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Anti-Human Immunodeficiency Virus Type 1 Activity of an Oligocationic Compound Mediated via gp120 V3 Interactions

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An oligocationic peptide compound (ALX40-4C) was developed for consideration in the treatment of human immunodeficiency virus type 1 (HIV-1) infection. This compound was designed to mimic the basic domain of the HIV-1 transactivation protein, Tat, and will competitively inhibit Tat binding to its specific RNA hairpin target (TAR [transactivation region]), found at the 5′ end of all HIV-1 transcripts. Blocking Tat-TAR interactions can abrogate HIV-1 replication. ALX40-4C was shown to inhibit replication of HIV-1_{NL4-3} in a range of cell types, including primary cells and transformed cell lines, by as much as 10^4-fold. In some experiments, virus rescue was not possible even after removal of ALX40-4C from the cultures. Strain-dependent resistance has been demonstrated for all antiretroviral agents tested; therefore, we tested for variable sensitivity to ALX40-4C. The cloned primary strains, HIV-1_{JR-CSF} and HIV-1_{JR-FL}, were less sensitive to ALX40-4C inhibition. Unexpectedly, determinants for efficient ALX40-4C inhibition were mapped by using recombinant virus strains to the V3 region of gp120 and were shown to act at early events in viral replication, which include viral entry. If entry and reverse transcription are bypassed by transfection, a more modest, virus strain-independent inhibition is shown; this inhibition is likely due to blocking of Tat-TAR interaction. Thus, the highly basic oligocationic Tat inhibitor ALX40-4C appears to interfere with initial virus-target cell interactions which involve HIV-1 gp120 V3 determinants, most efficiently for T-cell line-adapted strains.

Human immunodeficiency virus type 1 (HIV-1) inhibition by currently available antiretroviral therapy is modest, typically reducing levels of plasma HIV RNA by less than 1 log unit (40, 45, 51). Even the most potent HIV-1 inhibitors tested, the protease inhibitors, reduce levels of plasma viremia by only 1 to 2 log units despite levels of circulating virus as high as 10^6 to 10^7 virus particles per ml (24, 61). The duration of antiretroviral benefit is limited by the emergence of resistant strains, which arise as a consequence of mutation, and the high level of HIV-1 replication (47). Until recently, only inhibitors of reverse transcription were approved, but other retroviral targets, including virus binding, integration, and transactivation, could be exploited.

The HIV-1 regulatory protein, Tat, is an important potential target for pharmaceutical intervention. Tat acts to increase full-length transcripts following interaction with a viral RNA stem structure, TAR (transactivating region), which is found at the 5′ ends of all viral transcripts (3, 48, 56). Specific binding of Tat to TAR has been demonstrated by biochemical studies and by mutagenesis of both Tat and TAR (6, 18, 22, 37, 49, 64). Since expression of viral genes is required both for viral replication and for any cytopathic effects to be manifested, inhibitors of the action of Tat may provide an advantage over reverse transcriptase and protease inhibitors. Full suppression would, in theory, create a state of complete latency.

Binding to TAR is mediated by a short, linear peptide domain of Tat which is predominantly composed of basic amino acids. Basic peptides have been developed as potential competitors of this essential function. One such compound, *N*-α-acetyl-nona-D-arginine amide (ALX40-4C), was found to compete effectively with Tat for binding to TAR and to inhibit the transactivation and HIV-1 replication (57). In this report, we demonstrate that this polycationic peptide designed as a Tat inhibitor can also inhibit early events in HIV-1 replication, which include virus entry, in a strain-dependent fashion. Further, this inhibition appears to act by interference with target cell interactions that involve the V3 region of the HIV-1 envelope protein.

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MATERIALS AND METHODS

Virus and cells. Molecular cloning of the HIV-1 provirus from NL4-3 (1), JR-CSF and JR-FL (28), NFNSX (SX), NFNSM (SM), and NFNMX (MX) (41) was described previously. New recombinant HIV-1 strains were constructed by using fragments from molecular clones of HIV-1 JR-FL and HIV-1 NL4-3. For the recombinants substituting the V3 region, an *Mlu*I site was generated at nucleotide 7096 and an *Xba*I site was generated at nucleotide 7914 by site-directed mutagenesis without altering the amino acid sequence. *Mlu*I-to-*Xba*I fragments (0.1 kb) derived from JR-FL sequences by PCR were substituted into plasmid pKS+KXNL (39). Hybrid *Stu*I-to-*Xho*I fragments (2.1 kb) were substituted into pNL(*Stu*)*Xba*I to form full-length recombinant viral clones. Virus stocks were prepared following electroporation of peripheral blood mononuclear cells (PBMC) and coculture with phytohemagglutinin-stimulated peripheral blood lymphocytes (PBL) as previously described (10, 39). HUT 78 cells (19) were maintained in Iscove's medium containing 10% (vol/vol) fetal calf serum. Primary human PBL and mononuclear phagocytes were prepared from HIV-1-negative blood as previously described (41). Following 3 days of stimulation in RPMI 1640 medium containing 20% fetal calf serum and 1% phytohemagglutinin (Sigma), PBL were maintained in RPMI medium containing 20% fetal calf serum and 100 U of recombinant interleukin-2 (Amgen) per ml. Blood mononuclear phagocytes were purified by adherence to plastic tissue culture dishes and were maintained in Iscove's medium containing 20% fetal calf serum.

ALX40-4C. The development of ALX40-4C, a competitive inhibitor of the

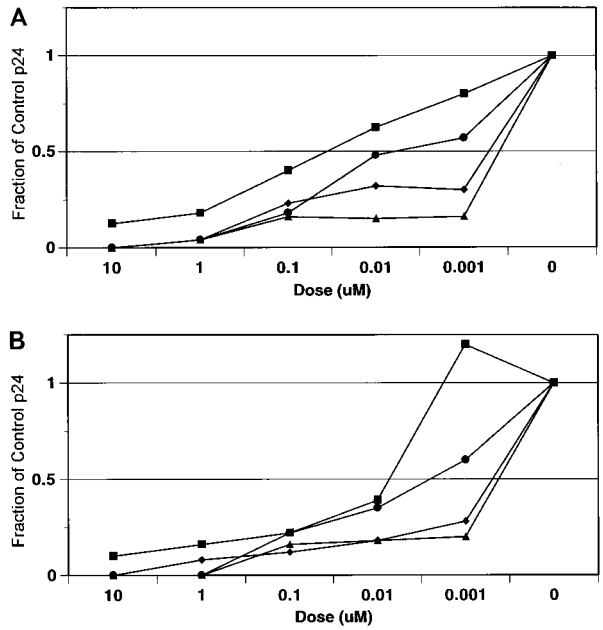

FIG. 1. Dose response of ALX40-4C inhibition in Hut 78 cells (A) or in PBL (B) infected with NL4-3. Cells were incubated with various concentrations of ALX40-4C from 0 to 10 μM for 24 h prior to HIV-1 infection. Virus production was assessed by measuring p24 production at day 4 (■), day 7 (●), day 10 (▲), or day 14 (◆) for each treatment condition.

binding of the HIV-1 Tat protein to its RNA target, TAR, has been described previously (57). This compound was synthesized by American Peptide Co. (Santa Clara, Calif.) by using standard solid support methods and was purified by reverse-phase high-pressure liquid chromatography to greater than 95% purity. Cytotoxicity is seen in culture systems at concentrations of 100 μM or greater (unpublished data).

