The fusion inhibitors mark the beginning of a new era in the management of human immunodeficiency virus type 1 (HIV-1) disease. With a unique mechanism of action that they represent a new fourth class of antiretrovirals. Enfuvirtide (T-20) has been shown to exert potent antiretroviral activity and is approved for treatment in combination with other antiretrovirals in treatment-experienced patients with evidence of virus replication despite ongoing antiretroviral therapy (22, 23).

HIV-1 entry is mediated by the HIV envelope glycoproteins gp120 and gp41. Upon binding of gp120 to CD4 and a cellular coreceptor (usually CCR5 or CXCR4), conformational changes occur in both the gp120 and gp41 subunits. Within gp41, the fusion peptide region becomes exposed and inserts into the cell membrane. Additional conformational changes result in the formation of a trimeric antiparallel coiled-coil structure between the HR-1 and HR-2 regions of gp41. The formation of the six-helix bundle is believed to bring the viral and cell membranes together and lead to viral entry (14, 42).

T-20 acts by binding to the HR-1 region of gp41, thereby preventing the interaction between the HR-1 and HR-2 domains of gp41 that is required for virus/host membrane fusion (3, 19). It is thought that T-20 can target the viral envelope only during a kinetic window that opens by CD4 and/or coreceptor binding and closes with the coalescence of HR-1 and HR-2 domains of gp41 forming a final six-helix bundle structure (1, 7, 14, 20, 27, 32). Although T-20 blocks fusion of both R5 and X4 strains of HIV-1, X4 strains are overall more sensitive to the drug (7, 44). Factors contributing to the greater sensitivity of X4 strains than of R5 strains to T-20 include the reduced affinity of CXCR4-gp120 interactions compared to that of CCR5-gp120 interactions (8, 9, 17), as well as the ability of T-20 to bind to gp120 of CXCR4 strains, thereby blocking gp120-CXCR4 interactions (44). Since R5 strains of HIV-1 are generally present throughout the course of HIV-1 infection (28), the reduced sensitivity of R5 strains to T-20 may decrease the overall efficacy of T-20-based treatment regimens.

Previous studies with cell lines have demonstrated that the sensitivity of R5 strains of HIV-1 to T-20 is influenced by CCR5 density levels, with higher CCR5 levels resulting in faster fusion kinetics and reduced T-20 sensitivity (31–33). We have recently extended these observations to primary CD4 T cells (16). These findings, together with our earlier observation that the drug rapamycin (RAPA) inhibits CCR5 surface expression on CD4 T cells (15), led us to postulate that RAPA might enhance the antiviral activity of T-20 against R5 strains of HIV-1. In the present study we have evaluated the effect of RAPA-mediated reduction of CCR5 expression on the antiviral activity of T-20.

**MATERIALS AND METHODS**

**Cell culture.** Peripheral blood mononuclear cells (PBMCs) from healthy donors were isolated by Ficoll-Hypaque density gradient centrifugation. PBMCs were cultured at 10⁶ cells/ml in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), antibiotics, and recombinant human interleukin-2 (IL-2; 100 U/ml) (Roche, Indianapolis, IN). Human HEK 293T cells were cultured in Dulbecco modified Eagle medium (DMEM) supplemented with 10% FBS, antibiotics, and G418 (0.5 mg/ml).

The numbers of viable cells in cultures were determined by trypan blue staining or by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction using a commercial kit (Roche). Briefly, for the MTT assay cells

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seeded in 96-well plates (100 μl) were incubated with 10 μl of MT solution for 4 h at 37°C. A solubilization solution was then added, and plates were incubated overnight at 37°C. The extent of MTT conversion to formazan by mitochondrial dehydrogenase, which indicates cell viability, was determined by measuring optical density at 490 nm.

RAPA was purchased from Calbiochem (San Diego, CA). A drug stock containing RAPA at 1 mM was prepared in dimethyl sulfoxide. Dilutions from this stock were made in RPMI medium, and the final concentration of dimethyl sulfoxide in the cultures was always <0.1%. The HIV-1 fusion inhibitor T-20 was obtained from Roche through the NIH AIDS Research and Reference Reagent Program (Germantown, MD).

