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Short communication

Inhibition of HIV-1 replication by the combined action of anti-gp41 single chain antibody and IL-16

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Abstract

HIV-1 replication is inhibited in T cells transfected with an anti-gp41 single chain antibody (ScFv) or IL-16. These two molecules target totally different events in the HIV-1 replication cycle. The present study shows that HIV-1 replication is inhibited to a substantially greater extent and for a longer duration in cells transfected with both anti-gp41 and IL-16 than with either molecule alone. It is concluded that anti-gp41 and IL-16 act in a synergistic fashion to inhibit HIV-1 replication.
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Keywords: HIV-1; IL-16; Anti-gp41; Single-chain antibody

Intracellular immunization of CD4+ T cells (Jurkat) with an anti-gp41 single chain antibody (ScFv) derived from a non-neutralizing antibody and targeted to the endoplasmic reticulum effectively inhibits HIV-1 replication (Zhou et al., 1998). Similarly, the intracellular expression of the C-terminus 130 amino acids of IL-16 with or without the genetically engineered signal peptide makes Jurkat cells resistant to HIV-1 infection (Zhou et al., 1997, 1999). These two molecules inhibit HIV-1 replication by targeting totally different events in the life cycle of the virus. Anti-gp41 ScFv acts intracellularly and blocks the maturation of gp120 and gp41. In contrast IL-16 inhibits the transcription of HIV-1 mRNA, and the degree of inhibition is dependent, in part, upon the extracellular concentration of IL-16 (Mackewicz et al., 1996; Zhou et al., 1999). To see whether the simultaneous expression of both molecules was more effective in inhibiting HIV-1 than either molecule alone, Jurkat cells were transfected with either anti-gp41 ScFv or IL-16 or the combination of the two, and then challenged with HIV-1.

To generate stable transfectants, Jurkat cells were electroporated with 20 μg of linearized IL-16 plasmid (without the signal peptide sequence used in our earlier experiments (Zhou et al., 1999)) and/or 20 μg of linearized human anti-gp41 ScFv-ER plasmid (anti-gp41 ScFv targeted to the endoplasmic reticulum, Zhou et al., 1997, 1998). Stable transfectants were selected with G418 (1.5 mg/ml) for 2–3 weeks and then single cell clones were isolated by limiting dilution. The intracellular expression of IL-16 (JIL) and anti-gp41 ScFv (JScFv) was analyzed by flow cytometry (FACS) (Prussin and Metcalfe, 1995). Jurkat cells transfected with the vector alone (JV) served as a negative control. FACS analysis revealed that when the transfected cells were stained with an anti-IL-16 antibody, only the cells transfected with IL-16 alone (JIL1) or in combination with anti-gp41 ScFv (JIS2) showed positive staining (Fig. 1A). Cells transfected with either the vector (JV1) alone or anti-gp41 ScFv (JScFv1) alone did not show positive staining. Similarly, only the cells transfected with anti-gp41 ScFv (JScFv1) alone or in combination with IL-16 (JIS2) showed positive staining with antibody to anti-gp41 ScFv. Cells transfected with either the vector (JV1) alone or IL-16

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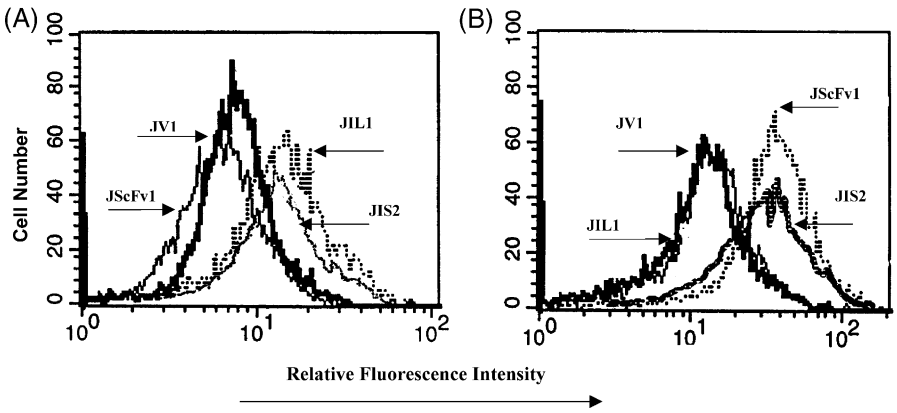