Infection and transfection. Virus infections were performed as described previously (39, 46). PBL, mononuclear phagocytes, or HUT 78 cells (10^6 cells, 24-well plates) were exposed to 300 μl of cell culture medium containing the virus inoculum at a multiplicity of infection of 0.01 (approximately 100 ng of p24). Following a 2-h adsorption period at 37°C, the virus-containing supernatants were removed, the cells were rinsed once in serum-free medium, and 1 ml of fresh medium was added. Cell supernatants were changed every 3 to 4 days and assessed for p24 content at day 7 (PBL) or at day 14 (mononuclear phagocytes and HUT 78 cells). HUT 78 cells were subjected to electroporation with full-length proviral clones as described previously (10). The culture supernatant was assessed for p24 content at 24 h.

Inhibition of HIV-1 by ALX40-4C. PBMC or HUT 78 cells were pretreated with or without various concentrations of ALX40-4C for 24 h prior to infection. A portion of the cells (10^6) was removed for PCR analysis (see below). The remaining cells were maintained in the same conditions used for pretreatment, with medium changes every 3 to 4 days.

Analysis of HIV-1-infected cell DNA by PCR. Twenty-four hours after infection, cells were washed once in phosphate-buffered saline and lysed in nonionic detergents and proteinase K as described previously (46). The samples were heated for 1 h at 60°C and then for 10 min at 95°C to inactivate the proteinase K. Each sample (2×10^5 cell equivalents) was subjected to amplification by PCR as previously described (39, 46), with 25 cycles of denaturation for 1 min at 94°C and annealing and extension for 2 min at 65°C. The HIV-1 primer pair M667-AA55 (66) was used for amplification. Amplified products were resolved by electrophoresis on an 8% acrylamide gel and analyzed directly by autoradiography. Quantitation was achieved by comparison with dilutions of cloned HIV-1 JR-CSF as previously described (41, 66).

RESULTS

Inhibition of NL4-3 replication by ALX40-4C. We assessed the ability of ALX40-4C-treated cells to support replication of HIV-1 NL4-3, a well-characterized, T-cell line-adapted strain. In HUT 78 cells, pretreatment for 24 h prior to infection and continuously thereafter resulted in marked reductions in virus replication at concentrations as low as 0.1 μM (Fig. 1A). Inhibition was greater at 1 μM, and up to a 10^4-fold reduction was seen in most experiments at 10 μM. Similar results were seen in PBMC (Fig. 1B) and with 1-h pretreatment of cells with ALX40-4C (data not shown). In these experiments, the 50% inhibitory dose at day 7 was approximately 0.003 μM. HIV-1 replication was completely abrogated at 10 μM ALX40-4C and did not return following removal of the inhibitor from cultures (Fig. 2). There was no difference in viable cell number in uninfected PBMC or HUT 78 cells cultured for 1 week in the absence or presence of ALX40-4C at concentrations of up to 10 μM. By using trypan blue exclusion and a range of ALX40-4C concentrations, cell cytotoxicity 50% was shown to be approximately 50 μM for PBMC and a variety of transformed cell lines (56a). In contrast, virus replication typically returns following removal of zidovudine from cultures (13). This may reflect the greater potency of ALX40-4C than of zidovudine, which has a 50% inhibitory dose of 0.1 μM for sensitive HIV-1 strains (35).

Some primary strains are resistant to inhibition by ALX40-4C. To determine whether ALX40-4C sensitivity is specific for NL4-3, we assessed the effect of ALX40-4C treatment on infection with primary HIV-1 isolates. Viral properties of strains selected for growth in transformed T-cell lines, such as NL4-3, may not adequately represent characteristics of primary strains obtained directly from patients. Differences in virus sensitivity to therapies for primary and T-cell line-adapted HIV-1 strains have been seen previously, most notably for soluble CD4 treatment, resulting in poor clinical efficacy (15, 53). In this case, sensitivity was found to be much greater for T-cell line-adapted strains than for primary strains, requiring at least 100-fold-lower concentrations of soluble CD4 to achieve comparable levels of neutralization. We therefore tested the efficacy of ALX40-4C inhibition on the molecularly cloned primary strains, JR-FL and JR-CSF. Although at early time points following infection of ALX40-4C-treated PBMC there was a modest reduction in virus production, this inhibition was not sustained, and virus production at days 10 and 14 postinfection was similar to that seen following infection of untreated cells (Fig. 3). To determine whether all primary strains demonstrated the diminished sensitivity to ALX40-4C inhibition seen with JR-FL and JR-CSF, we measured virus production following coculture of lymphocytes from patients in the presence or absence of ALX40-4C. Although the virus strains from two of these patients (patients 2 and 3) were somewhat resistant to ALX40-4C inhibition, another primary strain (from patient 1) was found to be as sensitive as NL4-3 to ALX40-4C. In contrast, essentially all primary HIV-1 strains exhibit soluble CD4 resistance (15, 21).

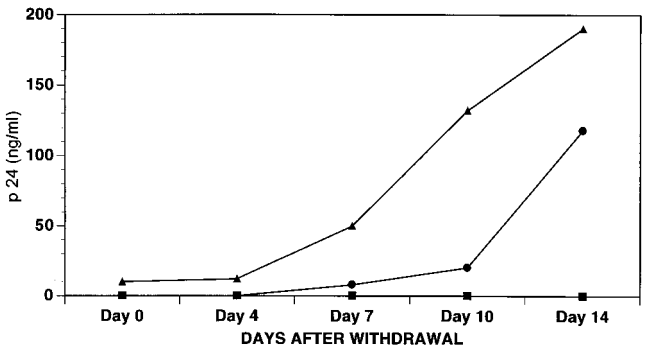

FIG. 2. Replication of NL4-3 in HUT 78 cells following withdrawal of ALX40-4C 1 day after infection. ■, 10 μM ALX40-4C; ●, 1 μM zidovudine; ▲, untreated.