Infectivity assays. The following HIV-1 strains were used in infection experiments: IIIB, ADA, 92RW008, 98BR029, and CC101.19. HIV-1 IIIB is a T-cell-line-adapted lab strain that uses CXCR4 for entry into cells, while the rest are CCR5-using isolates. 92RW008 and 98BR029 are clinical isolates derived from patients in Rwanda and Brazil, respectively. CC101.19 is an R5 isolate that is resistant to the CCR5 antagonist Antid101 (a precursor of SCH-4) (40). Viruses were obtained from the NIH AIDS Research and Reference Reagent Program (Germantown, MD), except for 92RW008 and 98BR029 (from the World Health Organization) and CC101.19 (from John Moore, Cornell Medical College, New York, NY).

For infection of PBMCs, cells were cultured in medium containing IL-2 (100 U/ml) and different concentrations of RAPA for 6 days. On day 6, cells were exposed to virus for 2 h. Nonadsorbed virus was removed by washing cells with phosphate-buffered saline (PBS) three times. Infected cells were cultured in IL-2 medium in the presence of drugs. Unless otherwise indicated, PBMCs were infected using a multiplicity of infection (MOI) of 0.001. Virus growth was monitored in culture supernatants by measuring p24 antigen levels by enzyme-linked immunosorbent assay (NCI, Frederick, MD) on day 4 after infection.

Quantification of CD4, CCR5, and CXCR4. Quantification of CCR5, CXCR4, and CD4 was done as described previously (38), using the following antibody clones: clone 45531 (CCR5), clone 12G5 (CXCR4), and clone RPA-T4 (CD4). For CCR5 and CXCR4, lymphocytes were first gated on CD3 (clone UCHT1) and CD4 (clone RPA-T4). For CD4, lymphocytes were gated using CD3 (clone UCHT1) in combination with CDR4 (clone SK1). All antibodies were from BD Biosciences (San Jose, CA) except for the CCR5 antibody, which was from R&D Systems (Minneapolis, MN). Prior to staining, PBMCs were washed twice with PBS and incubated in blocking buffer (PBS containing 2% human serum, 5% human albumin, and 0.1% sodium azide) for 30 min at room temperature. Cells were then stained with the antibodies for 30 min at room temperature, washed twice with PBS, and acquired on a FACSCalibur (BD Biosciences) using Cellquest software (BD Biosciences). Immunofluorescence intensity was measured as an estimate of the average number of molecules on the cell surface. Fluorescence was measured using the Quantikinet system (BD Biosciences), which produces a regression line from a series of Quantibrite-phycoerythrin (PE) standards (BD Biosciences). The correlation coefficient of the median-effect plot (r), the combination index (CI), and the dose reduction index (DRI) were determined by a nonlinear curve fit to the equation

\[ \frac{F_{\text{lower}}}{F_0} = \frac{1}{1 + \text{CI} \frac{F_{\text{upper}}}{F_0}} \]

where F is the percentage of fusion and CI is the inhibitor concentration. Curve fitting was done using Sigma Plot (version 9.0) software.

Real-time PCR. PBMCs were exposed to virus using an MOI of 0.01 to ensure that enough products of reverse transcription were synthesized after 3 h of infection. Pretreated infection was done on crude lysates of infected cells. Lysates were prepared by resuspending cells in lysis buffer (10 mM Tris, pH 8.0, 0.5 mM EDTA, 0.0001% sodium dodecyl sulfate, 100 μg/ml proteinase K) and incubating the reaction mixtures at 65°C for 1 h and 95°C for 15 min. DNA was amplified using primer pair R-U5 (45), which amplifies early HIV-1 products of reverse transcription. Amplification and detection were performed using SYBR green PCR Master Mix (Applied Biosystems, Foster City, CA) in an ABI Prism 7700 Sequence Detector System (Applied Biosystems). Duplicate 50-μl reaction mixtures contained a lysate amount corresponding to 25 × 10^3 cells and an 0.8 μM concentration of each primer. PCR was carried out by heating the reaction mixture at 95°C for 15 min, followed by 40 cycles of 94°C (15 seconds), 60°C (30 seconds), and 72°C (30 seconds). Quantification of PCR products was normalized according to the amount of genomic DNA by amplifying the housekeeping β-actin gene. Changes in transcript amounts between samples were estimated by the comparative cycle threshold method.

