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Design, intracellular expression, and activity of a human anti-human immunodeficiency virus type 1 gp120 single-chain antibody

(human immunodeficiency virus type 1 envelope protein/intracellular antibody/AIDS/CD4+ T cells/gene therapy)

WAYNE A. MARASCO*†, WILLIAM A. HASELTINE‡, AND SIYI CHEN‡

Departments of *Medicine and ‡Pathology, Division of Human Retrovirology, Dana–Farber Cancer Institute, Harvard Medical School, Harvard School of Public Health, 44 Binney Street, Boston, MA 02115

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ABSTRACT **A single-chain antibody, derived from a human monoclonal antibody that recognizes the CD4 binding region of the human immunodeficiency virus type 1 (HIV-1) envelope protein, has been designed for intracellular expression in eukaryotic cells. The single-chain antibody is composed of an immunoglobulin heavy-chain leader sequence and heavy- and light-chain variable regions that are joined by an interchain linker. The antibody is stably expressed and retained in the endoplasmic reticulum and is not toxic to the cells. The antibody binds to the envelope protein within the cell and inhibits processing of the envelope precursor and syncytia formation. The infectivity of the HIV-1 particles produced by cells that express the single-chain antibody is substantially reduced. These studies illustrate the feasibility of designing antibodies that bind and inactivate molecules intracellularly. Antibodies that act on target molecules within cells should provide a useful tool for research as well as for control of infectious and other diseases.**

Antibodies have the ability to recognize a very wide array of molecules. Upon synthesis, antibodies are normally secreted into the extracellular fluid or remain membrane bound on the B-cell surface as antigen receptors (1). Recent advances in antibody engineering have allowed antibody genes to be manipulated and antibody molecules to be reshaped (2). These advances raise the possibility that antibodies can be made to function intracellularly to bind and to inactivate molecules within cells. The feasibility of this principle is illustrated by using as a target the envelope glycoprotein specified by the human immunodeficiency virus type 1 (HIV-1). The observation that the nascent envelope protein binds to CD4 in the endoplasmic reticulum (ER) suggests that this strategy should be successful (3–5).

The envelope protein of HIV-1 is located on the exterior of the mature virus particle. The glycosylated envelope protein precursor gp160 is translocated into the ER after synthesis and is cleaved within the Golgi apparatus to yield a mature envelope protein composed of gp120 and gp41 (6–8). The exterior glycoprotein gp120 binds to the CD4 protein present on the surface of a subset of lymphocytes (9–12).

The antibody used for these studies is the broadly neutralizing human monoclonal antibody F105 that competes with CD4 for binding to gp120. Recent gp120 mutagenesis studies indicate that the binding sites on gp120 for CD4 and F105 overlap (13, 14). The F105 antibody binds to the envelope glycoprotein of multiple HIV-1 strains including several primary isolates. Moreover, the heavy- and light-chain variable regions (V_H and V_L) of the F105 antibody have been cloned and the sequence determined (15). The strategy followed was to create a correctly folded molecule that contains both V_H and V_L of the F105 antibody (designated sFv105) and to direct such a protein to the lumen of the ER. Such an antibody is designed to react with the nascent folded envelope protein within the ER and to prevent transit of the envelope antibody complex to the cell surface.

MATERIALS AND METHODS

Cloning and Construction of the Single-Chain Antibodies. The rearranged V_H and V_L genes of F105 were cloned and sequenced as described (15). To construct sFv105, a leader primer (15) and reverse J_H primer (J, joining region) (16), with an additional 45-nucleotide sequence encoding the (Gly-Gly-Gly-Gly-Ser)$_3$ interchain linker (17, 18) 5′-AGATCCGCCGCCACCGCTCCCACCACCTCCGGAGCCACCGCCACCTGAGGTGACCGTGACC(A/G)(G/T)GGT-3′, were used to amplify the leader signal, V_H, and interchain linker sequences by PCR as described (15). A framework 1 V_L primer (P-K), 5′-GGTGGCGGTGGCTCCGGAGGTGGTGGGAGCGGTGGCGGCGGATCTGAGCTC(G/C)(T/A)G(A/C)TGACCCAGTCTCCA-3′, and a reverse Ck primer with a stop codon, 5′-GGGTCTAGACTCGAGGATCCTTATTAACGCGTTGGTGCAGCCACAGT-3′, were used to amplify the V_L sequence (16). The fragments were digested with *Bsp*EI and then inserted into pRC/CMV (Invitrogen). The resultant pCMV-sFv105 contains the signal peptide, V_H, interchain linker, and V_L sequences. To construct sFv105-KDEL, the V_L was amplified with the P-K and a reverse Ck primer with an additional SEKDEL sequence. The V_L fragment in the pCMV-sFv105 was then replaced with the V_L-KDEL fragment. The sequence of the plasmids was confirmed by DNA sequence analysis (19).

