Susceptibility of HIV Type 1 to the Fusion Inhibitor T-20 Is Reduced on Insertion of Host Intercellular Adhesion Molecule 1 in the Virus Membrane

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**Background.** T-20 is a synthetic peptide that targets and blocks the entry of human immunodeficiency virus (HIV)–1 inside target cells. Data from clinical studies have shown that primary HIV-1 variants display a broad range of susceptibilities to T-20 that are mainly caused by mutations in the N-terminal heptad repeat of gp41 and by other determinants, such as envelope and coreceptor affinity, coreceptor specificity, and receptor density. We investigated whether sensitivity to this drug is affected by the incorporation of intercellular adhesion molecule (ICAM)–1 in HIV-1.

**Methods.** We used laboratory isolates of HIV-1 (X4- and R5-tropic) either lacking or bearing ICAM-1 and clinical variants.

**Results.** We demonstrate that ICAM-1–bearing virions are more resistant to T-20 than are isogenic HIV-1 particles without it. This effect is lost when ICAM-1/leukocyte function antigen–1 interactions are blocked by antibody. The mechanism through which virus-anchored host ICAM-1 is acting seems to be a reduction of the kinetic window during which the viral envelope is sensitive to T-20.

**Conclusions.** Altogether, these results demonstrate that the insertion of host-derived ICAM-1 into HIV-1 represents another factor that affects virus sensitivity to the entry inhibitor. These findings have potential implications for the therapeutic use of T-20.
sponsible for the differences in HIV-1 sensitivity to T-20. Virus strains bearing a mutation in the HR1 sequence that results in a modification of the peptide binding site show strong variability in their sensitivity to the inhibitor [4, 5]. Resistant field variants of HIV-1 isolated from patients undergoing therapy with T-20 have been reported to carry some of these mutations [6]. However, factors other than polymorphism in the HR1 region seem to be largely responsible for the wide range of susceptibilities of primary strains of HIV-1 isolated from entry inhibitor–naive patients [7]. Among these, it has been proposed that modifications in the Env V3 loop affect the inhibitory property [8]. Moreover, receptor density and fusion kinetics also alter viral sensitivity to the drug [8]. However, given that the above-mentioned determinants do not fully explain the extended range of susceptibilities to T-20 and considering the increasing number of entry inhibitors under investigation, it is of foremost importance to identify other possible cellular and/or viral determinants that modulate the antiviral efficacy of T-20.

Viral budding is thought to occur primarily through a restrictive process that excludes the majority of proteins of cellular origin [9]. However, a less-harsh protein-sorting mechanism seems to operate for retroviruses. It has been shown in a large number of studies that HIV-1 accommodates several host cell-surface constituents within its membrane [10–12]. Surprisingly, viral Env proteins have been shown to constitute as little as 0.1% of total viral protein, compared with as much as 20% for host-derived HLA-DR [13]. More importantly, it has been demonstrated that host proteins incorporated into mature HIV-1 particles retain their functionality. For example, the insertion of some specific host cell-surface constituents can affect the rate and efficiency of virus entry and confer resistance to neutralization [12]. Among these acquired host molecules, intercellular adhesion molecule type 1 (ICAM-1 or CD54) has been demonstrated to markedly affect the virus life cycle. This cell-surface molecule is acquired by clinical strains of HIV-1 that bear various coreceptor usages once they are expanded in primary human cells [14–18]. The presence of this adhesion molecule considerably increases virus infectivity and diminishes sensitivity to neutralization when target cells express leukocyte function antigen (LFA)-1, a natural counterligand for ICAM-1 [19–21]. It is now well established that lymphocytes can transiently bear an activated form of LFA-1 that is due to conformational changes in surface domains, which enables higher affinity binding to its natural ligand. Of interest, the activation state of LFA-1 seems to be essential for cell-mediated adhesion [22–25]. Thus, it is not surprising to find that HIV-1 infectivity is more significantly augmented when ICAM-1–bearing virions are used to infect human T lymphocytes that express activated LFA-1 on their surface [26].

