 3 days before the number of ZsGreen<sup>+</sup> cells was determined. To generate gene-modified HIV target cells, 1.2×10<sup>5</sup> U373-MAGI-CCR5E cells or 1.2×10<sup>5</sup> U373-MAGI-CXCR4<sub>end</sub> cells were transduced with 9.6×10<sup>5</sup> TU of LVX or LVX-sscFv<sub>PRO140</sub> vector particles following the same protocol (level of gene-modification, ~95%). Mixed populations of gene-modified and unmodified cells were generated by diluting the gene-modified cells with unmodified cells (level of gene-modification, ~30%) or by transducing U373-MAGI-CCR5E cells at a multiplicity of infection of 1-2 (level of gene-modification, ~50%).

Protein purification and quantification

Gene-modified 293T cells were grown to 95% confluence in 10 cm dishes and the medium was replaced by 8 ml DMEM containing 1% FBS. The cells were incubated for 5 days before the culture supernatants were harvested, filtered through 0.45 μm filters, and loaded onto HisFALON cobalt columns (Clontech) under native conditions as per manufacturer’s instructions. The eluted fractions were analyzed by 12% SDS-PAGE, followed by Coomassie Blue staining. The concentration of the purified proteins was determined using the Bradford protein assay (Bio-Rad, Hercules, California) according to the manufacturer’s instructions. To quantify the concentration of sscFv<sub>PRO140</sub> present in the culture supernatant, serial dilutions of purified sscFv<sub>PRO140</sub> and culture supernatant were analyzed in parallel by Western blot using 1 μg/ml anti-6xHis tag-HRP conjugated to horse radish peroxidase (anti-6xHis-tag-HRP; Clontech) in phosphate buffered saline (PBS). The band intensities of purified sscFv<sub>PRO140</sub> and of sscFv<sub>PRO140</sub> from the culture supernatant were compared using the software ImageJ [18].

CCRV binding assay

293T or U373-MAGI-CCR5E cells were grown to 100% confluence in 6 well plates and fixed with 4% paraformaldehyde. PBS or purified sscFv<sub>PRO140</sub> (70 nM) were added to the cells for 1 hour, followed by 6 washes with PBS and incubation with the anti-6xHis-tag-HRP mAb (2 μg/ml) for 1 hour. After 6 additional washes with PBS, HRP activity was detected using 3,3'5,5'-Tetramethylbenzidine liquid substrate (Sigma-Aldrich). Product formation was measured using a standard spectrophotometer at a wavelength of 650 nm.

Production of Env-pseudotyped reporter vector particles and single-round infection assays

The reporter vector particles contained the HIV<sub> styl</sub> or HIV<sub> env</sub> glycoproteins on their envelope and encapsidated LJM2 vector RNA encoding dsRed instead of HIV genomic RNA. The reporter vector particles were generated by calcium phosphate co-transfection of 293T cells with 9 μg of pLJM2, 6 μg of psPAX2, and 6 μg of pJRFL-env or pHXB2-env 16 hours after the transfection, the culture supernatants were exchanged with 6.5 ml of cDMEM. The culture supernatants containing the reporter vector particles were harvested 30 hours after the medium change. Subsequently, the culture supernatants were filtered (0.45 μm) and aliquots were stored at -80°C. For single-round infection assays, 1.2×10<sup>5</sup> U373-MAGI-CCR5E cells were seeded into 12 well plates and incubated for 24 hours. On the day of the infection, sscFv<sub>PRO140</sub> was added to U373-MAGI-CCR5E cells. 1 hour later, reporter vector particles and 8 μg/ml polybrene were added to each well. Single-round infection assays in the presence of sCD4 or F<sub>I-20</sub> were performed by pre-incubating the reporter vector particles with the protein-based inhibitors for 30 minutes before adding the mixture and polybrene to the cells. The medium was changed after 24 hours and the cells were cultured for an additional 4-5 days before the number of dsRed<sup>+</sup> cells was analyzed by flow cytometry or fluorescence microscopy. For infection of gene-modified U373-MAGI-CCR5E or U373-MAGI-CXCR4<sub>end</sub> cells, 1×10<sup>4</sup> cells were seeded in a 12 well dish and incubated for 2 days. The old medium was replaced with medium containing 8 μg/ml polybrene and HIV<sub> JRFL</sub> or HIV<sub> HXB2</sub> Env-pseudotyped reporter vector particles. The cells were incubated for 24 hours. The medium was changed the next day and the cells were incubated for additional 4-5 days before analysis by flow cytometry or fluorescence microscopy.