To construct the anti-tat single-chain variable region fragment (sFv) the genes of V_H and V_L domains of a murine anti-HIV-1 tat hybridoma cell line were cloned and the DNA was sequenced (data not shown). A heavy-chain leader primer (P-L), 5′-TTTAAGCTTACCATGAACTTCGGGCTC-3′, and reverse primer (P-J), 5′-TG(A/C)GGAGACGGTGACC(A/G)(A/T)GGTCCCT-3′, were used to amplify the leader and rearranged heavy-chain sequences by PCR as described (15, 16). A V_L primer (P-K), 5′-GAGCTCGTGCTCAC(C/A)CA(G/A)(T/A)CTCCA-3′, and a reverse Ck primer, 5′-GGGTCTAGACTCGAGGATCCTTATTATACAGTTGGTGCAGCATC-3′, were used to amplify the V_L sequence (16). An interchain linker was amplified by using primers complementary to the P-J and P-K primers and containing the internal interchain linker sequence. The anti-tat

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Abbreviations: HIV-1, human immunodeficiency virus type 1; ER, endoplasmic reticulum; V_H, heavy-chain variable region; V_L, light-chain variable region; sFv, single-chain variable region fragment; PTV, punta toro virus.

†To whom reprint requests should be addressed.

sFv was produced by overlap extension (20). It was cloned into pRC/CMV and the DNA sequence was confirmed (19).

Construction of Transformed Cell Lines. COS-1 cells were transfected with 10 μg of pCMV-sFv105, pCMV-sFv105-KDEL, or vector DNAs with lipofectin (BRL) as described (21). Two hours after transfection cells were incubated for 48 hr in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. The transformed cells were selected in DMEM with 10% fetal bovine serum containing G418 (500 μg/ml) (BRL).

Radiolabeling and Immunoprecipitation. The COS-1 cells or transformed COS vector, COS sFv105, or COS sFv105-KDEL cells were metabolically labeled by incubation for 30 min in cysteine-free medium with 100 μCi of [^{35}S]cysteine (1 Ci = 37 GBq). The cells were washed and incubated in DMEM containing 10 mM unlabeled cysteine. Proteins were immunoprecipitated from the cell lysates or culture medium. Proteins were resolved by electrophoresis on SDS/polyacrylamide gels and visualized by autoradiography (21).

Syncytium Formation Assay. CD4$^+$ HeLa cells were cotransfected with 3 μg of pSVIIIENV and 15 μg of pCMV vector, pCMV-sFv105, pCMV-sFv105-KDEL, or pCMV-sFvtat. Syncytia were counted 30 hr posttransfection. COS cells transfected with pSVIIIENV were incubated with phosphate-buffered saline containing 50 mM EDTA at 37°C for 40 min after 48 hr. The cells were removed from the plate, washed, resuspended in DMEM supplemented with 10% fetal calf serum, and then added to 2 × 10^6 SupT1 lymphocytes. After 12 hr at 37°C, syncytia were scored.

Detection of Infectious Virus Production. COS vector, COS sFv105, or COS sFv105-KDEL cells were transfected with 5 μg of infectious pSVIIIB DNA. The presence of viral particles in the supernatant was measured after 4 days by using a radioimmunoassay for the HIV-1 p24 capsid antigen protein (DuPont/NEN) following the manufacturer's instructions. Equal amounts of p24 in each of the supernatants were used to infect 2 × 10^6 SupT1 cells for 16 hr.