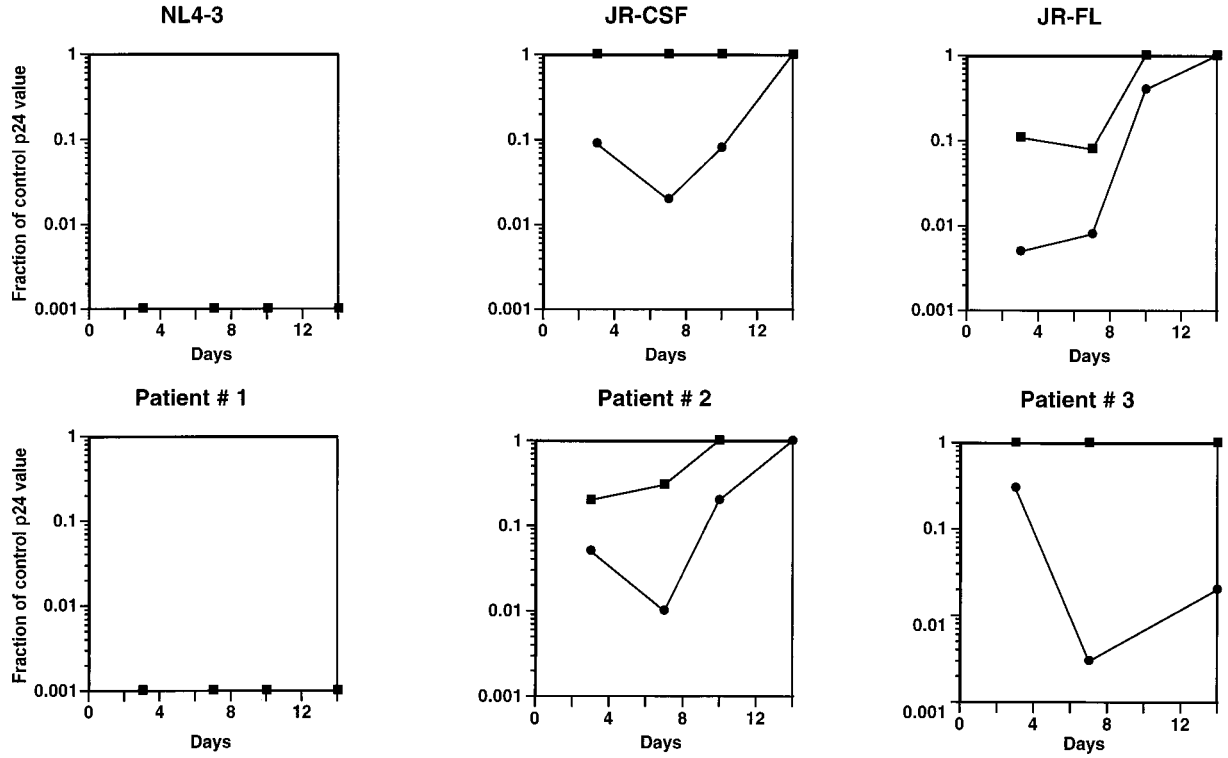

FIG. 3. Inhibition of molecularly cloned and primary HIV-1 strains recovered by PBMC coculture following 24 h of pretreatment of PBMC with ALX40-4C at 1 μM (■) and 10 μM (●) in PBL, expressed as a fraction of HIV-1 extracellular p24 production in untreated cells.

Resistance to ALX40-4C inhibition occurs at early steps in HIV replication, which include virus entry. If inhibition by ALX40-4C was mediated exclusively by interference with Tat-TAR interactions, we would expect to see comparable levels of new viral DNA formation by reverse transcription following virus entry, in the absence of viral spread. The effects of the Tat inhibition would result in diminished levels of HIV-1-specific RNA levels only as a result of interference with transactivation. To test this hypothesis, we analyzed viral DNA formation in a quantitative PCR assay 24 h after infection. Surprisingly, inhibition of virus replication for NL4-3 or for the strain from patient 1 was accompanied by corresponding decreases in new viral DNA formation (Fig. 4). As expected, viral DNA formation was not affected by ALX40-4C treatment in resistant strains. This finding suggests that for NL4-3 and the primary strain sensitive to ALX40-4C, the mechanism of inhibition appeared to be at virus entry, prior to reverse transcription.

To further examine the mechanism of ALX40-4C inhibition, we bypassed entry by transfecting HUT 78 cells with full-length proviral clones. Modest three- to fivefold decreases in virus production were seen in cells pretreated with ALX40-4C for 1 day prior to electroporation. This decrease is consistent with a modest inhibition of transactivation and is less than the decrease in virus replication exhibited when strains are blocked at entry (Table 1).

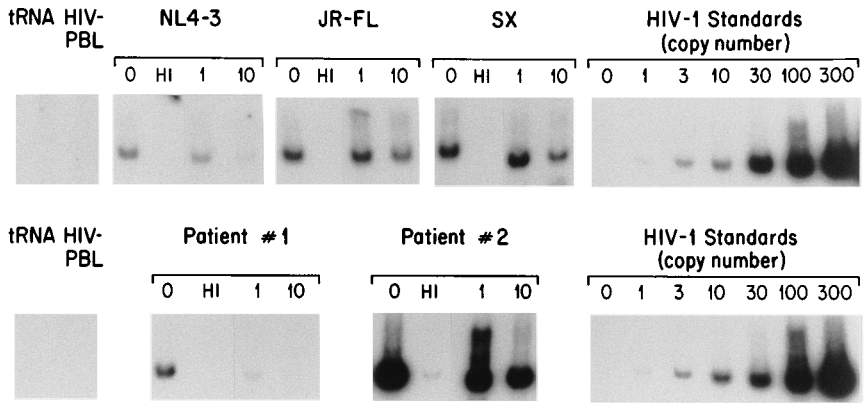

FIG. 4. Viral DNA formation by PCR 24 h after infection with NL4-3, JR-FL, SX, or two primary strains recovered from patient blood. Viruses from patients 1 and 2 are the same as those shown in Fig. 3. PBMC were cultured in the absence (lanes 0) or presence of 1 μM (lanes 1) or 10 μM (lanes 10) ALX40-4C for 24 h prior to infection. Heat-activated (HI) (60°C for 1 h) virus supernatants were used as controls for input viral DNA removal by DNase treatment.

TABLE 1. Virus production by HUT 78 cells cultured in the presence or absence of 5 μM ALX40-4C for 24 h prior to electroporation with 25 μg of proviral DNA

HIV-1 strain	p24 production (pg/ml)	
	ALX40-4C-treated cells	Untreated cells
NL4-3	211	715
NFN-SM	104	858
JR-CSF	215	998

Genetic determinants of ALX40-4C resistance. To identify viral genetic determinants for sensitivity to ALX40-4C inhibition, recombinant virus strains generated between HIV-1 NL4-3 and HIV-1 JR-FL were used. Chimeras between these strains (SX and SM) have previously been used to map macrophage tropism and soluble CD4 resistance (39, 41).