Flow cytometry and fluorescence microscopy

Cells were fixed with 4% paraformaldehyde. ZsGreen<sup>+</sup> and dsRed<sup>+</sup> cells were analyzed by flow cytometry using Coulter Epics XL MCL (Beckman Coulter, Brea, California). DNA was stained with 4',6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich). Fluorescence microscope images were acquired with an inverted microscope (Axiovvert 135; Carl Zeiss, Jena, Germany) equipped with a charge coupled device camera (Orca R2, Hamamatsu, Quorum Technologies Inc., Guelph, Canada). All figures were colored using Adobe Photoshop.

Statistics

Data are presented as the mean with standard error bars.
representing the standard deviation. Data were analyzed by Student’s t-test for significance and considered significantly different if p < 0.05.

Results

Design of sscFV\textsubscript{PRO140}

Smaller scFvs are favored over mAbs due to improved tissue penetration and the limited capacities of vectors used for gene delivery [19]. sscFV\textsubscript{PRO140} was designed by connecting the variable heavy (V\textsubscript{H}) and light (V\textsubscript{L}) chains of the parent mAb via a flexible three repeat GGGGS linker (Figure 1c). Lentiviral vector particles were generated using pLVX and pLVX-sscFV\textsubscript{PRO140}. 293T cells were transduced with LVX and LVX-sscFV\textsubscript{PRO140} vector particles to engineer producer cells. PCR analysis of genomic DNA and RT-PCR analysis of cellular RNA revealed that the gene encoding sscFV\textsubscript{PRO140} integrated into the chromosome and generated full-length transcripts (data not shown). 99% of the cells transduced with LVX and 97% of the cells transduced with LVX-sscFV\textsubscript{PRO140} were positive for ZsGreen1 expression.

Purification and characterization of sscFV\textsubscript{PRO140}

The codon-optimized gene encoding sscFV\textsubscript{PRO140} was cloned into the lentiviral transfer vector pLVX, which expresses the fluorescent marker protein ZsGreen1 from a bicistronic mRNA under the control of the constitutive cytomegalovirus immediate early promoter (Figure 1c). Lentiviral vector particles were generated using pLVX and pLVX-sscFV\textsubscript{PRO140}. 293T cells were transduced with LVX and LVX-sscFV\textsubscript{PRO140} vector particles to engineer producer cells. PCR analysis of genomic DNA and RT-PCR analysis of cellular RNA revealed that the gene encoding sscFV\textsubscript{PRO140} integrated into the chromosome and generated full-length transcripts (data not shown). 99% of the cells transduced with LVX and 97% of the cells transduced with LVX-sscFV\textsubscript{PRO140} were positive for ZsGreen1 expression.

To determine whether sscFV\textsubscript{PRO140} binds to surface CCR5, we used cells that either lacked or expressed CCR5 (293T or U373-MAGI-CCR5 cells, respectively). Binding of sscFV\textsubscript{PRO140} to cells was analyzed by flow cytometry, and the OD\textsubscript{650} (optical density at 650 nm) was increased when sscFV\textsubscript{PRO140} was added to U373-MAGI-CCR5E cells, indicating that sscFV PRO140 binds to surface CCR5 (Figure 2b). To quantify the concentration of sscFV\textsubscript{PRO140} present in the culture supernatant of 293T cells transduced with LVX-sscFV\textsubscript{PRO140}, vector particles, culture supernatant from gene-modified cells and serial dilutions of purified sscFV\textsubscript{PRO140} were analyzed by Western blot with an anti-6xHis-tag antibody (Figure 2c). Cell culture supernatant from gene-modified 293T cells revealed a distinct band correlating with the expected molecular weight; this band was absent in the control sample. In comparison to the band intensities of the purified sscFV\textsubscript{PRO140} standard, the concentration of sscFV\textsubscript{PRO140} in the culture supernatant was estimated to be 90 nM. The V\textsubscript{H} and V\textsubscript{L} chains of sscFV\textsubscript{PRO140} are connected with a 15 amino acid linker, which may increase the sensitivity of sscFV PRO140 to proteolytic cleavage. We incubated the supernatants containing sscFV\textsubscript{PRO140} for 7 days at 37°C and analyzed samples at different time points for proteolytic cleavage by performing a Western blot (Figure 2d). No significant difference in the band intensity of day 0 to day 7 samples was observed, indicating that sscFV\textsubscript{PRO140} is stable.

sscFV\textsubscript{PRO140}-mediated inhibition of R5 HIV but not X4 HIV infection