Fig. 1. Intracellular expression of anti-gp41 ScFv and IL-16. Jurkat cells transfected with vector alone (JV1), IL-16 alone (JIL1), anti-gp41 ScFv alone (JScFv1), and IL-16 and anti-gp41 ScFv (JIS2). (A) FACS analysis of cells stained with affinity purified rabbit anti-IL-16 polyclonal antibody. (B) FACS analysis of cells stained with rabbit anti-human kappa light chain antibody that reacts with anti-gp41 ScFv. The secondary antibody was FITC conjugated goat anti-rabbit IgG.

(JIL1) alone did not show positive staining (Fig. 1B). The double transfectants expressed lower amounts of IL-16 and anti-gp41 ScFv than the single transfectants expressing either IL-16 or anti-gp41 ScFv alone, presumably due to the fact that the same CMV promoter was used by both IL-16 and anti-gp41 ScFv. Culture supernatants from cells transfected with IL-16 alone (JIL1, JIL2) or in combination with anti-gp41 ScFv (JIS1, JIS2) contained comparable amounts of IL-16 (approximately 250 pg/ml), but only about one-fourth the amount found in supernatants of cell lines transfected with IL-16 constructs carrying a genetically engineered signal peptide (Zhou et al., 1997, 1999). The growth kinetics and the expression of CD4 was essentially the same in all the transfected Jurkat cell lines (Figs. 2 and 3). Taken together these experiments show that the double transfectants express both IL-16 and anti-gp41 ScFv within the same cell.

Transfected cells were then infected with HIV-1, strain IIIB, using two concentrations of virus, a low dose corresponding to 6000 cpm RT units [multiplicity of infection (MOI): 0.001] and a ten times higher dose corresponding to 60,000 cpm RT units (MOI: 0.01). Viral replication was

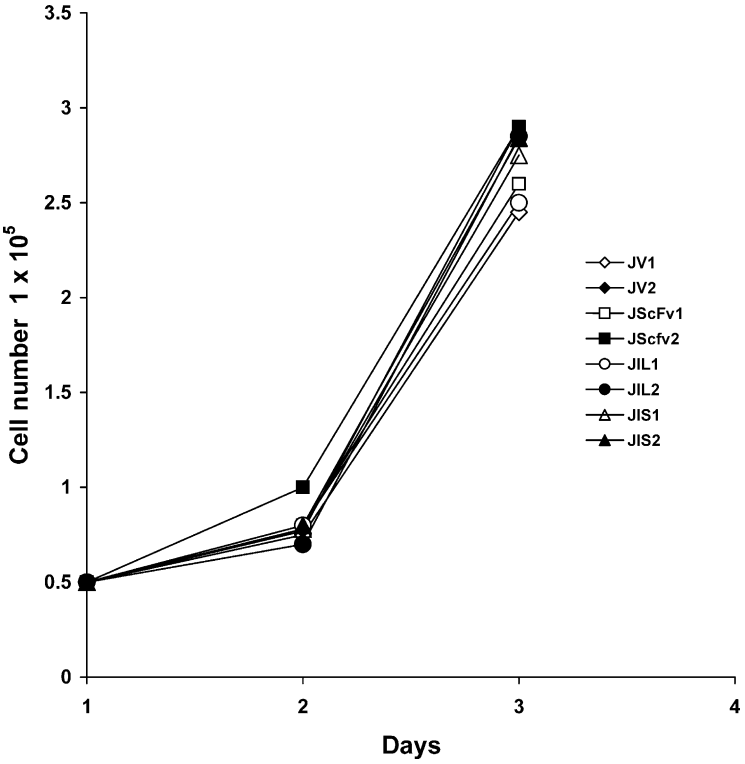

Fig. 2. Cell growth kinetics. Jurkat cells transfected with vector alone (JV1, JV2); anti-gp41 ScFv alone (JScFv1, JScFv2); IL-16 alone (JIL1, JIL2); or IL-16 and anti-gp41 ScFv (JIS1, JIS2). The viable cells were counted by trypan blue staining.