It has been postulated that the kinetic window during which HIV-1 Env is sensitive to T-20 is brief and only exists between virus binding to CD4 and coreceptor attachment [2, 3]. Hence, factors that accelerate membrane fusion will shorten the kinetic window during which viral Env is sensitive to the entry inhibitor. Recently, we provided evidence that virus binding and uptake are both enhanced on the incorporation of ICAM-1 [27]. This has prompted us to define whether the insertion of ICAM-1 within HIV-1 particles can affect its susceptibility to neutralization by T-20. In the present study, we observed that ICAM-1–bearing virions are more resistant to the inhibitory activity of the drug than are isogenic HIV-1 particles without host ICAM-1. Experiments performed with clinical strains of HIV-1 expanded in primary human cells revealed that virus replication is more potently reduced when the entry inhibitor is administered in combination with a blocking anti–LFA-1 antibody. Furthermore, data from kinetic infection studies suggest that additional interactions between virus-anchored host ICAM-1 and cell-surface LFA-1 are most likely accelerating the coreceptor engagement, which results in a shorter window of T-20 action.

### MATERIALS AND METHODS

**Cells and virus preparations.** The parental human embryonic kidney cell line 293T (ICAM-1 negative) and a 293T transfectant stably expressing a high surface level of ICAM-1 (Hi–ICAM-1) [28] were used to produce the prototypic X4 and R5 by use of laboratory molecular clones NL4-3 and JR-CSF, respectively. Both cell lines were maintained in Dulbecco’s modified Eagle medium (DMEM; Gibco BRL) supplemented with 10% fetal bovine serum (FBS; HyClone), glutamine (2 mmol/L), penicillin G (100 U/mL), and streptomycin (100 µg/mL). Primary HIV-1 isolates 92HT599 (X4) and 92US657 (R5) were provided by the AIDS Repository Reagent Program (Rockville, MD) and were amplified in peripheral blood mononuclear cells (PBMCs) from healthy donors. PBMCs were isolated from whole blood, and cultures were maintained in RPMI 1640 supplemented with 10% FBS, 2 mmol glutamine/L, 100 µg streptomycin/mL, 3 µg phytohemagglutinin-P/mL (PHA-P; Sigma), and 50 U of recombinant human interleukin-2/mL at 37°C in a 5% CO₂ atmosphere before virus infection. The virus-containing supernatant was filtered through a 0.45-µm pore filter and purified by Centricon Plus-20 (Millipore). Isolated viruses were stored in 200-µL aliquots at −85°C before use. The infectious titer of each virus stock was individually determined in PBMCs by measuring p24 production at day 7 by use of a sensitive double-antibody ELISA specific for the major viral core protein p24 [14] in wells that contained 10-fold serial virus dilutions. The TCID₅₀ was calculated with the Spearman–Karber formula.

**T-20, antibodies, and vectors.** The T-20 peptide was pro-
vided by Roche Biocscience. The anti–ICAM-1 R6.5 was supplied by R. Rothlein (Boehringer Ingelheim). The activating LFA-1 antibody MEM-83 was obtained from V. Horejsi (Institute of Molecular Genetics, Prague, Czech Republic), and the blocking anti–LFA-1 antibody MEM-25 was purchased from EXBIO Praha. Fluorescein isothiocyanate–conjugated goat anti–mouse antibody was purchased from Jackson ImmunoResearch Laboratories. The infectious molecular clones of HIV-1 pNL4-3 [29] and pYK-JR-CSF [30] were obtained from the AIDS Repository Reagent Program.

Flow cytometry. Cells were washed twice with PBS, centrifuged at 1000 g for 5 min at 4°C, and incubated for 30 min on ice with a saturating concentration of the biotinylated anti–ICAM-1 R6.5 (1 µg/10⁶ cells). The cells were then washed twice in PBS and incubated for 5 min on ice with a saturating concentration of R-phycoerythrin–conjugated goat anti–mouse antibody. Finally, cells were washed twice in PBS and resuspended in 500 µL of PBS that contained 1% paraformaldehyde before flow-cytometric analysis (EPICS Xl; Coulter). Cells incubated with a biotinylated irrelevant isotype-matched control antibody (i.e., IgG2a) were used as a negative control.

Virus-capture test and infection studies. The presence of host ICAM-1 in progeny virus was monitored by use of an established procedure that is based on immunomagnetic beads [31]. Beads coated with the biotinylated R6.5 anti–ICAM-1 antibody and a biotinylated irrelevant isotype-matched control antibody (i.e., IgG2a) were used in the present study.