Evaluation of synergy. Analysis of synergy, additivity, or antagonism between RAPA and T-20 was performed using fixed ratios of drug combinations in antiviral assays according to the median-effect principle of Chou (4), using CalcuSyn software (Biosoft, Ferguson, MO). To this end, RAPA and T-20 were tested individually and in a fixed molar ratio combination over a range of serial dilutions. When tested individually, each drug was tested using twofold dilutions (0.125 to 2 nM range for RAPA and 2.5 to 40 nM range for T-20). When used in combination, a 1:20 RAPA:T-20 molar ratio was used. The software calculated the values of the doses required for 50% inhibition (Dm) by each of the two drugs (alone and in combination), the cooperativity coefficient (m), the linear correlation coefficient of the median-effect plot (r), the combination index (CI), and the dose reduction index (DRI). The CI value reflects the nature of the interaction between the drugs. A CI of ~1 indicates synergy, a CI of 1 indicates additivity, and a CI of >1 indicates antagonism. The DRI is a measure of how much the dose of each drug in a synergistic combination is reduced at a given effect level compared with the doses for each drug alone.

RESULTS

RAPA reduces CCR5 density levels and inhibits R5 HIV-1 replication in PBMCs. We have previously shown that treatment of PBMCs with RAPA (0.1 to 1 nM) results in reduced expression of CCR5 (RNA and protein levels) and inhibition of HIV-1 R5 strains in cultured PBMCs (15). We now wished to evaluate the effects of RAPA on the densities of CCR5, CXCR4, and CD4 and relate these effects to its antiviral activity. To this end, donor PBMCs were cultured in the presence of IL-2 and RAPA (0.1 to 1 nM) for 6 days and then subjected to quantitative fluorescence-activated cell sorting analysis and infectivity assays. To control for cell viability, donor PBMCs were cultured in IL-2 medium in the presence of RAPA for 14 days. Results are shown in Fig. 1a, and they indicate that RAPA concentrations of 0.03 to 1 nM did not affect cell viability. RAPA concentrations of 0.1 to 1 nM resulted in reduced CCR5 density on CD4 T cells compared to non-RAPA-treated cells (Fig. 1b, P ≤ 0.01) while having no effect on CXCR4 density (Fig. 1c). Regarding the effect of RAPA on CD4, an ~15% decrease in CD4 density levels was observed at 1 nM RAPA (P = 0.049 compared to the untreated control) (Fig. 1d). Consistent with the observed decrease in CCR5 density in the presence of RAPA, drug-treated cells produced lower p24 levels of R5 HIV-1 (ADA strain) than did untreated cells on day 4 after infection (Fig. 1e). In contrast, the effect of RAPA on X4 (HIV-1 IIIB) infection showed considerable variability among different donors (Fig. 1f).
RAPAMYCIN POTENTIATES T-20 ACTIVITY AGAINST R5 HIV-1

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the case of HIV-1 R5 strain ADA, the EC\textsubscript{50} values for RAPA and T-20 used as single drugs were 0.15 nM and 10 nM, respectively. When 10 nM T-20 was combined with RAPA concentrations in the 0.125 to 1 nM range, virus replication was inhibited by \( \geq 80\% \) (Fig. 4a, left). For HIV-1 X4 strain IIIb, the T-20 EC\textsubscript{50} value was 2.5 nM and RAPA inhibited virus replication at 1 nM only. However, RAPA effects on T-20 activity against HIV-1 X4 strain IIIb were variable among donors, and RAPA did not impact T-20 antiviral effect in a consistent manner (Fig. 4a, right). We further evaluated the effect of RAPA on T-20 antiviral activity using the R5 clinical isolates 92RW008 and 93BR029. Similar to the results obtained with HIV-1 strain ADA, addition of RAPA to 10 nM T-20 inhibited virus replication by \( > 90\% \) in both isolates (Fig. 4b). Moreover, the RAPA–T-20 combination was also effective against HIV-1 CC101.19, a virus strain that uses CCR5 for viral entry and is resistant to the CCR5 antagonist AD101 (40) (Fig. 4b). Cell viability was not affected by the drug concentrations used (data not shown).

In order to characterize the nature of the drug interaction (i.e., additivity, synergy, or antagonism) between RAPA and T-20, we set up PBMC infectivity assays using drug concentrations that allow data analysis according to the median-effect principle. Thus, RAPA and T-20 were used alone and in combination at fixed molar ratios. Data obtained upon infection with HIV-1 R5 strain ADA were analyzed using CalcuSyn software, and results from four different experiments using cells from four donors with measurements done in duplicate. For X4 HXB2 Env, each data point represents the mean ± standard deviation of two different experiments using cells from two donors with measurements done in duplicate.