To test the effect of sscFV\textsubscript{PRO140} on HIV entry, we performed single-round infection assays with HIV Env-pseudotyped reporter vector particles encoding the fluorescent protein dsRed as a marker for infection. Since the reporter vector particles are incapable of producing progeny virus particles, a reduction in the number of dsRed+ cells would indicate the inhibition of viral entry.
directly correlated with inhibition of viral entry. Initially, we transduced U373-MAGI-CCR5E and U373-MAGI-CXCR4CEM cells with LVX or LVX-sscFvPRO140 vector particles and generated a mixed population of gene-modified and unmodified cells as described in the Materials and Methods section (level of gene-modification, ~30%). Transduced U373-MAGI-CCR5E and U373-MAGI-CXCR4CEM cells were infected with R5 HIVJRFL and X4 HIVHXB2 Env-pseudotyped reporter vector particles, respectively. As sscFvPRO140 targets CCR5, only R5 HIV infection was expected to be inhibited, while entry of X4 HIV which utilizes CXCR4 as a co-receptor should be unaffected. A fluorescence microscopic analysis revealed that gene-modified U373-MAGI-CCR5E cells expressing sscFvPRO140 were protected from infection, but not gene-modified U373-MAGI-CXCR4CEM cells expressing sscFvPRO140 (Figure 2).

sscFvPRO140-mediated inhibition of HIV infection of gene-modified target cells

To further assess the antiviral effect of sscFvPRO140 in gene-modified HIV target cells, LVX and LVX-sscFvPRO140 vector particles were used to transduce U373-MAGI-CCR5E cells at a high (~95%) transduction efficiency. Single-round infection assays were performed with the gene-modified HIV target cells, and the number of cells infected with the HIVJRFL Env-pseudotyped reporter vector particles was determined by flow cytometry (Figures 4a and 4b). Infection of gene-modified target cells expressing sscFvPRO140 was reduced by >85% (p<0.05). This result was further confirmed by fluorescence microscopy; representative images are shown in Figure 4c.

Figure 2: Characterization of sscFvPRO140
(a) Purification of sscFvPRO140. 50 ml of culture supernatant from gene-modified 293T cells expressing sscFvPRO140 was loaded onto a HiTalon cobalt column. The flow through (FT), washes (W1 and W2), and the sequential elution fractions from the same column (E1, E2, E3 and E4) were collected and equal volumes were separated by SDS-PAGE, followed by Coomassie blue staining. (b) Binding of sscFvPRO140 to cells expressing CCR5. 293T cells (CCR5- or U373-MAGI-CCR5E-CCR5+ cells) were incubated with purified sscFvPRO140 or PBS, followed by incubation with an anti-6xHis mAb/HRP conjugate. OD650 was measured after addition of HRP substrate; n = 4. (c) Quantification of sscFvPRO140 in culture supernatants. 1×10^6 cells transduced with LVX vector particles was also analyzed (Sn 1). Decreasing concentrations of purified sscFvPRO140 were analyzed in parallel to generate a protein standard curve (black bar, from left to right: 560, 280, 140, 70, and 35 nM). (d) Stability of sscFvPRO140. Culture supernatant from gene-modified 293T cells expressing sscFvPRO140 was incubated at 37°C. Aliquots were taken at the indicated time intervals and analyzed by Western blot.

sscFvPRO140-mediated inhibition of HIV infection of unmodified target cells

Pre-incubation of unmodified HIV target cells (U373-MAGI-CCR5E) with varying concentrations of purified sscFvPRO140 reduced infection of R5 HIVJRFL Env-pseudotyped reporter vector particles in a dose-dependent manner (Figure 5a). However, the antiviral effect...
HIV entry in the presence of culture supernatant containing 90 nM sscFvPRO140 or in the unmodified target cells incubated with sscFv PRO140-mediated toxicity was observed in the gene-modified cells expressing sscFvPRO140 or in the unmodified target cells incubated with sscFvPRO140 (data not shown).

sscFvPRO140-mediated inhibition of HIV infection in comparison to other protein-based entry inhibitors

While sscFvPRO140 binds to surface CCR5 on the target cells, sCD4 binds to R5 and X4 tropic HIV Env glycoprotein gp120. In order to compare the antiviral potency of the two secreted proteins, we designed a lentiviral vector for the expression of sCD4 (LVX-sCD4) and transduced U373-MAGI-CCR5E cells with vector particles encoding sscFvPRO140 or sCD4 at a low multiplicity of infection, resulting in a mixed population of gene-modified and unmodified target cells (level of gene-modification, ~50%). Cells transduced with LVX served as a control. Subsequently, we performed single-round infection assays with R5 HIVJRFL Env-pseudotyped reporter vector particles expressing dsRed. The number of infected cells in the gene-modified population and in the unmodified population was quantified by flow cytometry. Relative infections in comparison to the control cells are presented in Figure 6a. Both unmodified and gene-modified control cells were susceptible to infection. The gene-modified HIV target cells expressing sscFvPRO140 were protected from infection (>90% reduction of infection), while only a modest reduction of entry was observed in the neighboring unmodified target cells (~25% reduction of infection). In contrast, HIV entry was significantly inhibited in both the gene-modified HIV target cells expressing sCD4 and neighboring unmodified target cells (>80% inhibition).