The V3 region of HIV-1 gp120 has been shown to be crucial for macrophage tropism, strain-specific antibody neutralization, and syncytium induction (16, 25, 41, 50, 55, 62). Syncytium-inducing (SI) strains emerge in some patients during the course of disease and are believed to be more pathogenic, resulting in greater T-cell killing (14, 27, 47, 54, 59), although this is controversial (36, 65). These strains are identified by the ability to replicate in and cause fusion between MT-2 cells, a transformed T-cell line. Virus strains cloned following prolonged propagation in T-cell lines, such as NL4-3, are typically SI and replicate poorly in mononuclear phagocytes. In contrast, primary strains cloned after short-term culture in PBL, such as JR-CSF and JR-FL, are often non-SI (NSI) and typically replicate poorly in T-cell lines such as HUT 78. Although variable, most NSI strains replicate to some extent in mononuclear phagocytes. The biochemical basis of the SI phenotype seems to be related to a lower virion envelope density and lower retention of the extracellular envelope glycoprotein, gp120 (42, 63). These biochemical properties and corresponding phenotypes have been mapped to sequences in the V3 region (16, 17, 63).

As shown above, ALX40-4C is particularly effective against NL4-3 but less effective against JR-FL and JR-CSF. For these studies, we generated chimeric strains having substitutions of only the region encoding the 33-amino-acid V3 loop, with or without larger contributions from downstream envelope sequences (Fig. 5). These strains replicate in mononuclear phagocytes with only slightly less efficiency than strains with

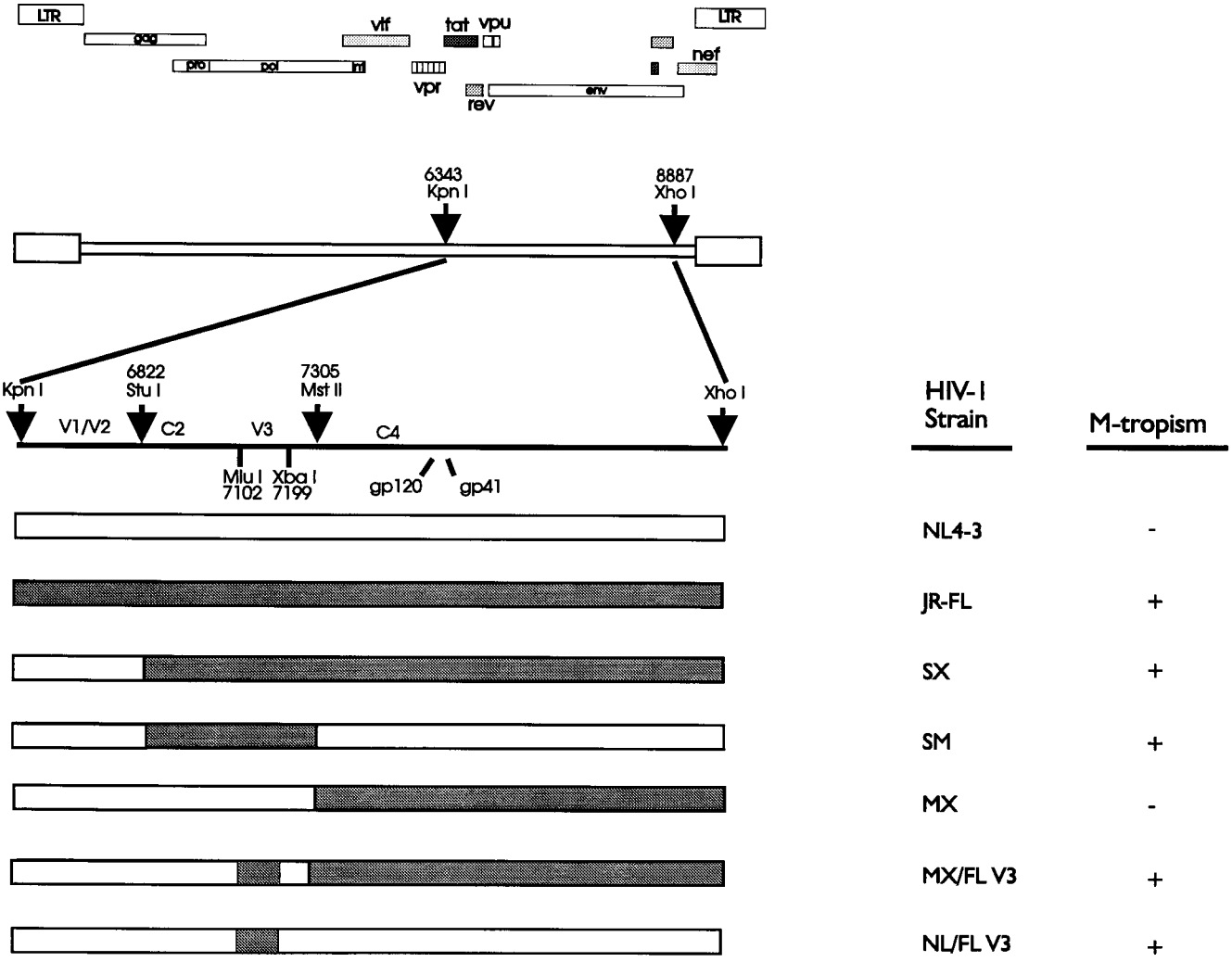

FIG. 5. Schematic depiction of HIV-1 recombinant virus strains. LTR, long terminal repeat.

TABLE 2. Replication of parental and V3 recombinants in PBL (day 7) and in MP and HUT 78 cells (day 14)

HIV strain	p24 production (ng/ml)					
	Expt 1			Expt 2		
	PBL	MP	HUT 78	PBL	MP	HUT 78
JR-FL	34	18	0	180	35	0
NL4-3	258	0.7	199	240	0.9	86
NL-FLV3	31	4.8	0	210	14	0
MX-FLV3	64	5.3	0	190	16	0

larger substitutions of the JR-FL envelope (Table 2). The contrast in replication was greater in the T-cell line HUT 78, in which case there was nearly complete restriction to productive infection for chimeric strains containing JR-FL V3 sequences. Therefore, we have confirmed the importance of the V3 loop in conferring the macrophage-tropic phenotype (12, 25), in this case using JR-FL V3 sequences in the background of NL4-3.