Before HIV-1 infection, PBMCs were incubated for 20 min in the absence or presence of T-20 (0.008, 0.04, 0.2, 1, or 5 µg/mL) or zidovudine (0.005, 0.05, 0.5, or 5 µmol/L) in duplicate wells. A similar amount of each virus stock, standardized in terms of infectivity (i.e., MOI, 0.04; 10⁴ TCID₅₀/10⁶ target cells) for different periods (10, 30, 60, 120, or 180 min), was added to the cells and left to incubate at 37°C for either 6 (NL4-3, JR-CSF, and 92HT599) or 12 (92US657) days after treatment with the drug. It should be noted that T-20 was left for the duration of the experiment. In some experiments, PBMCs were treated with MEM-25, MEM-83, or an isotype-matched antibody for 20 min before HIV-1 infection (5 µg/mL/10⁶ target cells). The infectivity of NL4-3 and JR-CSF viruses either lacking or bearing host ICAM-1 was determined by inoculating PHA-activated PBMCs, was added to the cells and left to incubate at 37°C for either 6 (NL4-3, JR-CSF, and 92HT599) or 12 (92US657) days after treatment with the drug. It should be noted that T-20 was left for the duration of the experiment. In some experiments, PBMCs were treated with MEM-25, MEM-83, or an isotype-matched antibody for 20 min before HIV-1 infection (5 µg/mL/10⁶ target cells). The infectivity of NL4-3 and JR-CSF viruses either lacking or bearing host ICAM-1 was determined by inoculating PHA-activated PBMCs with equal levels of each virus stock, standardized in terms of p24 (10 ng of p24/10⁶ target cells). For kinetic studies, primary human cells were incubated with similar amounts of each NL4-3 preparation (10 ng of p24/10⁶ target cells) for different periods (10, 30, 60, 120, or 180 min), washed, and then treated with either 1.9 µg T-20/mL or 1 µmol zidovudine/L—these doses of each drug were sufficient to decrease by 95% the replication of NL4-3 either lacking or bearing host ICAM-1 (figure 1A).

Statistical analysis. To determine the T-20 dose corresponding to the IC₅₀ and a 95% confidence interval, a linear dose-response curve was drawn for each condition analyzed. The exponential growth function in Microsoft Excel was used to estimate the IC₅₀ values. Mean IC₅₀ values were calculated by use of all replicates for each virus preparation and are expressed as ±SD. Statistically significant differences between groups were computed by analysis of variance. P value calculations were done with Student’s t test in Microsoft Excel, and P < .05 was considered to be statistically significant.

RESULTS

To define whether the phenomenon of ICAM-1 incorporation can affect the sensitivity of HIV-1 to the fusion inhibitor T-20, we made use of an established transient expression system that allows the production of isogenic HIV-1 differing only in the absence or the presence of virion-bound ICAM-1 [20]. The X4-tropic NL4-3 and R5-tropic JR-CSF molecular clones were introduced into parental 293T and Hi–ICAM-1 cells to generate the tested virus preparations. The host cell-surface constituent was found to be inserted to a similar degree in NL4-3 and in JR-CSF, as monitored by the virus-capture test (data not shown).

Sensitivity of HIV-1 either lacking or bearing host ICAM-1 to the fusion inhibitor T-20. To monitor the sensitivity of the studied viruses to T-20, comparable amounts of each virus stock, standardized in terms of infectivity titer, were used to inoculate mitogen-stimulated PBMCs from different healthy donors. Infections were performed and maintained in the presence of increasing concentrations of T-20. A representative series of investigations is depicted in figure 1 and indicates that the incorporation of ICAM-1 reduces the sensitivity of both NL4-3 and JR-CSF strains to the fusion inhibitor. Indeed, when PBMCs from different healthy donors were used as targets, the mean IC₅₀ calculated for the NL4-3 lacking the adhesion molecule was 111 ng/mL, compared with 205 ng/mL for isogenic NL4-3 virus bearing host ICAM-1 (table 1). Similar observations were made for JR-CSF—the mean IC₅₀ calculated for virus lacking the molecule was 20 ng/mL, compared with 44 ng/mL for ICAM-1–bearing virions. Although there were some variations in sensitivity to T-20, depending on the donor source, statistically significant differences were obtained for both NL4-3 and JR-CSF (P = .015 and JR-CSF (P = .003). Differences in the drug IC₅₀ between NL4-3 and JR-CSF were in agreement with those in the literature. In fact, NL4-3 bears a mutation in its heptad region that considerably reduces its sensitivity to the inhibitor, compared with other X4 viruses [4, 5]. As expected, NL4-3 and JR-CSF virions either lacking or bearing foreign ICAM-1 show a similar sensitivity to zidovudine, an antiviral agent that inhibits a more downstream event in the virus life cycle than T-20 (i.e., reverse transcription) (figure 1C and 1D).