Immunofluorescence Staining. The sFv105 or vector-transformed cells were grown on coverslips and fixed in 95% (vol/vol) ethanol/5% (vol/vol) acetic acid at −20°C for 5 min. The sFv105-KDEL-transformed cells were transfected with pSVIIIENV plasmid for 48 hr and then fixed. The cells were incubated with anti-human κ or rat anti-BiP monoclonal antibody at 37°C for 30 min followed by fluorescein isothiocyanate-conjugated anti-rabbit or anti-mouse IgG. The cells were then mounted and observed on a Nikkon microscope with fluorescence optics.

RESULTS

Binding Activity of sFv105-B Produced in *Escherichia coli*. The single-chain antibody sFv105-B derived from the F105 antibody was first expressed in bacteria. The sFv105-B protein contains a leader polypeptide that directs the nascent single-chain antibody into the periplasmic space. The sFv105-B protein made in bacteria is recognized by antiserum to both the human heavy and light κ chains. The secreted sFv105-B protein binds to purified gp120 as determined by ELISA in which gp120 is fixed to a plastic surface. Binding of the sFv105-B protein in this assay is blocked by competition with soluble CD4. The sFv105-B protein binds to and can be eluted from a gp120 affinity column (data not shown).

Expression of sFv105 and sFv105-KDEL in Mammalian Cells. Two plasmids were made that are suitable for expression of the single-chain antibody in eukaryotic cells. The coding sequences of both proteins were modified to include an N-terminal immunoglobulin signal sequence to create the protein designated sFv105 (15) (Fig. 1). A derivative of sFv105 was made by addition of sequences that specify six C-terminal amino acids, SEKDEL, to yield the sFv105-KDEL protein. The added sequence is predicted to specify an ER retention signal (22). The ability of these proteins to be expressed in mammalian cells was determined by transient transfection of COS-1 cells and a HeLa cell line that constitutively express the CD4 protein HeLa CD4 (23, 24). It was

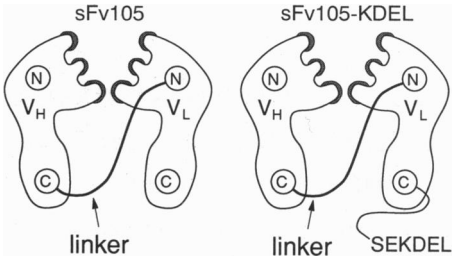

FIG. 1. Diagram of structures of sFv105 and sFv105-KDEL of F105.

