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The θ-Defensin, Retrocyclin, Inhibits HIV-1 Entry

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ABSTRACT

Retrocyclin is a circular antimicrobial 18-residue peptide encoded in the human genome by a θ-defensin pseudogene. In the human genome, the gene for retrocyclin is inactivated by an in-frame stop codon in its signal sequence but its mature coding sequence is intact. The peptide corresponding to the processed human retrocyclin, generated by solid phase peptide synthesis, inhibited replication of R5 and X4 strains of HIV-1 in human cells. Luciferase reporter virus and Vpr-BLaM entry assays were used to demonstrate that retrocyclin specifically blocked R5 and X4 HIV-1 replication at entry. Surface plasmon resonance demonstrated that retrocyclin bound to soluble CD4 and gp120, but gp120 cell-binding assays revealed that retrocyclin did not fully inhibit the binding of soluble CD4 to gp120. A fluorescent retrocyclin congener localized in cell-surface patches either alone or colocalized with CD4, CXCR4, and CCR5. In the aggregate, these results suggest that retrocyclin blocks an entry step in HIV-1 replication. Retrocyclin represents a new class of small molecule HIV-1 entry inhibitors.

INTRODUCTION

ANTIMICROBIAL PEPTIDES AND PROTEINS are among the most ancient effectors of innate immunity.[1] Defensins, a family of cysteine-rich, cationic antimicrobial peptides, are expressed by the leukocytes and epithelial cells of birds and mammals.[2–5] The α- and β-subfamilies of defensins are the most widespread in nature, and have been extensively studied (reviewed in Lehrer and Ganz[6]). Recently, members of a new defensin subfamily, the θ-defensins, were isolated from the leukocytes and bone marrow of the rhesus macaque.[7–9] θ-Defensins are circular, 18-residue peptides derived from two precursor peptides, each of which contributes nine residues (including three cysteines) to the mature peptide.[7–9] Intracellular posttranslational head-to-tail ligation of the peptide backbone of two nine-residue precursors renders the mature 18-residue peptide circular, and an internal tridisulfide ladder renders it tetracyclic. Because precursors from identical or different genes can be ligated together, this enables θ-defensins to have a unique element of structural diversity without requiring alternative RNA splicing or genome expansion.[7,9]

Unlike α- and β-defensins, whose mature peptides are produced by rhesus monkeys and humans, only monkeys produce mature θ-defensin peptides.[8] Humans instead express θ-defensin precursor (pseudo)genes, but a stop codon within the signal sequence arrests translation prematurely.[10] Consequently, humans have not been found to produce mature θ-defensin peptides. Instead we used solid-phase peptide synthesis based on the cDNA sequence to generate a human θ-defensin, called retrocyclin, and characterized its properties.[10]

Recent studies[11] suggested that human α-defensins may be a component of the CD8 antiviral factor (CAF)—an elusive low-molecular-weight, noncytolytic antiviral factor secreted by stimulated $CD8^+$ lymphocytes, originally described by Walker and colleagues.[12] Not only are θ-defensin (DEFT) genes the evolutionary descendents of α-defensin (DEFA) genes,[13] but θ-defensin peptides including retrocyclin can also protect primary T cells from *in vitro* infection by HIV-1.[10] Phylogenetic evidence indicates that human retrocyclins were silenced after the orangutan and hominid lineages had diverged, approximately 7.5–10 million years ago. Given retrocyclin's antiviral properties against HIV-1, the evolutionary loss of retrocyclin may contribute to HIV-1 susceptibility in modern humans.

Experiments herein extend studies of our discovery of retrocyclin[10] by further exploring the mechanism by which retrocy-

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clin inhibits HIV-1 infection. We report here that retrocyclin inhibits HIV-1 replication at entry by blocking envelope glycoprotein-mediated fusion to the target cell membrane.