FIG. 2. RAPA increases the activity of T-20 in a cell-cell fusion assay. Target cells (6-day RAPA-treated CD8-depleted PBMCs) were stained with calcein, and effector cells (HIV-1 envelope-transfected 293T cells) were loaded with CMTMR. Target and effector cells were then cocultured at a 1:3 ratio in the absence and presence of T-20 for 2.5 h. Fused cells (positive for both markers) were detected by fluorescence microscopy, and the extent of fusion was determined as indicated in Materials and Methods. (a) Effect of RAPA pretreatment of PBMCs on R5 and X4 Env-induced fusion. (b) Effect of RAPA pretreatment of PBMCs on T-20 inhibition of R5 Env-induced fusion. (c) Effect of RAPA pretreatment of PBMCs on T-20 inhibition of X4 Env-induced fusion. (d) Net RAPA potentiation effect of T-20 inhibitory activity in R5 and X4 Env-induced fusion. For R5 JRFL Env, each data point represents the mean ± standard deviation from four different experiments using cells from four donors. For X4 HXB2 Env, each data point represents the mean ± standard deviation of two different experiments using cells from two donors with measurements done in duplicate.

FIG. 3. RAPA potentiates the R5 antiviral activity of T-20 as determined by quantification of early products of HIV-1 transcription. PBMCs cultured for 6 days in the presence of IL-2 and RAPA were exposed to an MOI of 0.01 of a DNase-treated HIV-1 ADA (R5) stock in the presence of T-20 for 3 h. DNA was then extracted and amplified by real-time PCR using R-U5 primers for detection of early viral transcripts and β-actin primers for amplification of a housekeeping gene. R-U5 signals were normalized for total DNA content using the β-actin amplification signal. Each data point represents the mean ± standard deviation of three experiments, each using PBMCs from a different donor.
When the drugs were used in combination at a RAPA/T-20 ratio of 1:20, the EC₅₀ values of RAPA and T-20 were reduced to 0.12 and 2.48 nM, respectively. These reductions in EC₅₀ values suggested a synergistic interaction between RAPA and T-20 in antiviral activity against R5 strains of HIV-1. To determine the degree and nature of the drug interaction, the CI values of the RAPA–T-20 combination were calculated and shown to be < 1, thus indicating that the drug combination was synergistic.

**FIG. 4.** RAPA potentiates T-20-mediated inhibition of R5 HIV-1 replication in PBMC infectivity assays. (a) Differential effect of RAPA on T-20 antiviral activity against HIV-1 R5 strain ADA and HIV-1 X4 strain IIIb. RAPA-pretreated PBMCs were infected with HIV-1 ADA and HIV-1 IIIb at an MOI of 0.001 in the presence of different concentrations of T-20. Infected cells were cultured in medium containing RAPA and T-20, and p24 production in the culture supernatants was determined on day 4 after infection. Results are means ± standard deviations of three independent experiments. (b) Effect of RAPA on the antiviral activity of T-20 against R5 clinical isolates 92RW008 and 93BR029 and against the CCR5-using AD101-resistant CC101.19 strain. Infections were carried out as described for panel a. Results are means ± standard deviations of two independent experiments.

**TABLE 1.** Dose-effect relationship parameters of RAPA and T-20 alone and in combination for the replication of R5 HIV-1 in PBMCs^a^