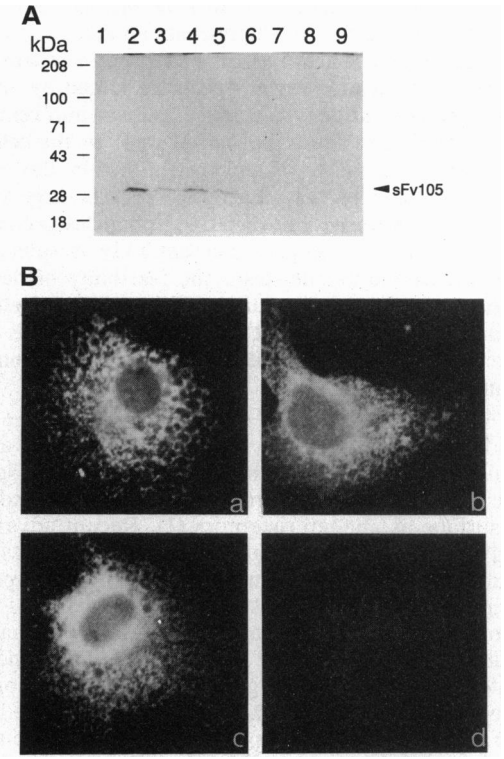

FIG. 2. Transformed cells constitutively expressing sFv105 or sFv105-KDEL retained in the ER. (*A*) Immunoprecipitation of sFv105 expressed in COS cells. Proteins were immunoprecipitated with anti-human immunoglobulin κ-chain antibody (Boehringer Mannheim) from the cell lysates or culture medium. Proteins were resolved by electrophoresis on a SDS/12.5% polyacrylamide gel (25). Positions of protein markers are shown. Lanes: 1, cell lysates of COS vector cells, 60-min chase; 2–5, samples immunoprecipitated from cell lysates of COS sFv105; 6–9, precipitated from the medium of COS sFv105. Lanes 2 and 6, 30-min chase; lanes 3 and 7, 60-min chase; lanes 4 and 8, 120-min chase; lanes 5 and 9, 360-min chase. (*B*) Immunofluorescent staining of the sFv105 and sFv105-KDEL cells. (*a*) COS sFv105 cells stained with anti-human κ-chain antibody followed by incubation with fluorescein isothiocyanate-conjugated anti-rabbit IgG. (*b*) COS sFv105-KDEL cells transfected with the envelope protein expressor plasmid pSVIIIenv (26, 27) stained as in *a*. (*c*) Neomycin-resistant COS cells selected to contain the vector pRC/CMV alone (COS vector-transformed control) stained with a rat anti-BiP monoclonal antibody (28–31) followed by incubation with fluorescein isothiocyanate-conjugated goat anti-mouse IgG, which displays 60% cross-reactivity with rat IgG (Boehringer Mannheim). (*d*) COS vector-transformed control stained as in *a* and *b*. (×500.)

found that whereas abundant amounts of the sFv105 protein are precipitated by anti-human heavy- and light-chain antibodies, very little of the sFv105-KDEL protein is detected in the transient expression assay (data not shown).

Cells that constitutively express the sFv105 and sFv105-KDEL proteins (COS sFv105 and COS sFv105-KDEL) were made by transfection of COS-1 cells with the two plasmids followed by selection for neomycin resistance. Fig. 2*A* shows that abundant sFv105 protein accumulates intracellularly. Pulse–chase experiments using radioactive amino acids demonstrate that the half-life of the sFv105 protein is >6 hr, indicating that sFv105 is stably expressed in the COS-1 cells. The morphology and growth rate of cells that express sFv105 are not affected. The sFv105 protein is not detected in cell supernatant fluids. The amount of sFv105-KDEL in the cells stably transformed with the expression plasmid was too low to detect by these methods.

The location of the sFv105 protein within the cell was determined by immunofluorescence of fixed cells using an anti-human κ-chain antibody. This antibody stains a tubular network throughout the cytoplasm typical of an ER resident protein (Fig. 2*Ba*). This pattern is the same as that obtained with an antibody to the ER resident protein immunoglobulin heavy-chain-binding protein BiP (28–31) (Fig. 2*Bc*) in the parental cell. Coimmunoprecipitation experiments were performed with a rat monoclonal antibody to the ER chaperone protein BiP. The sFv105 protein is precipitated by using this rat monoclonal antibody to the BiP protein (data not shown).

Inhibition of HIV-1 Envelope Protein Processing and Activity by Transformed Cells Expressing sFv105 and sFv105-KDEL Proteins. The ability of cell lines that constitutively express the sFv105 or sFv105-KDEL proteins to inhibit HIV-1 envelope protein processing and activity was determined by transfection of the COS sFv105 and COS sFv105-KDEL cells with a vector that expresses high levels of the envelope protein. Pulse–chase analysis followed by immunoprecipitation of the envelope protein shows that a significant fraction of gp160 is cleaved to gp120 in the parental cell line during the 4-hr chase (Fig. 3*A*). Although similar amounts of gp160 are made in the parental and COS sFv105 cells, very little gp120 is evident after the 4-hr chase (Fig. 3 *A* and *C*). The gp160 protein present in the COS sFv105 cells can be coprecipitated by using an anti-human κ-chain antibody. This antibody does not precipitate the gp160 protein made in the parental COS-1 cell line. An antibody to the HIV-1 envelope glycoprotein also coprecipitates the sFv105 protein in cells that express gp160 (Fig. 3*A*).