The protective effect on the unmodified cells was further compared in single-round infection experiments in the presence of culture supernatants containing sscFvPRO140 or sCD4. In the presence of culture supernatants from gene-modified 293T cells transduced with LVX-sscFvPRO140 or LVX-sCD4 vector particles, infection was inhibited by >40% and >85% respectively (Figure 6b).

Next, we compared the antiviral effect of purified sscFvPRO140 and sCD4 at two different concentrations for inhibition of HIV entry in the unmodified U373-MAGI-CCR5E target cells. Additionally, we used the Food and Drug Administration (FDA)-approved fusion inhibitor FIT20, which consists of a peptide that binds to gp41 and inhibits membrane fusion [21]. Results are shown in Figure 6c. Under the tested conditions, sCD4 provided the strongest protection to unmodified target cells at a concentration of 30 nM (>95% inhibition of entry), followed by FIT20 (~60% inhibition of entry) and sscFvPRO140 (~40% inhibition of entry).

Discussion

sscFvPRO140 was used to block surface CCR5 on HIV target cells in order to render the receptor unavailable for R5 HIV binding and inhibit HIV entry. Gene-modified cells secreted sscFvPRO140, which was shown to bind to CCR5+ cells. We have further demonstrated that R5 HIV entry in both gene-modified and unmodified HIV target cells is reduced.

We observed a stronger inhibition of R5 HIV infection of gene-modified HIV target cells expressing sscFvPRO140 (~85%, Figure 4) than of unmodified target cells in the presence of sscFvPRO140 (~40%, Figure

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The increased inhibition of R5 HIV entry into the gene-modified target cells may be due to intracellular binding and retention of CCR5 by sscFv PRO140, despite the lack of a retention sequence in sscFv PRO140 (Figure 7a). Indeed, it has previously been described that scFvs without a retention sequence can still retain their target in the rER, probably as a consequence of their association with the binding immunoglobulin protein (BIP) [22]. Hence, reduced surface CCR5 expression on gene-modified target cells could have rendered these cells more resistant to HIV entry than the unmodified target cells, which are only protected by sscFv PRO140 to inhibition of HIV infection by sCD4 and FDA-approved FIT20, both of which target R5 and X4 HIV. In single-round infections, sCD4 and FIT20 surpassed sscFv PRO140-mediated inhibition of R5 HIV entry in unmodified target cells (Figures 6b and 6c). As expected, sCD4 conferred similar protection to the gene-modified target cells from which it was secreted and to the unmodified neighboring target cells, whereas sscFv PRO140 protected the gene-modified cells better than the unmodified neighboring target cells (Figure 6a). sCD4 was also shown to inhibit infection of peripheral blood mononuclear cells by primary HIV strains (unpublished results).

Figure 7: Potential modes of action of sscFv PRO140 (a) sscFv PRO140 binds to CCR5 intracellularly. Co-expression of sscFv PRO140 and CCR5 in the same cell could result in binding of sscFv PRO140 to CCR5 in the rER. The binding immunoglobulin protein (BIP) may bind to the sscFv PRO140/CCR5 complex and retain it in the rER, resulting in diminished surface expression of CCR5 and stronger inhibition of R5 HIV entry in the gene-modified compared to unmodified target cells. (b) sscFv PRO140 binds to CCR5 extracellularly. The sscFv PRO140 is secreted and masks surface CCR5, which prevents R5 HIV entry. If CCR5-, HIV target cells are used to secrete sscFv PRO140, the sscFv PRO140 could bind to surface CCR5 on gene-modified target cells upon release from the cell.

In conclusion, we have shown that sscFvs targeting CCR5 can protect gene-modified HIV target cells and have the potential to prevent infection of unmodified target cells. In the future, more potent scFvs or mAbs targeting surface CCR5 may be designed to confer protection to unmodified target cells. Gene therapy using vectors expressing these proteins may provide a feasible alternative to injections of purified mAb PRO140 to inhibition of HIV infection by sCD4 and FDA-approved FIT20. Alternatively, the full-length mAb PRO 140 could be expressed as a polyprotein with a self-cleavable peptide 2A in between the heavy and light chains. This approach has been used by others and was shown to allow efficient expression of another mAb [26].

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