Antiviral property of T-20 when ICAM-1–bearing virions are used to infect target cells expressing activated LFA-1.

In an attempt to test whether T-20 sensitivity can be more severely
Resistance to T-20 by ICAM-1 bearing HIV-1

Figure 1. Sensitivity to inhibition by T-20 of isogenic NL4-3 and JR-CSF either lacking or bearing host intercellular adhesion molecule (ICAM)-1. Mitogen-stimulated primary human cells were exposed to similar amounts, standardized in terms of infectivity (MOI, 0.04), of isogenic NL4-3 (A and C) and JR-CSF (B and D) either lacking or bearing ICAM-1 in the presence of increasing concentrations of T-20 (A and B) or zidovudine (C and D). Levels of p24 in the cell-free supernatants were measured 6 days after infection and were used for calculating virus infectivity relative to that of the control (vertical axes) and IC_{50} values for each virus stock. Shown here are the results for 1 donor (table 1; donor A for NL4-3 and donor E for JR-CSF). Results are the means ± SD of duplicate samples and are representative of 2 independent experiments. AZT, zidovudine.

affected by interactions between virus-associated ICAM-1 and LFA-1 under a conformational activated state on target cells, primary human cells were first incubated with the anti-LFA-1 antibody MEM-83, which is known to induce activation of the β2 integrin LFA-1 [32, 33]. PBMCs were next infected with isogenic ICAM-1–negative and –positive HIV-1 particles, and virus production was monitored by assessing p24 levels at a defined time point after infection. As expected, the combined action of virus-bound ICAM-1 and cell-surface–activated LFA-1 augmented HIV-1 infectivity for both NL4-3 and JR-CSF (figure 2). These findings are perfectly in line with our previous observations that HIV-1 infectivity toward PBMCs is increased on the acquisition of the adhesion molecule [26]. Of interest, the ~2-fold increase in virus infectivity that is conferred by host ICAM-1 seems to correlate with the modulation of sensitivity to T-20 (table 1). A more significant augmentation was seen when PBMCs were induced to express an activated LFA-1 form (i.e., a 4.7-fold increase for NL4-3 and a 4.1-fold increase for JR-CSF vs. ICAM-1–deficient virus). These data represent the first evidence that the biology of a macrophage-tropic isolate of HIV-1 is affected positively by the association between virus-anchored host ICAM-1 and cell-surface LFA-1 in an activated state. More importantly, viral sensitivity to the drug is severely reduced after the activation of LFA-1 on PBMCs for HIV-1 particles bearing the molecule (figure 3). Indeed, a statistically significant 4–5-fold difference (P < .016) was obtained when the sensitivity to T-20 of NL4-3 virus lacking ICAM-1 was compared with that of ICAM-1–bearing progeny virus when target cells were treated with MEM-83 (mean IC_{50} 45 vs. 213 ng/mL) (figure 3A). Similar results were obtained when MEM-83–treated target cells were infected with JR-CSF virions either lacking or bearing host-encoded ICAM-1 (mean IC_{50} 23 vs. 94 ng/mL) (P < .007) (figure 3B).

Effect on the duration of the kinetic window of sensitivity
to T-20 of the interaction between virally embedded ICAM-1 and cell-surface LFA-1. To gain insight into the possible biological mechanism by which the incorporation of ICAM-1 can decrease viral sensitivity to T-20, the rapidity with which fusion is occurring for viruses either lacking or bearing host ICAM-1 was studied by treating PBMCs with T-20 at specific time points after the addition of NL4-3. To this end, we used a final concentration of the drug corresponding to the mean IC₅₀ for NL4-3 virions either lacking or bearing host ICAM-1 that was calculated for donors A–D (table 1). As indicated in figure 4, virus production was not affected when T-20 was added until 60 min after infection with NL4-3 viruses either lacking or bearing host ICAM-1. However, ICAM-1–bearing virions were found to be more resistant to T-20 than were isogenic viruses lacking host ICAM-1 when the fusion inhibitor was added at later time points (i.e., 120 and 180 min after virus exposure), which suggests that ICAM-1–bearing viruses fuse more rapidly than do isogenic virions lacking host ICAM-1.