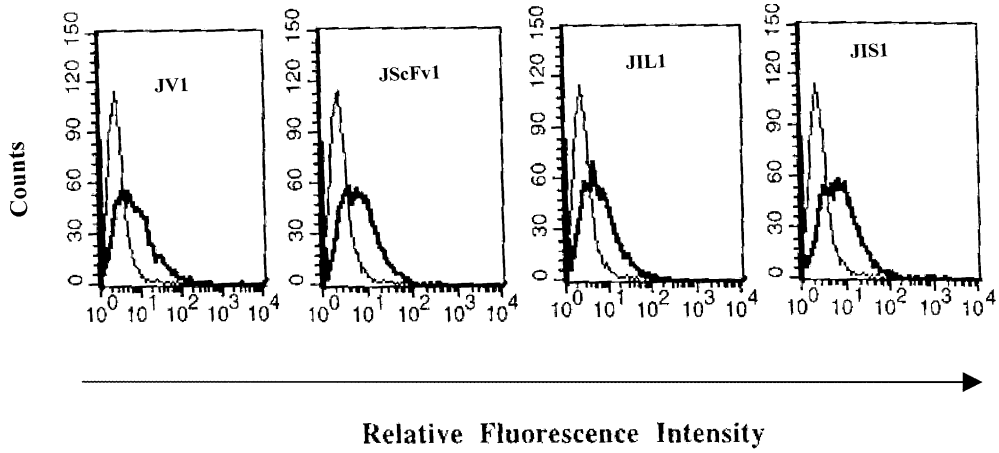

Fig. 3. Surface expression of CD4. Jurkat cells transfected with vector alone (JV1), anti-gp41 ScFv alone (JScFv1), IL-16 alone (JIL1), or IL-16 and anti-gp41 ScFv (JIS1) were stained with an anti-CD4 antibody (dark line) or isotype matched (control) mouse IgG1 antibody (light line) and examined by FACS.