In the COS sFv105-KDEL cells, processing of gp160 to gp120 is partially inhibited (Fig. 3*B*). The amount of sFv105-KDEL protein precipitated by an anti-human κ-chain antibody is increased by the presence of gp160. The gp160 protein present in the COS sFv105-KDEL cells is also precipitated by an anti-κ-chain antibody. Antiserum to gp160 also precipitates the sFv105-KDEL protein. The intracellular distribution of the sFv105-KDEL protein in cells that express gp160 is similar to the distribution of the sFv105 protein in COS sFv105 cells (Fig. 2*Bb*). Evidently, the sFv105-KDEL protein is stabilized by binding of gp160.

Specificity of sFv105 Binding to the HIV-1 Envelope Protein. To determine whether sFv105-mediated inhibition of processing of the envelope protein of HIV-1 is specific, the ability of cells that express a single-chain antibody to inhibit processing of a mutant of the env protein that is incapable of binding sFv105 was examined. For this purpose, the COS sFv105 cells were transfected with a plasmid that expresses a mutant of the envelope protein in which glutamic acid has been substituted with aspartic acid at position 370. This mutation has been previously shown to eliminate detectable binding of the envelope protein by the sFv105 parental antibody (33). The data of Fig. 3*D* (lane 1) show that in contrast to the absence of processing of the parental envelope protein in COS sFv105 cells, the mutant gp160 envelope protein is processed normally.

The specificity of sFv105 binding to the HIV-1 envelope protein was examined by transfecting the COS sFv105 cells with a vector expressing envelope proteins of the punta toro virus (PTV), which have been previously shown to be processed in the ER (21). The PTV envelope proteins were not