Table 1. Sensitivity of isogenic virus preparations either lacking or bearing host-derived intercellular adhesion molecule (ICAM)-1.

<table>
<thead>
<tr>
<th>Virus strain, PBMC donors</th>
<th>IC₅₀ ± SD, ng/mL</th>
<th>Fold change in IC₅₀</th>
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<tbody>
<tr>
<td></td>
<td>Without ICAM-1</td>
<td>With ICAM-1</td>
</tr>
<tr>
<td>NL4-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>69 ± 10</td>
<td>195 ± 20</td>
</tr>
<tr>
<td>B</td>
<td>102 ± 7</td>
<td>222 ± 24</td>
</tr>
<tr>
<td>C</td>
<td>112 ± 13</td>
<td>192 ± 19</td>
</tr>
<tr>
<td>D</td>
<td>162 ± 16.2</td>
<td>209 ± 25</td>
</tr>
<tr>
<td>Mean</td>
<td>111 ± 13</td>
<td>205 ± 22</td>
</tr>
<tr>
<td>JR-CSF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>19 ± 3</td>
<td>51 ± 8</td>
</tr>
<tr>
<td>F</td>
<td>16 ± 2</td>
<td>42 ± 4</td>
</tr>
<tr>
<td>G</td>
<td>25 ± 5</td>
<td>53 ± 5</td>
</tr>
<tr>
<td>H</td>
<td>18 ± 3</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>Mean</td>
<td>20 ± 3</td>
<td>44 ± 5</td>
</tr>
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NOTE. Sensitivity of the tested virus stocks to T-20 was monitored as described in Materials and Methods. PBMC, peripheral blood mononuclear cells.

* IC₅₀ for ICAM-1–bearing virus was divided by IC₅₀ for virus lacking ICAM-1.

DISCUSSION

Previous works have shown that, despite its potent antiviral capacity, T-20 exhibits differences in its efficiency against primary isolates of HIV-1 that are more variable than the efficacy of other antiretroviral agents [5, 6, 34]. For example, the estimated T-20 concentrations needed to inhibit HIV-1 field strains can fluctuate by as much as ~2.5 log₁₀, and such variations in the inhibitory properties of T-20 sometimes involve mutations in the HR1 domain of gp41, in addition to factors that have not yet been identified [7]. Given that T-20 inhibits fusion by binding to the HR1 domain of gp41 and preventing the formation of the 6-helix bundle, any factor that will influence the kinetics of coreceptor engagement will affect sensitivity to the drug [2, 3]. The ICAM-1 adhesion molecule, once found embedded within mature HIV-1 particles, constitutes a good candidate in this re-
Resistance to T-20 by ICAM-1 bearing HIV-1

Figures 3 and 4. Effect of the leukocyte function antigen (LFA)-1 activated state on sensitivity of isogenic NL4-3 and JR-CSF either lacking or bearing host intercellular adhesion molecule (ICAM)-1 to inhibition by T-20. Mitogen-stimulated primary human cells were either left untreated or treated with the activating anti-LFA-1 antibody MEM-83 (3.0 μg/10^6 cells). Next, target cells were inoculated with similar levels, standardized in terms of infectivity (MOI, 0.04), of isogenic NL4-3 (A) and JR-CSF (B) particles either lacking or bearing ICAM-1 in the presence of increasing concentrations of T-20. Infection was allowed to proceed for 6 days before measuring p24 values, which were used to calculate virus infectivity relative to that of the control (vertical axis), and IC_50 values for each virus stock. Shown here are the results of donor D for NL4-3 and donor F for JR-CSF. Results are the means ± SD of duplicate samples and are representative of 2 independent experiments.