Using quantitative PCR analysis 24 h following infection, we found that infection with recombinant HIV-1 strains unable to replicate efficiently in mononuclear phagocytes was associated with decreased levels of new viral DNA (data not shown). Thus, the restriction to replication for the V3 chimeras was blocked for early events, which include virus entry into the cell. These strains were then used in inhibition assays with ALX40-4C to determine whether resistance to ALX40-4C inhibition was determined by these domains. As shown in Fig. 6, resistance to ALX40-4C in PBL is conferred by the V3 region alone, with results similar to those seen with JR-FL or with chimeric strains having larger substitutions of JR-FL envelope. Thus, ALX40-4C appears to interfere with interactions which involve the V3 region and target cells. Previous studies have shown that these viral determinants are important for virus entry and neutralization (39, 41, 50).

DISCUSSION

In this report, we demonstrate HIV-1 strain-dependent inhibition of HIV-1 replication by pretreatment of target cells with the cationic compound ALX40-4C. The cationic compound ALX40-4C effectively inhibits replication of T-cell line-adapted strains and some primary strains but does not block macrophage-tropic strains. Although this compound was developed as an inhibitor of the Tat-TAR interaction, unexpectedly, we found that strain-dependent differences in sensitivity mapped to the V3 region and were blocked at early events in HIV-1 replication. The mapping of ALX40-4C sensitivity to the V3 region supports the concept that inhibition is exerted at the level of entry, not at other phases of virus replication. Our results demonstrate the importance of HIV-1 gp120 V3 interactions for entry into target cells and suggest that the high net positive charge in V3 found in most SI strains is important for HIV-1 entry into transformed T-cell lines (as well as PBL) and impairs entry into mononuclear phagocytes.

The HIV-1 envelope protein is crucial for initiation of infection and mediates both binding to the surface of cells that express CD4 and fusion of the virus and plasma membranes. In particular, the V3 region of gp120 has been shown to be responsible for differences in the efficiency of entry into mononuclear phagocytes for various HIV-1 strains (11, 25, 41, 55, 62). Virus strains selected for efficient entry into transformed T-cell lines by prolonged propagation in culture generally are inefficient at entering mononuclear phagocytes. A characteristic of the V3 region of these T-cell line-adapted HIV-1 strains is a relatively high net positive amino acid charge, averaging +8 to +9, compared with that seen in primary, highly macrophage-tropic strains, which have a net positive charge in the V3 region of +3 to +4. Since both macrophage-tropic and T-cell line-adapted HIV-1 strains require interaction with CD4, it is believed that other interactions in addition to CD4 binding are required for virus and plasma membrane fusion and virus entry. HIV-1 envelope interactions which involve CD4 precede entry during productive infection of both mononuclear phagocytes and $CD4^+$ lymphocytes. CD4 alone is not sufficient, however, since transfection of CD4 into CD4-negative cell lines does not universally confer susceptibility to infection. Following binding of HIV-1 gp120 to CD4, there are conformational changes in gp120 which result in exposure of previously occult epitopes, including V3 (32, 52). In some cases, monoclonal antibodies directed to these novel epitopes can inhibit syncytium formation (20, 26, 52). Since the V3 region is not specifically involved in binding to CD4 (29, 43, 60), it is possible that V3 is involved in post-CD4 binding target cell interactions.

Domain 1 of CD4 contains an immunoglobulin-like, CDR2-like motif which contains all of the determinants necessary for binding to gp120 (2). Conformation-dependent V3 interactions with the CDR3 region of CD4 domain 1 were considered to play a role in membrane fusion preceding virus entry (9, 38), but studies involving extensive mutagenesis of the CDR3 region suggest that this is unlikely (7). Of note, CDR3 has a net negative charge; peptides and monoclonal antibodies directed to this region can interfere with fusion, but this inhibition may be nonspecific, as judged from charge.

Sulfated polyanionic saccharides, such as dextran sulfate, were shown to be potent inhibitors of replication of T-cell line-adapted strains in vitro (4, 34). Since dextran sulfate interfered with binding of V3 loop monoclonal antibodies (5, 8, 23, 33), it was proposed that this class of compounds interfered with V3-mediated interactions associated with fusion. In fact, dextran sulfate was shown to inhibit HIV-1 replication without interfering with gp120-CD4 binding (8), suggesting that interference was restricted to post-CD4 binding events. In some experimental systems, polyanions also disrupted gp120-CD4 binding (30, 31), but post-CD4 binding conformational changes can increase the block to V3-specific monoclonal antibodies (23). It seems likely that polyanionic saccharides block HIV-1 replication of T-cell line-adapted strains by competitively blocking the interaction with negatively charged cell surface receptors, specifically by binding to V3. Macrophage-tropic strains are resistant to inhibition by these compounds (30),

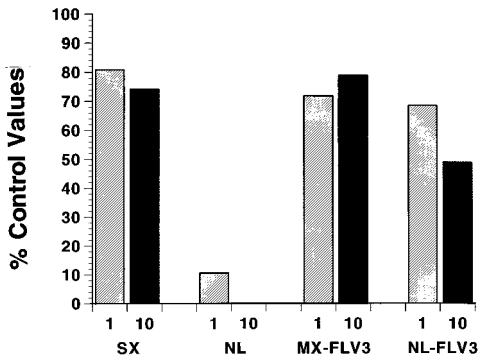

FIG. 6. Inhibition of recombinant HIV-1 strains by ALX40-4C in PBL at day 7, expressed as the fraction of p24 production in cells treated with 1 μM (bars 1) or 10 μM (bars 10) ALX40-4C over that in untreated cells for each virus strain.

suggesting that the auxiliary receptor on macrophages is less negatively charged or that the V3 domain of macrophage-tropic strains is bound less well by the polyanionic compounds.

The finding that a polycationic peptide can efficiently block infection of an SI strain but not that of a macrophage-tropic NSI strain supports the premise that the ability of V3 to interact with its target is charge dependent. Moreover, the secondary target cell interaction may involve distinct cell surface molecules for different HIV-1 subtypes. Since the side chains of ALX40-4C have a net charge of +9, it is possible that this compound competes with the V3 loop for binding to its receptor target on the cell surface. Indeed, our proposal is that the second receptor on T-cell lines is negatively charged. Others have proposed that this receptor is cell surface heparan sulfate (44). Macrophage-tropic strains, which do not enter transformed T cells, are unable to interact with this second receptor because of the lower net V3 loop charge for these strains. In PBMC, macrophage-tropic strains are partially inhibited by ALX40-4C but break through because the inhibition is weak and the virus is able to spread through the culture. Our PCR data support the premise that ALX40-4C-resistant strains overcome the block at virus entry. The failure to efficiently inhibit entry of macrophage-tropic strains suggests that ALX40-4C does not interact with the secondary target cell receptor utilized by JR-FL and other NSI strains or at least competes less well with the V3 of those strains for binding to the receptor.