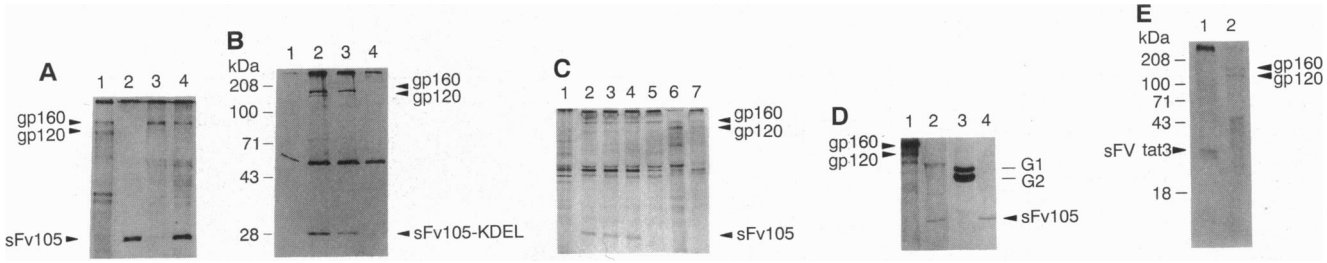

FIG. 3. sFv105 or sFv105-KDEL specific binding to the HIV-1 glycoprotein in cells. (*A* and *B*) Autoradiograms showing that sFv105 (*A*) or sFv105-KDEL protein (*B*) is coprecipitated with the HIV-1 glycoprotein. Transformed cells were transfected with 10 μg of pSVIIIENV plasmid DNA and 2 μg of pSVIIItat expressing tat protein (27, 32), pulse labeled with [^{35}S]cysteine, and chased for 4 hr. (*A*) Lanes: 1, lysate of COS vector cells transfected with the envelope expressor plasmid pSVIIIENV and precipitated with anti-gp120 antiserum (AIDS Research and Reference Program); 2, lysate of mock-transfected COS sFv105 cells precipitated with a mixture of anti-gp120 and anti-κ-chain antisera; 3 and 4, lysates of COS sFv105 cells transfected with pSVIIIENV; 3, precipitated with anti-gp120 antiserum; 4, precipitated with anti-κ-chain antiserum. (*B*) Lanes: 1, lysates of mock-transfected COS-1 cells precipitated with a mixture of anti-gp120 and anti-κ-chain antisera; 2 and 3, lysates of COS sFv105-KDEL cells transfected with pSVIIIENV; 2, precipitated with anti-gp120 antiserum; 3, precipitated with anti-κ-chain antiserum; 4, lysate of COS sFv105-KDEL cells precipitated with a mixture of anti-gp120 and anti-κ-chain antisera. (*C*) Processing of HIV-1 glycoprotein is inhibited in the COS sFv105 cells. COS sFv105 or COS vector cells were transfected with pSVIIIenv and pSVIIItat and pulse labeled for 30 min. Cell lysates were immunoprecipitated with polyclonal sheep anti-gp120 antiserum. Lanes: 1, lysate of COS vector cells; 2–4, lysates of transfected COS sFv105 cells; 5–7, lysates of transfected COS vector cells. Lanes 1, 2, and 5, 0-min chase; lanes 3 and 6, 4-hr chase; lanes 4 and 7, 6-hr chase. (*D*) sFv105 is not coprecipitated with unrelated proteins. COS sFv105 cells were pulse labeled for 30 min and then chased for 4 hr. Lanes 1 and 2, COS sFv105 cells were transfected with 10 μg of a mutant HIV-1 glycoprotein expressor 370E/D (33). Proteins were immunoprecipitated with either anti-HIV-1 glycoprotein (lane 1) or anti-κ-chain antibody (lane 2). Lanes 3 and 4, COS sFv105 cells were infected with vaccinia virus encoding T7 polymerase (multiplicity of infection, 5) (22) for 2 hr and then transfected with 10 μg of plasmid DNA PTV-G1–G2, which contains the genes of G1 and G2 glycoproteins of punta toro virus under control of T7 polymerase (21). Lanes 3 and 4, lysates of PTV-G1–G2-transfected cells immunoprecipitated with anti-PTV glycoprotein (21) (lane 3) or anti-κ-chain (lane 4). (*E*) An intracellularly retained anti-tat sFv does not bind HIV-1 glycoprotein. COS cells were cotransfected with 10 μg of pSVIIIENV and 10 μg of pRC/CMV-sFv-tat plasmid DNA, pulse labeled for 30 min, and then chased for 4 hr. Proteins were immunoprecipitated with anti-mouse immunoglobulin (lane 1) or sheep anti-gp120 (lane 2) antisera.

precipitated with anti-human κ-chain antibodies that are shown to coprecipitate the sFv105 HIV-1 gp160 complex. Fig. 3*D* also shows that the PTV envelope proteins are processed in COS sFv105 cells at a level comparable (data not shown) to that of COS vector cells.

In addition, the ability of a single-chain antibody that does not bind to gp160 to interfere with processing of the envelope protein was examined. This single-chain antibody is derived from a murine monoclonal antibody that recognizes the HIV-1 tat protein. The processing of gp160 to gp120 was unaffected by cotransfection of the HIV-1 glycoprotein expressor with the plasmid that expresses the anti-tat sFv. Moreover, an antiserum that precipitates the anti-tat sFv does not coprecipitate the HIV-1 envelope protein (Fig. 3*E*).

sFv105- and sFv105-KDEL-Expressing Cells Inhibit HIV-1 Envelope-Mediated Syncytia Formation. The ability of the sFv105 and sFv105-KDEL proteins to inhibit the function of the envelope protein was determined by measurement of the ability of cells transfected with the envelope gene to induce syncytium formation of CD4+ cells. In one set of experiments, the parental COS vector cells as well as the COS sFv105 and COS sFv105-KDEL cells were transfected with a plasmid that expresses a functional envelope glycoprotein. At two days posttransfection the cells were mixed at a ratio of ≈1:10 with a human CD4+ T-cell line, SupT1, that is susceptible to envelope-mediated fusion. The extent of envelope-mediated syncytium formation was reduced by 80–90% in cells that express either the sFv105 or sFv105-KDEL proteins (Fig. 4*A*). Similar amounts of gp160 were made in all three lines as determined by metabolic labeling and precipitation of the transfected cultures (data not shown). Reduction in syncytium formation was also observed upon cotransfection of the HeLa CD4+ cell line with a plasmid that expresses a functional envelope glycoprotein along with a second plasmid that expresses either the sFv105 or sFv105-KDEL proteins (Fig. 4*A*). In contrast, there was no significant reduction in syncytium formation with the second plasmid that expresses the anti-tat sFv (Fig. 4*A*).