<table>
<thead>
<tr>
<th>Drug dose (nM)</th>
<th>Fa</th>
<th>m</th>
<th>Dm (nM)</th>
<th>r</th>
<th>CI</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>RAPA</td>
<td>T-20</td>
<td></td>
<td></td>
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<tr>
<td>RAPA alone</td>
<td></td>
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<tr>
<td>0.125</td>
<td>0.34 ± 0.11</td>
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<tr>
<td>0.250</td>
<td>0.66 ± 0.07</td>
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<tr>
<td>0.5</td>
<td>0.78 ± 0.02</td>
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<tr>
<td>1</td>
<td>0.85 ± 0.07</td>
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<tr>
<td>2</td>
<td>0.92 ± 0.02</td>
<td>1.03 ± 0.13</td>
<td>0.18</td>
<td>0.98</td>
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<tr>
<td>T-20 alone</td>
<td></td>
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<tr>
<td>2.5</td>
<td>0.23 ± 0.09</td>
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<td>5</td>
<td>0.50 ± 0.11</td>
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<td>10</td>
<td>0.57 ± 0.14</td>
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<tr>
<td>20</td>
<td>0.73 ± 0.17</td>
<td>0.84 ± 0.12</td>
<td>12.93</td>
<td>0.94</td>
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<tr>
<td>40</td>
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<tr>
<td>RAPA plus T-20 (1:20 ratio)</td>
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<tr>
<td>0.125/2.5</td>
<td>0.47 ± 0.10</td>
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<tr>
<td>0.25/5</td>
<td>0.74 ± 0.10</td>
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</tr>
<tr>
<td>0.5/10</td>
<td>0.91 ± 0.06</td>
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<td></td>
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<tr>
<td>1/20</td>
<td>0.94 ± 0.06</td>
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<td></td>
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<tr>
<td>2/40</td>
<td>0.99 ± 0.01</td>
<td>1.37 ± 0.11</td>
<td>0.12</td>
<td>2.48</td>
<td>0.99</td>
</tr>
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^a^ Fa, fractional inhibition; m, slope coefficient of the curve; Dm, dose at 50% inhibition (equivalent to EC₅₀ value); r, linear correlation coefficient of the median-effect plot; CI, combination index. Data are means ± standard deviations of four independent experiments using PBMCs from different donors.
infectivity assays using the HIV-1 X4 strain IIIb yielded CI values of ~1, suggesting that RAPA does not synergize with T-20 in inhibition of X4 HIV-1. Thus, these results demonstrate that the RAPA–T-20 drug combination has synergistic antiviral activity against R5 strains of HIV-1.

**DISCUSSION**

HIV-1 entry in cells requires a receptor (CD4) and a coreceptor (mainly CCR5 or CXCR4) on the cell surface (2, 6, 10, 11). CCR5 serves as the main coreceptor for transmitting strains of HIV-1, and CCR5 strains are generally present at all stages of infection (28). Individuals homozygous for the Δ32 mutation in the CCR5 gene are highly resistant to infection (29), whereas individuals carrying the mutation in a heterozygous state progress to AIDS more slowly than do individuals carrying the wild-type gene (5, 28). Moreover, CCR5 density levels (molecules/cell) on CD4+ T cells have been shown to correlate with RNA viral load (34) and progression to AIDS (35) in untreated HIV-1-infected individuals. Together, these findings highlight the importance of CCR5 for HIV-1 entry and suggest that CCR5 levels may be limiting for HIV-1 infection.

In the present study we demonstrate that treatment of PBMCs with RAPA results in reduced CCR5 density levels and significant inhibition of R5, but not X4, HIV-1 replication. The fact that RAPA concentrations in the 0.1 to 1 nM range had a modest effect in reducing CCR5 density levels while significantly suppressing R5 strains of HIV-1 supports the notion that physiological CCR5 levels are limiting for R5 HIV-1 infection and that threshold levels are required for efficient infection (16, 30, 31, 43). Accordingly, small decreases in CCR5 density can result in significant reductions in R5 HIV-1 replication. Moreover, Platt et al. (30) showed that CCR5 density levels below a threshold of ~2 × 10^3 CCR5 receptors/cell led to inefficient replication of R5 strains of HIV-1. It is interesting that 1 nM RAPA treatment of PBMCs in our study resulted in CCR5 density levels close to that threshold, leading to ~80% suppression of R5 HIV-1 replication.

In addition to their role in viral replication, CCR5 density levels also influence the antiviral activity of the fusion inhibitor T-20. Reeves et al. (32, 33) have shown that “low” CCR5 density levels on cell lines lead to slower kinetics of fusion and increased susceptibility to T-20. In agreement with these studies, we have recently demonstrated that CCR5 density levels on primary CD4+ T cells influence the T-20 susceptibility of R5 HIV-1 strains (16). We now report that RAPA-mediated reduction in CCR5 density levels increases T-20 activity against R5 strains of HIV-1, as determined by a cell-cell fusion assay and by quantification of early reverse transcription products in infected PBMCs. Moreover, in PBMC infectivity assays, drug interaction analysis of the RAPA–T-20 combination using the median-effect principle revealed synergy between the two drugs. These results, showing increased antiviral activity of T-20 on cells with reduced CCR5 density, are consistent with a previous report demonstrating synergistic antiviral activity between T-20 and a CCR5 antagonist in PBMC infectivity assays (39).