ALX40-4C readily penetrates cells and also can interfere with Tat-TAR interactions (57). Despite biochemical evidence for a potent effect on this interaction, our transfection data suggest that this effect on HIV-1 replication is weaker than the effect mediated at entry for NL4-3. Notably, this compound has also been shown to block herpesvirus infection at the level of entry (58). The ability to effectively inhibit entry of SI strains, which are associated with increased pathogenicity, suggests that this compound may have an important role in combination antiretroviral therapy. Development of new compounds which have more effect on post-CD4 binding interactions of macrophage-tropic strains may ultimately demonstrate greater clinical utility. This class of compounds may also prove to be useful reagents to identify the second receptor on primary cells and lead to development of new strategies to prevent HIV-1 entry.

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REFERENCES

1. **Adachi, A., E. Gendelman, S. Koenig, T. Folks, R. Willey, A. Rabson, and M. A. Martin.** 1986. Production of acquired immunodeficiency syndrome-associated retrovirus in human and non-human cells transfected with an infectious molecular clone. J. Virol. **59:**284–291.
2. **Arthos, J., K. C. Deen, M. A. Chaikin, J. A. Fornwald, G. Sathe, Q. J. Sattentau, P. R. Clapham, R. A. Weiss, J. S. McDougal, C. Pietropaolo, R. Axel, A. Truneh, P. J. Maddon, and R. W. Sweet.** 1989. Identification of the residues in human CD4 critical for the binding of HIV. Cell **57:**469–481.
3. **Arya, S. K., C. Guo, S. F. Josephs, and F. Wong-Staal.** 1985. Trans-activator gene of human T-lymphotropic virus type III (HTLV-III). Science **229:**69–73.
4. **Baba, M., R. Pauwels, J. Balzarini, J. Arnout, J. Desmyter, and E. DeClercq.** 1988. Mechanism of the inhibitory effect of dextran sulfate and heparin on replication of human immunodeficiency virus *in vitro*. Proc. Natl. Acad. Sci. USA **85:**6132–6136.
5. **Batinic, D., and F. A. Robey.** 1992. The V3 region of the envelope glycoprotein of human immunodeficiency virus type 1 binds sulfated polysaccharides and CD4-derived synthetic peptides. J. Biol. Chem. **267:**6664–6671.
6. **Berkhout, B., and K.-T. Jeang.** 1989. *trans* activation of human immunodeficiency virus type 1 is sequence specific for both the single-stranded bulge and loop of the *trans*-acting-responsive hairpin: a quantitative analysis. J. Virol. **63:**5501–5504.
7. **Brand, D., K. Srinivasan, and J. Sodroski.** 1995. Determinants of human immunodeficiency virus type 1 entry in the CDR2 loop of the CD4 glycoprotein. J. Virol. **69:**166–171.
8. **Callahan, L. N., M. Phelan, M. Mallinson, and M. A. Norcross.** 1991. Dextran sulfate blocks antibody binding to the principal neutralizing domain of human immunodeficiency virus type 1 without interfering with gp120-CD4 interactions. J. Virol. **65:**1543–1550.
9. **Camerini, D., and B. Seed.** 1990. A CD4 domain important for HIV-mediated syncytium formation lies outside the principal virus binding site. Cell **60:**747–754.
10. **Cann, A. J., Y. Koyanagi, and I. S. Y. Chen.** 1988. High efficiency transfection of primary human lymphocytes and studies of gene expression. Oncogene **3:**123–128.
11. **Chesebro, B., J. Nishio, S. Perryman, A. Cann, W. O'Brien, I. S. Y. Chen, and K. Wehrly.** 1991. Identification of human immunodeficiency virus envelope gene sequences influencing viral entry into CD4-positive HeLa cells, T-cell leukemia cells, and macrophages. J. Virol. **65:**5782–5789.
12. **Chesebro, B., K. Wehrly, J. Nishio, and S. Perryman.** 1992. Macrophage-tropic human immunodeficiency virus isolates from different patients exhibit unusual V3 envelope sequence homogeneity in comparison with T-cell-tropic isolates: definition of critical amino acids involved in cell tropism. J. Virol. **66:**6547–6554.
13. **Chow, Y.-K., M. S. Hirsch, D. P. Merrill, L. J. Bechtel, J. J. Eron, J. C. Kaplan, and R. T. D'Aquila.** 1993. Use of evolutionary limitations of HIV-1 multidrug resistance to optimize therapy. Nature (London) **361:**650–654.
14. **Connor, R. I., H. Mohri, Y. Cao, and D. D. Ho.** 1993. Increases viral burden and cytopathicity correlate temporally with $CD4^+$ T-lymphocyte decline and clinical progression in human immunodeficiency virus type 1-infected individuals. J. Virol. **67:**1772–1777.
15. **Daar, E. S., X. L. Li, T. Moudgil, and D. D. Ho.** 1990. High concentrations of recombinant soluble CD4 are required to neutralize primary HIV-1 isolates. Proc. Natl. Acad. Sci. USA **87:**6574–6578.
16. **de Jong, J.-J., A. de Ronde, W. Keulen, M. Tersmette, and J. Goudsmit.** 1992. Minimal requirements for the human immunodeficiency virus type 1 V3 domain to support the syncytium-inducing (SI) phenotype: analysis by single amino acid substitution. J. Virol. **66:**6777–6780.
17. **de Jong, J.-J., J. Goudsmit, W. Keulen, B. Klaver, W. Krone, M. Tersmette, and A. deRonde.** 1992. Human immunodeficiency virus type 1 clones chimeric for the envelope V3 domain differ in syncytium formation and replication capacity. J. Virol. **66:**757–765.
18. **Garcia, J. V., and A. D. Miller.** 1991. Serine phosphorylation independent downregulation of cell-surface CD4 by nef. Nature (London) **350:**508–511.
19. **Gazdar, A. F., D. N. Carney, P. A. Bunn, E. K. Russell, E. S. Jaffe, G. P. Schecter, and J. G. Guccion.** 1980. Mitogen requirements for the in vitro propagation of cutaneous T-cell lymphomas. Blood **55:**409–417.
20. **Gershoni, J. M., G. Denisova, D. Raviv, N. I. Smorodinsky, and D. Buyaner.** 1993. HIV binding to its receptor creates specific epitopes for the CD4/gp120 complex. FASEB J. **7:**1185–1187.
21. **Groenink, M., J. P. Moore, S. Broersen, and H. Schuitemaker.** 1995. Equal levels of gp120 retention and neutralization resistance of phenotypically distinct primary human immunodeficiency virus type 1 variants upon soluble CD4 treatment. J. Virol. **69:**523–527.
22. **Harrich, D., C. Hsu, E. Race, and R. B. Gaynor.** 1994. Differential growth kinetics are exhibited by human immunodeficiency virus type 1 TAR mutants. J. Virol. **68:**5899–5910.
23. **Harrop, H. A., D. R. Coombe, and C. C. Rider.** 1994. Heparin specifically inhibits binding of V3 loop antibodies to HIV-1. AIDS **8:**183–192.
24. **Ho, D. D., A. U. Neumann, A. S. Perelson, W. Chen, J. M. Leonard, and M. Markowitz.** 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature (London) **373:**123–126.
25. **Hwang, S. S., T. J. Boyle, H. K. Lyerly, and B. R. Cullen.** 1991. Identification of envelope V3 loop as the primary determinant of cell tropism in HIV-1. Science **253:**71–74.
26. **Kang, C.-Y., K. Hariharan, P. L. Nara, J. Sodroski, and J. P. Moore.** 1994. Immunization with a soluble CD4-gp120 complex preferentially induces neutralizing anti-human immunodeficiency virus type 1 antibodies directed to conformation-dependent epitopes of gp120. J. Virol. **68:**5854–5862.
27. **Koot, M., I. P. M. Keet, H. V. Vos, R. E. Y. DeGoede, M. T. L. Roos, R. A. Coutinho, F. Miedema, P. T. A. Schellekens, and M. Tersmette.** 1993. Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4+ cell depletion and progression to AIDS. Ann. Intern. Med. **118:**681–688.
28. **Koyanagi, Y., S. Miles, R. T. Mitsuyasu, J. E. Merryl, H. V. Vinters, and I. S. Y. Chen.** 1987. Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms. Science **236:**819–822.
29. **Lasky, L. A., G. Nakamura, D. H. Smith, C. Fennie, C. Shimasaki, E. Patzer, P. Berman, T. Gregory, and D. J. Capon.** 1987. Delineation of a region of the