sFv105- and sFv105-KDEL-Transformed Cells Inhibit Production of Infectious HIV-1. To examine the ability of the sFv105 proteins to inhibit production of infectious virus, COS vector, COS sFv105, and COS sFv105-KDEL cells were transfected with a plasmid that contains a copy of the entire viral genome (26, 34). Four days posttransfection, the virus in the culture supernatant fluids was used to initiate infection of the sensitive indicator cell line SupT1. The supernatants of all three transfected cell lines were shown to contain similar amounts of the viral capsid protein, p24. Release of capsid proteins into the cell supernatant has previously been shown to occur in the absence of synthesis of the envelope glycoprotein as well as in the presence of envelope glycoproteins that contain processing defects and are therefore retained in the ER (27, 32). Fig. 4*B* shows that virus replication in SupT1 cells initiated by supernatants from the transfected COS sFv105 or COS sFv105-KDEL cells is delayed ≈5 days relative to that initiated by virus produced by a control COS-1

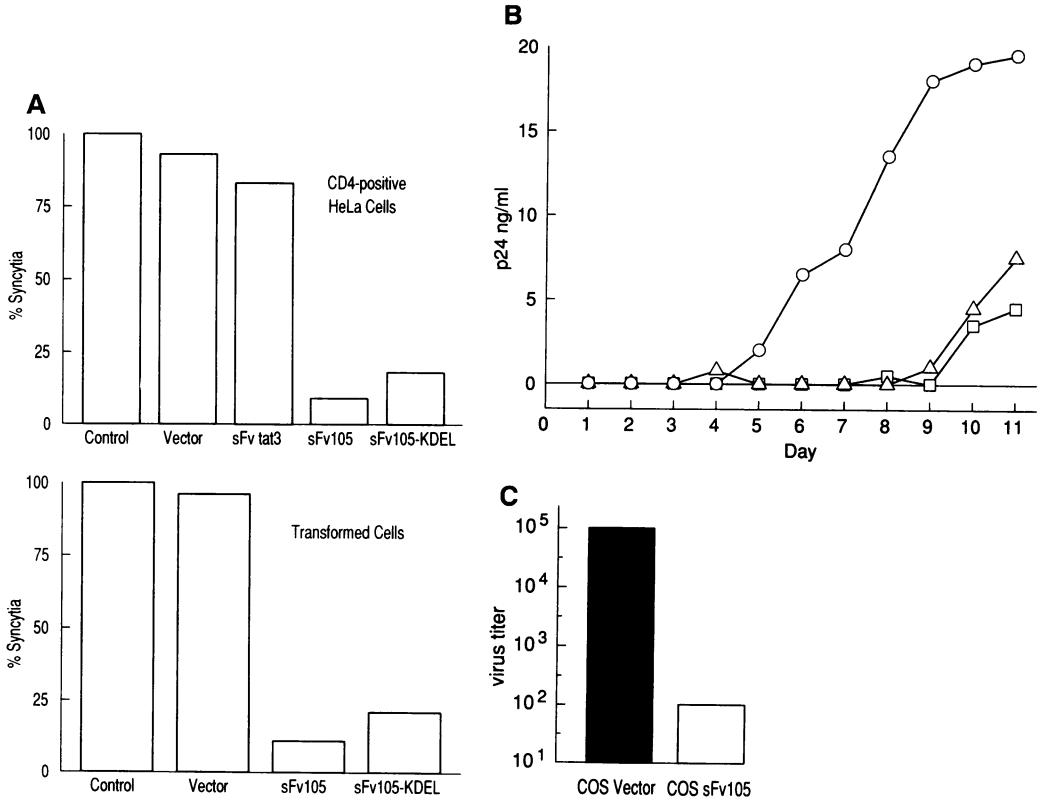

FIG. 4. Inhibition of syncytium formation and production and titers of infectious HIV-1 in cells expressing sFv105 or sFv105-KDEL. (*A*) Significant reduction of syncytium formation in the sFv105- and sFv105-KDEL-transfected CD4+ HeLa cells and the COS sFv105 or sFv105-KDEL cells compared to the CD4+ HeLa cells (control) or the vector-transformed COS cells (vector) transfected with pSVIIIENV is shown. (*B*) Virus yield by infected SupT1 cells. COS vector, COS sFv105-, and COS sFv105-KDEL-transformed cells were transfected with 5 μg of pSVIIIB plasmid DNA containing an infectious HIV-1 proviral DNA of the HXBc2 strain (26, 34). Amounts of p24 antigen detected in the supernatants of transfected cells were 1.2 ng/ml (COS vector), 1.0 ng/ml (COS sFv105), and 1.4 ng/ml (COS sFv105-KDEL), respectively. Amounts of p24 from the medium of SupT1 cells infected by supernatants harvested from COS vector (○), COS sFv105 (□), and COS sFv105-KDEL (△) cells are shown. (*C*) Virus titer by syncytium formation in SupT1 cells. COS vector and COS sFv105 cells were transfected with 4 μg of pSVIIIB plasmid DNA and after 48 hr the supernatants were harvested and used in serial dilutions to infect SupT1 cells for 16 hr. After 8 days, syncytia were counted in five high-power fields (HPF) in each well. One or more syncytia in five HPF counts as positive for the dilution.

cell line that contains the vector but not the sFv105 sequences. When serial dilutions of the supernatants were used to infect SupT1 cells, there was a $>10^3$-fold reduction in syncytium formation (Fig. 4*C*). This delay in replication of virus produced by COS sFv105 cells and the decrease in infectious titer are attributed to low infectivity of the virus relative to that of virus produced by the control cell line.

DISCUSSION