Potentiation of T-20 activity against R5 strains of HIV-1 in our studies was more apparent when assessing p24 values in PBMC infectivity assays than by measuring levels of cell-cell fusion or amounts of early products of reverse transcription. It is possible that the modest RAPA effects observed in the cell-cell fusion and PCR assays could be amplified and become more evident after several rounds of virus replication in the PBMC infectivity assay. In addition, it should be noted that CCR5 density levels are known to impact not only the efficiency of virus entry but also the magnitude of G protein signaling occurring upon R5 HIV-1 binding to CCR5, a signaling event that modulates the efficiency of postentry steps in the virus cycle (24–26). Thus, these data suggest that RAPA-mediated decreases in CCR5 levels result in both a reduced level of fusion and a lower magnitude of CCR5-dependent intracellular signaling, thereby impacting the overall process of virus replication.

It is interesting that RAPA partially inhibited cell-cell fusion induced by X4 HIV-1 Env without causing detectable changes in CXCR4 expression levels. However, the 50% inhibitory concentration values for X4 Env were >10 times greater than those for R5 Env. These results, together with a trend towards reduced CD4 levels on RAPA-treated cells (Fig. 1d), lead us to speculate that RAPA may partially inhibit X4 Env fusion (and perhaps R5 Env fusion as well) through decreases in CD4 density levels. In this regard, Platt et al. (30) have shown that CCR5 density levels that allow maximal infection in HeLa cell lines expressing ~4 × 10^5 CD4 molecules/cell become insufficient for infection when CD4 levels are reduced to ~10^4 CD4 molecules/cell. Alternatively, RAPA-mediated inhibition of X4 Env fusion may be related not to changes in CD4 levels but to interference with constitutive or HIV-1 Env-mediated colocalization of CD4 and coreceptor on the cell surface, as shown elsewhere with some HIV-1 fusion inhibitors (12, 13, 41). Further studies are required to determine the role of a mechanism(s) other than reduction in CCR5 density to explain the differential activity of the RAPA–T-20 combination in R5 versus X4 HIV-1 inhibition.

In summary, these results demonstrate that pretreatment of PBMCs with RAPA results in decreased CCR5 density levels which, in turn, lead to lower replication levels and increased T-20 sensitivities of R5 strains. Roy et al. have shown that RAPA also inhibits basal HIV-1 long terminal repeat-driven gene expression and that this antiviral activity inhibits both R5 and X4 strains to similar extents (36). Importantly, reduction in CCR5 density levels and inhibition of virus long terminal repeat gene expression occur at RAPA concentrations that do not inhibit cell proliferation (Fig. 1a) (15, 36). At present, RAPA is used as an immunosuppressant for the treatment of acute kidney rejection at dosages of 2 and 5 mg/day, which
result in approximate plasma trough levels of 9 and 19 nM, respectively (Rapamune package insert, Wyeth, 2002). In vitro data support the use of low concentrations of RAPA in the treatment of HIV-1 infection. Although therapeutic alteration of CCR5 levels is expected to be safe because individuals who congenitally lack CCR5 are otherwise healthy, the potential consequences of reduced CCR5 expression in individuals carrying wild-type CCR5 are unknown. However, the facts that inhibition of CCR5 function with antagonist blockers (in both HIV-1-infected and uninfected individuals) has been well tolerated in several studies (37) and that low RAPA doses (plasma trough levels of 5 to 10 nM) in combination with highly active antiretroviral therapy were safely used for 2 years in HIV-1-infected individuals receiving kidney transplants (21) suggest that inhibition of CCR5 expression by RAPA will be safe in patients.

In conclusion, these results, together with those of others (21, 36), suggest that low doses of RAPA may help to control HIV-1 replication in patients as well as enhance the antiviral activity of T-20 against the less-sensitive R5 strains. Moreover, based on our previous work demonstrating that RAPA enhances the antiviral activity of the CCR5 antagonist TAK-779 (15), early clinical evaluation of entry inhibitor therapy targeting CCR5 in combination with RAPA is warranted and would be a prudent way to attempt to minimize the toxicity of these important new agents.

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