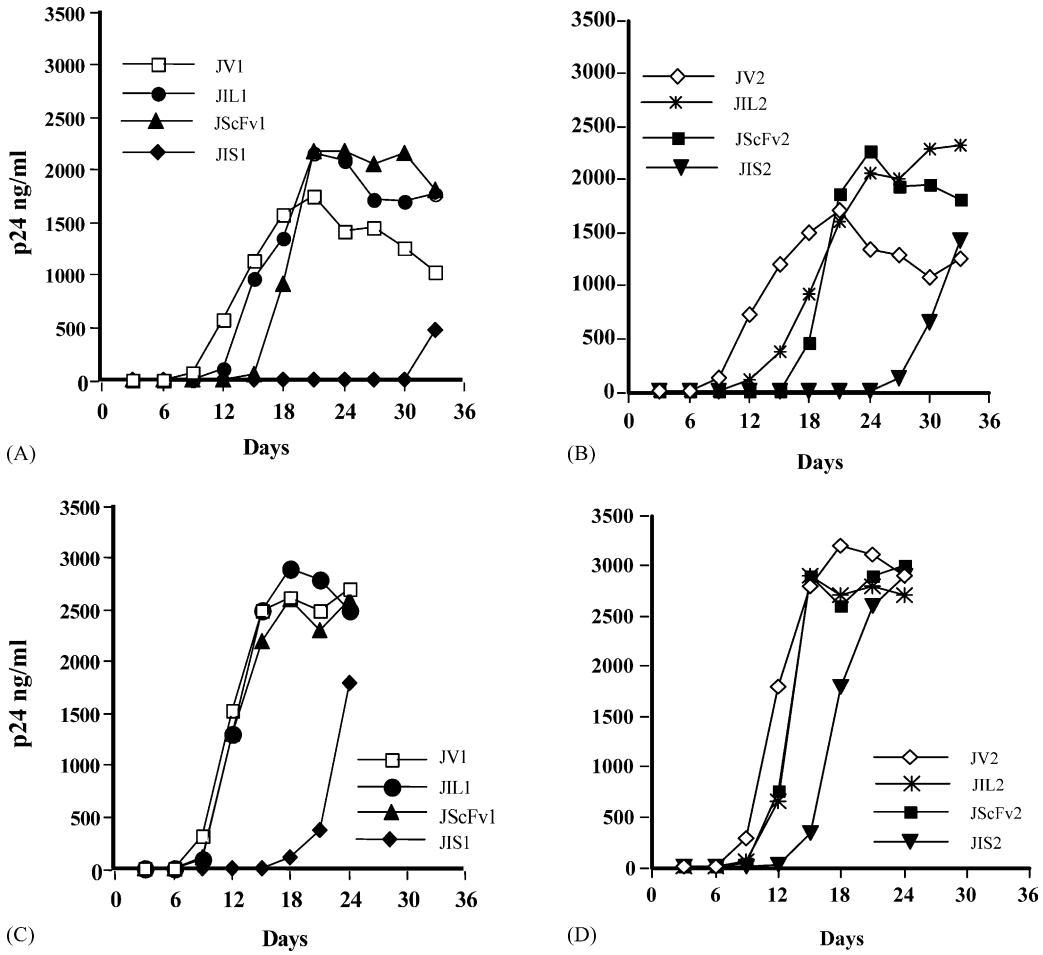

Fig. 4. Inhibition of HIV-1 replication. Cells infected with (A, B) a low dose (MOI: 0.001) or (C, D) a high dose (MOI: 0.01) of HIV-1 (IIIB). HIV-1 replication was quantitated by assessing HIV-1 p24 antigen levels in the culture supernatants by ELISA. The data are representative of at least two sets of independent experiments.

monitored by measuring the p24 content in the culture supernatants. Inhibition of HIV-1 replication in the JIL, JScFv, and JIS lines was apparent as early as 6 days post infection (Fig. 4A and B), when the cells were infected with a low dose of virus. On day 12, all the transfected cell lines tested showed 80–90% inhibition of HIV-1 replication as compared to the vector controls. However, by day 21, the cell lines transfected only with anti-gp41 ScFv or IL-16 showed no inhibition of viral replication. In contrast, the Jurkat cell lines transfected with both anti-gp41 ScFv and IL-16 (JIS1, JIS2) showed marked inhibition of HIV-1 replication, equal to or greater than 90–99%, for as long as 27–30 days after infection. When the cells were infected with a 10-fold higher dose of virus, the modest inhibition of HIV-1 replication produced by cells expressing IL-16 alone or anti-gp41 ScFv alone was lost (Fig. 4C and D). However, cells expressing both IL-16 and anti-gp41 ScFv (JIS1, JIS2) inhibited HIV-1 replication by over 85–90% for as long as 15–18 days post infection.

Recently, we (Tewari et al., 2003; Zhou et al., unpublished data, 2003) and others (Mhashilkar et al., 1999; Maurice et al., 2002) showed that transfection of primary human and macaque T cells with anti-HIV-1 single chain antibody inhibited HIV/SHIV infection. The findings described here and elsewhere (Rondon and Marasco, 1997; Ho et al., 1998; Ranga et al., 1998; Tewari et al., 1998; Center et al., 2000) raise the possibility that introduction of antiviral genes into lymphoid stem cells, from HIV-1 infected patients, might render these cells resistant to HIV infection when they mature into HIV-susceptible CD4+ T cells. Concomitant intracellular expression of anti-HIV ScFv and IL-16 could be used in conjunction with conventional antiviral therapy to maintain a T cell pool in infected individuals that is resistant to HIV infection.

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