Figure 4. Kinetics studies of the sensitivity to inhibition by T-20 of isogenic NL4-3 either lacking or bearing host intercellular adhesion molecule (ICAM)-1. Mitogen-stimulated primary human cells were first exposed for the indicated periods to equal quantities of isogenic NL4-3 either lacking or bearing ICAM-1 in the presence of increasing concentrations of T-20. Infection was allowed to proceed for 6 days before measuring p24 values, which were used to calculate virus infectivity relative to that of the control (vertical axis), and IC_50 values for each virus stock. Shown here are the results of donor D for NL4-3 and donor F for JR-CSF. Results are the means ± SD of duplicate samples and are representative of 3 independent experiments.

After the demonstration that ICAM-1–bearing virions are less susceptible to T-20 than are isogenic viruses lacking host ICAM-1, we next investigated the mechanism through which this resistance could be achieved. We speculated that the kinetics of the membrane fusion process is faster for ICAM-1–bearing HIV-1 particles. This hypothesis was confirmed by showing by the insertion of a host cell-surface component in HIV-1. Furthermore, our results show that the conformational state of LFA-1 represents an additional factor that significantly affects the efficacy of this entry inhibitor. LFA-1 is a natural ligand for ICAM-1 and can undergo conformational changes that will convert it from a nonligand-binding to a ligand-binding state. Using the activating LFA-1 antibody MEM-83, we observed a 4–5-fold decreased sensitivity to the drug. This finding has some physiological significance when one considers that several chemokines trigger the activation of LFA-1 binding to ICAM-1, a process that is responsible for the vascular arrest of circulating lymphocytes. For example, the secondary lymphoid-tissue chemokine (SLC) and EBI1-ligand chemokine (ELC) are known to activate LFA-1 and to be constitutively expressed in secondary lymphoid tissues [35, 36]. Because it is now well established that persistent HIV-1 infection takes place in secondary lymphoid organs and that the replication of both X4- and R5-tropic virions is enhanced by SLC and ELC [37], it can be speculated that the antiviral efficacy of T-20 is most likely reduced in such natural viral reservoirs.

gard, on the basis of its reported capacity to increase virus infectivity and to confer resistance to neutralization by agents that block the initial interactions between the virion and the cell surface [19–21]. We have reported here that the incorporation of ICAM-1 results in an ∼2-fold decrease in sensitivity to the fusion inhibitor when X4- and R5-tropic viruses and primary human cells are used as targets. To the best of our knowledge, it is the first demonstration that viral sensitivity to T-20 is affected
that progeny viruses carrying host-encoded ICAM-1 are less vulnerable to the action of T-20 once the entry inhibitor is added ≥120 min, but not 60 min, after HIV-1 infection. These results suggest that the incorporation of ICAM-1 leads to a more rapid coreceptor linkage with gp120, an event that culminates in a reduced accessibility of T-20 to its action site. The possibility that virions bearing host ICAM-1 are fusing more rapidly than progeny virus lacking ICAM-1 is supported by the results of a recent study that showed that the presence of host ICAM-1 in mature HIV-1 particles promotes fusion at the cellular membrane [27]. This observation was made with an enzyme-based assay that detects the fusion of HIV-1 with target cells through the use of HIV-1 virions labeled with β-lactamase–Vpr chimerical proteins in combination with cells loaded with CCF2/AM dye, a fluorescent substrate of β-lactamase.

The additional interaction between ICAM-1 and LFA-1 can thus be proposed as being responsible, to some extent, for the wide range of susceptibilities to T-20 for HIV-1 plasma viruses originating from treatment-naïve patients. The likelihood of this possibility is reinforced by the demonstration that ICAM-1 has been found to be embedded in all field isolates of HIV-1 with distinct tropisms after production in primary human cells [14–18]. It is interesting to note that foreign ICAM-1 is also acquired by T- and macrophage-tropic variants of HIV-1 when they are expanded in human lymphoid tissue cultured ex vivo without exogenous stimulation [14]. Given that it is now accepted that key pathogenesis events and most viral replication take place in lymphoid tissues, it can thus be expected that ICAM-1 is most likely also incorporated in natural infection.