human immunodeficiency virus type 1 gp120 glycoprotein critical for interaction with the CD4 receptor. Cell **50:**975–985.
30. **Lynch, G., L. Low, S. Li, A. Sloane, S. Adams, C. Parish, B. Kemp, and A. L. Cunningham.** 1994. Sulfated polyanions prevent HIV infection of lymphocytes but do not inhibit monocyte infection. J. Leukocyte Biol. **56:**266–272.
31. **McClure, M. O., J. P. Moore, D. F. Blanc, P. Scotting, G. M. Cook, R. J. Keynes, J. N. Weber, D. Davies, and R. A. Weiss.** 1992. Investigations into the mechanism by which sulfated polysaccharides inhibit HIV infection in vitro. AIDS Res. Hum. Retroviruses **8:**19–26.
32. **McKeating, J. A., J. Cordell, C. J. Dean, and P. Balfe.** 1992. Synergistic interaction between ligands binding to the CD4 binding site and V3 domain of human immunodeficiency virus type 1 gp120. Virology **191:**732–742.
33. **Meshcheryakova, D., S. Andreev, S. Tarasova, M. Sidorava, M. Vafina, G. Kornilaeva, E. Karamov, and R. Khaitov.** 1993. CD4-derived peptide and sulfated polysaccharides have similar mechanisms of anti-HIV activity based on electrostatic interactions with positively charged gp120 fragments. Mol. Immunol. **30:**993–1001.
34. **Mitsuya, H., D. J. Looney, S. Kuno, R. Ueno, F. Wong-Staal, and S. Broder.** 1988. Dextran sulfate suppression of viruses in the HIV family: inhibition of virus binding to CD4+ cells. Science **240:**646–649.
35. **Mitsuya, H., K. J. Weinhold, P. A. Furman, et al.** 1985. 3′-Azido-3′-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus in vitro. Proc. Natl. Acad. Sci. USA **82:**7096–7100.
36. **Mosier, D. E., R. J. Gulizia, P. D. MacIsaac, B. E. Torbett, and J. A. Levy.** 1993. Rapid loss of CD4+ T cells in human-PBL-SCID mice by noncytopathic HIV isolates. Science **260:**689–692.
37. **Muller, W. E. G., T. Okamoto, P. Reuter, D. Ugarkovic, and H. C. Schroder.** 1990. Functional characterization of Tat protein from human immunodeficiency virus. J. Biol. Chem. **265:**3803–3808.
38. **Nara, P. L., K. M. Hwang, D. M. Rausch, J. D. Lifson, and L. E. Eiden.** 1989. CD4 antigen-based antireceptor peptides inhibit infectivity of human immunodeficiency virus in vitro at multiple stages of the viral life cycle. Proc. Natl. Acad. Sci. USA **86:**7139–7143.
39. **O'Brien, W. A., I. S. Y. Chen, D. D. Ho, and E. S. Daar.** 1992. Human immunodeficiency virus type 1 resistance to soluble CD4 is conferred by domains of gp120 that determine cell tropism. J. Virol. **66:**3125–3130.
40. **O'Brien, W. A., K. Grovit-Ferbas, A. Namazi, S. Ovcak-Derzic, H.-J. Wang, J. Park, C. Yeramian, S.-H. Mao, and J. A. Zack.** 1995. HIV-1 replication can be increased in peripheral blood of seropositive patients following influenza vaccination. Blood **86:**1082–1089.
41. **O'Brien, W. A., Y. Koyanagi, A. Namazie, J.-Q. Zhao, A. Diagne, K. Idler, J. A. Zack, and I. S. Y. Chen.** 1990. HIV-1 tropism for mononuclear phagocytes can be determined by regions of gp120 outside the CD4-binding domain. Nature (London) **348:**69–73.
42. **O'Brien, W. A., A. Namazi, S.-H. Mao, H. Kalhor, J. A. Zack, and I. S. Y. Chen.** 1994. Kinetics of human immunodeficiency virus type 1 reverse transcription in blood mononuclear phagocytes are slowed by limitations of nucleotide precursors. J. Virol. **68:**1258–1263.
43. **Olshevsky, U., E. Helseth, C. Furman, J. Li, W. Haseltine, and J. Sodroski.** 1990. Identification of individual human immunodeficiency virus type 1 gp120 amino acids important for CD4 receptor binding. J. Virol. **64:**5701–5707.
44. **Patel, M., M. Yanagishita, G. Roderiquez, D. C. Bou-Habib, T. Oravecz, V. C. Hascall, and M. A. Norcross.** 1993. Cell-surface heparan sulfate proteoglycan mediates HIV-1 infection of T-cell lines. AIDS Res. Hum. Retroviruses **9:**167–174.
45. **Piatak, M., M. S. Saag, L. C. Yang, S. J. Clark, J. C. Kappes, K.-C. Luk, B. H. Hahn, G. M. Shaw, and J. D. Lifson.** 1993. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science **259:**1749–1754.
46. **Rich, E. A., I. S. Y. Chen, J. A. Zack, M. L. Leonard, and W. A. O'Brien.** 1992. Increased susceptibility of differentiated mononuclear phagocytes to productive infection with human immunodeficiency virus expression. Proc. Natl. Acad. Sci. USA **87:**176–183.
47. **Richman, D. D., D. Havlir, J. Corbeil, D. Looney, C. Ignacio, S. A. Spector, J. Sullivan, S. Cheeseman, K. Barringer, and D. Pauletti.** 1994. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. J. Virol. **68:**1660–1666.
48. **Rosen, C. A., J. G. Sodroski, and W. A. Haseltine.** 1985. The location of cis-acting regulatory sequences in the human T cell lymphotropic virus type III (HTLV-III/LAV) long terminal repeat. Cell **41:**813–823.