The results of these experiments demonstrate that cells can produce antibodies that function intracellularly. The sFv105 single-chain antibody when expressed in human cell lines binds to the envelope glycoprotein made in the same cell and inhibits env protein maturation and function as judged by inhibition of gp160 to be cleaved to gp120 and by reduction in envelope protein-mediated syncytia formation. Virus produced by cells that make the single-chain antibody are less infectious than those made in a control cell line.

The inhibition of gp160 maturation and function is the likely result of direct binding of the sFv105 antibody to the envelope protein within the ER. The two proteins can be coprecipitated. Coprecipitation is not observed when an envelope protein mutant in the sFv105 binding site is used. Processing of the binding site mutant protein is normal in cells that constitutively express the anti-envelope antibody. The HIV-1 envelope protein is not coprecipitated with a single-chain anti-tat antibody expressed intracellularly, nor does such an antibody inhibit HIV-1 envelope protein processing. The unrelated PTV envelope proteins are not coprecipitated with the sFv105 antibody protein, and the anti-HIV-1 antibody does not inhibit processing of PTV envelope proteins. Evidently, retention of the sFv105 antibody in the ER does not interfere with the normal trafficking of proteins.

The sFv105 remains stably in the ER, is not secreted into the medium, and is functionally active in the presence of the env protein. Retention of sFv105 in the ER is probably a consequence of association with BiP. Binding of the single-chain antibody to BiP does not impair the specific binding activity. Normal immunoglobulin heavy or light chains expressed in the absence of the appropriate counterpart light or heavy chains also bind to BiP and can be retained in the ER. These normal proteins are not exported until the correct partner is introduced (25–28). Moreover, a point mutation in the framework of a V_L, similar to F105 light-chain framework mutation, was also shown to result in ER retention of the light chain (15, 30, 31). The defect in secretion of the light chain was not due to misfolding of the light chain as the light chains assembled into functional antibodies when coexpressed with an immunoglobulin heavy chain (31).

The results presented here raise the possibility that single-chain antibodies may provide another class of active molecules for gene therapy. Cells resistant to infection by specific microorganisms may be made by introduction of single-chain antibodies that recognize critical epitopes. Single-chain antibodies can also be made that recognize certain cellular proteins including the products of oncogenes. This approach is particularly well suited to inhibition of the activity of proteins that are processed by the ER, the subcellular compartment in which antibodies themselves are made and properly folded (2). The observation that active antibodies can be targeted to specific subcellular compartments by linkage to appropriate signal sequences (35, 36) raises the additional possibility that single-chain antibodies can be made that bind target molecules in other subcellular compartments including the nucleus, nucleolus, and cytoplasm.

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