It has been reported that the interaction between Env and coreceptor represents another determinant that affects sensitivity to the T-20 inhibitor. In one study performed by Reeves et al. [8], a single amino-acid mutation in Env that reduced affinity for CCCR5 also increased T-20 sensitivity by ∼30-fold. Although this effect is much more prominent than those observed in the present work, some differences in experimental methodologies may account for the different results. For example, Reeves et al. were testing T-20 sensitivity in a cell-to-cell fusion assay that involved QT6 target cells expressing CD4 and CCR5, whereas we studied sensitivity to T-20 using a virus/cell fusion system. Moreover, it is of importance to note that our findings were made by use of experimental conditions that are closer to physiological conditions—that is, viruses produced in primary human cells and natural target cells. Although the present study focused on the effect of a single virus-anchored host protein on the sensitivity of HIV-1 to T-20, supplementary interactions between virus-bound host proteins other than ICAM-1 and their ligands (see below for more details) might even further accelerate the virus–cell membrane fusion process and reduce viral sensitivity to T-20 in a more dramatic fashion.

It is imperative to more fully characterize the host molecular signature present on the virion surface to identify proteins that might reduce the overall efficacy of the recently developed class of entry inhibitors. For example, the acquisition of HLA-DR, LFA-1, and CD28 might also influence sensitivity to T-20, because the insertion of such host cell-surface components into mature virions increases virus infectivity [38–40]. However, our understanding of the various cellular and viral factors that can qualitatively and quantitatively affect the process of incorporation is still fragmentary, and more efforts are needed to shed light on this topic. Nevertheless, the remarkable ability of HIV-1 to evade the pressure of pharmacological agents, as has been seen in clinical trials with T-20, confirms that combined therapy represents the most appropriate strategy for controlling viremia. Our findings that the replication of X4- and R5-tropic primary isolates of HIV-1 is more significantly diminished when the drug is administered in combination with an agent that abrogates ICAM-1/LFA-1 interactions is an indication that inhibitors of this interaction should be considered as an adjuvant to the arsenal of drugs currently in use for the treatment of patients infected with HIV-1. Newly developed LFA-1 antagonists—such as BIRT-377, which inhibits the induction of the mAb24 reporter epitope on activated LFA-1—constitute promising therapeutic agents [41]. BIRT-377 exerts its inhibitory effects by preventing LFA-1 from adopting its activated conformational state [42]. Moreover, other antagonist peptides or small-molecule inhibitors of ICAM-1/LFA-1 infections have been identified and could also be considered as putative can-

<table>
<thead>
<tr>
<th>Virus strain, PBMC donors</th>
<th>Without MEM-25</th>
<th>With MEM-25</th>
<th>Fold change in IC_{so} a</th>
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<tr>
<td>92HT599 (X4-tropic)</td>
<td></td>
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<tr>
<td>1</td>
<td>12 ± 3</td>
<td>5 ± 1</td>
<td>2.4</td>
</tr>
<tr>
<td>2</td>
<td>17 ± 3</td>
<td>7 ± 2</td>
<td>2.4</td>
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<tr>
<td>4</td>
<td>12 ± 2</td>
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<td>2.9</td>
</tr>
<tr>
<td>Mean</td>
<td>12 ± 2</td>
<td>5 ± 1</td>
<td>2.5</td>
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<tr>
<td>92US657 (R5-tropic)</td>
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<tr>
<td>5</td>
<td>17 ± 5</td>
<td>5 ± 3</td>
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<tr>
<td>8</td>
<td>10 ± 4</td>
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<tr>
<td>Mean</td>
<td>13 ± 5</td>
<td>3 ± 2</td>
<td>4.4</td>
</tr>
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</table>

NOTE. Clinical isolates of HIV-1 were used to infect peripheral blood mononuclear cells (PBMCs) that were either left untreated or treated with the anti–leukocyte function antigen–1 antibody MEM-25 before treatment with increasing concentrations of T20 (0–10 μg/mL). Levels of p24 were estimated at 6 and 12 days after infection for 92HT599 and 92US657, respectively.

a IC_{so} of untreated samples was divided by the IC_{so} of samples treated with MEM-25.

b Not detectable.
candidates for combined therapy with T-20 [43]. The high cost and limited bioavailability of this drug, coupled with the development of hypersensitivity symptoms in patients undergoing T-20 treatment, support the use of antagonists of ICAM-1/LFA-1 ligation in the care of HIV-1-infected individuals.

Acknowledgments

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