49. **Roy, S., N. T. Parkin, C. Rosen, J. Itovitch, and N. Sonenberg.** 1990. Structural requirements for *trans* activation of human immunodeficiency virus type 1 long terminal repeat-directed gene expression by *tat*: importance of base pairing, loop sequence, and bulges in the *tat*-responsive sequence. J. Virol. **64:**1402–1406.
50. **Rusche, J. R., K. Javaherian, C. McDanal, J. Petro, D. L. Lynn, R. Grimaila, A. J. Langlois, R. C. Gallo, L. O. Arthur, P. J. Fischinger, D. P. Bolognesi, S. D. Putney, and T. J. Matthews.** 1988. Antibodies that inhibit fusion of human immunodeficiency virus-infected cells and a 24-amino acid sequence of the viral envelope, gp120. Proc. Natl. Acad. Sci. USA **84:**6924–6928.
51. **Saag, M. S., E. A. Emini, O. L. Laskin, J. Douglas, W. I. Lapidus, W. A. Schleif, R. J. Whitley, C. Hildebrand, V. W. Byrnes, and J. C. Kappes.** 1993. A short-term clinical evaluation of L-697,661, a non-nucleoside inhibitor of HIV-1 reverse transcriptase. L-697,661 Working Group. N. Engl. J. Med. **329:**1065–1072.
52. **Sattentau, Q. J., and J. P. Moore.** 1991. Conformational changes induced in the human immunodeficiency virus envelope glycoprotein by soluble CD4 binding. J. Exp. Med. **174:**407–415.
53. **Schooley, R. T., T. C. Merigan, P. Gaut, M. S. Hirsch, M. Holodniy, T. Flynn, S. Liu, R. E. Byington, S. Henochowicz, E. Gubish, D. Springgs, D. Kufe, J. Schindler, A. Dawson, D. Thomas, D. L. Hanson, B. T. Liu, J. Gulinello, S. Kennedy, R. Fisher, and D. D. Ho.** 1990. A phase I/II escalating dose trial of recombinant soluble CD4 therapy in patients with AIDS or AIDS-related complex. Ann. Intern. Med. **112:**247–253.
54. **Schuitemaker, H., M. Koot, N. A. Kootstra, M. W. Dercksen, R. E. Y. DeGoede, R. P. Van Steenwijk, J. M. A. Lange, S. Eeftink, J. K. M., F. Miedema, and M. Tersmette.** 1992. Biological phenotype of human immunodeficiency virus type 1 clones at different stages of infection: progression of disease is associated with a shift from monocytotropic to T-cell-tropic virus populations. J. Virol. **66:**1354–1360.
55. **Shioda, T., J. A. Levy, and C. Cheng-Mayer.** 1991. Macrophage and T cell-line tropisms of HIV-1 are determined by specific regions of the envelope gp120 gene. Nature (London) **349:**167–169.
56. **Sodroski, J., R. Patarca, C. Rosen, F. Wong-Staal, and W. Haseltine.** 1985. Location of the trans-activating region of the genome of human T-cell lymphotropic virus type III. Science **229:**74–77.
56a. **Sumner-Smith, M.** Unpublished data.
57. **Sumner-Smith, M., E. D. Blair, B. Dabek, W. Lin, P. Yungblut, E. M. Twist, and S. C. Climie.** Arginine-rich oligopeptides inhibit HIV-1 TAR-dependent transactivation. Submitted for publication.
58. **Sumner-Smith, M., Y. Zheng, Y. P. Zhang, E. M. Twist, and S. C. Climie.** 1995. Antiherpetic activities of N-α-acetyl-nona-d-arginine amide acetate. Drugs Exp. Clin. Res. **21:**1–6.
59. **Tersmette, M., R. A. Gruters, F. de Wolf, R. E. Y. de Goede, J. M. A. Lange, P. T. A. Schellekens, J. Goudsmit, H. G. Huisman, and F. Miedema.** 1989. Evidence for a role of virulent human immunodeficiency virus (HIV) variants in the pathogenesis of acquired immunodeficiency syndrome: studies on sequential HIV isolates. J. Virol. **63:**2118–2125.
60. **Thali, M., C. Furman, D. D. Ho, J. Robinson, S. Tilley, A. Pinter, and J. Sodroski.** 1992. Discontinuous, conserved neutralization epitopes overlapping the CD4-binding region of human immunodeficiency virus type 1 gp120 envelope glycoprotein. J. Virol. **66:**5635–5641.
61. **Wei, X., S. K. Ghosh, M. E. Taylor, V. A. Johnson, E. A. Emini, P. Deutsch, J. D. Lifson, S. Bonhoeffer, M. A. Nowak, B. A. Hahn, M. S. Saag, and G. M. Shaw.** 1995. Viral dynamics in human immunodeficiency virus type 1 infection. Nature (London) **373:**117–122.
62. **Westervelt, P., H. E. Gendelman, and L. Ratner.** 1991. Identification of a determinant within the HIV-1 surface envelope glycoprotein critical for productive infection of cultured primary monocytes. Proc. Natl. Acad. Sci. USA **88:**3097–3101.
63. **Willey, R. L., T. S. Theodore, and M. A. Martin.** 1994. Amino acid substitutions in the human immunodeficiency virus type 1 gp120 V3 loop that change viral tropism also alter physical and functional properties of the virion envelope. J. Virol. **68:**4409–4419.
64. **Wu, F., J. Garcia, D. Sigman, and R. Gaynor.** 1991. tat regulates binding of the human immunodeficiency virus trans-activating region RNA loop-binding protein TRP-185. Genes Dev. **5:**2128–2140.
65. **Yu, X., M. F. McLane, L. Ratner, W. A. O'Brien, R. Collman, M. Essex, and T. Lee.** Direct killing of primary T-cells by HIV-1. Proc. Natl. Acad. Sci. USA, in press.
66. **Zack, J. A., S. J. Arrigo, S. R. Weitsman, A. S. Go, A. Haislip, and I. S. Y. Chen.** 1990. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. Cell **61:**213–222.