MATERIALS AND METHODS

Peptides

Peptides were synthesized at a 0.25 mmol scale as described previously.[10] The crude peptides were reduced under nitrogen until purified by reverse-phase high-performance liquid chromatography (HPLC). After this step, the peptides appeared homogeneous and their masses by MALDI-TOF MS agreed well with their theoretical masses. The reduced peptides (0.1 mg/ml) were oxidized, cyclized, purified, and characterized as previously described.[8,10]

Cells and viruses

Primary $CD4^+$ lymphocytes from HIV-1-seronegative donors were generated from freshly purified peripheral blood mononuclear cells (PBMC) stimulated with a CD3–CD8 bispecific monoclonal antibody (generously provided by Dr. Johnson T. Wong, Massachusetts General Hospital[14–16]). After approximately 7 days, when >98% of these cells coexpressed CD3 and CD4 (data not shown), they were subjected to HIV-1 infection assays. $CD4^+$ PBMC were maintained in R10-50 medium [RPMI containing 10% fetal calf serum (FCS) supplemented with 2 mM glutamine, 100 U of penicillin/ml, 10 μg of streptomycin/ml, and 50 U of interleukin-2/ml].

HIV infections with $CD4^+$ PBMC

Peptides (final concentration, 20 μg/ml) were added to 2.5×10^5 $CD4^+$ PBMC in 100 μl R10-50 medium, and the cells were challenged with an inoculum of HIV-1 that gave a multiplicity of infection (MOI) of 0.01–0.05. After incubation for 3 hr at 37°C, the cells were washed twice with R10-50 and added to 48-well plates at 2.5×10^5 cells/well in 1 ml of R10-50 medium containing peptides at 20 μg/ml final concentrations. At 3 day intervals, 0.5 ml of supernatant was removed from each well for HIV-1 $p24^{gag}$ antigen quantitation by an enzyme-linked immunosorbent assay (ELISA; DuPont, Boston, MA) and replaced with 0.5 ml of fresh medium supplemented with peptide at the final concentration. Peptides (20 μg/ml) were not cytotoxic after 9 days of infection as measured by trypan blue exclusion.

Luciferase replication assay

Adherent cell lines, HOS and 293T, were cultured in Dulbecco's modified Eagle medium (DMEM), 10% fetal bovine serum (FBS). hCD4, hCXCR4, and hCCR5 were introduced stably as previously described.[17] HIV-1 single-cycle luciferase reporter viruses were produced by cotransfecting 293T cells with pNL-LucR^-E^-, and JR.FL, ADA, SF33 (a gift from Cecilia Cheng-Mayer), JC2, amphotropic MLV, or VSV-G Env expression vector.[18] Viruses were quantitated by p24 antigen ELISA. Then 4×10^3 cells/96-well plate were preincubated with 50 μl culture medium containing RC100 or RC106 in 2× concentration for 1 hr at 37°C. Luciferase reporter viruses (1 ng p24 for HIV and ampho env, 0.1 ng p24 for VSV-G pseudotypes) were added in 50 μl. Luciferase activity was measured 3 days later with Luc-Lite (Packard) according to the manufacturer's directions on a Packard TopCount luminometer.

Entry assay

β-Lactamase-containing virions were produced by cotransfection of 293T cells with equal amounts of pNL4.3 or pNL-Bal (NL4.3 with the env of BaL)[19] and pMM310, a vector that encodes a β-lactamase-Vpr fusion protein (provided by Michael Miller, Merck, Inc.). Cells (2.0×10^5) in six-well dishes were infected with β-lactamase-loaded virus at an MOI of 1.0 in 2 ml. After 4–5 hr, the cells were washed twice with phosphate-buffered saline (PBS) and incubated with 2 μM CCF2/AM (GeneBLAzer Loading Kit, Aurora Biosciences, La Jolla, CA), 1% probenecid in 1.0 ml serum-free DMEM/1 mM Hepes for 16–18 hr at 25°C 5% CO_2. The cells were then trypsinized, fixed in 1% paraformaldehyde/PBS for 10 min, and analyzed on a BD LSR 3 analytical flow cytometer (Becton Dickinson) with a UV laser and 470-nm long-pass dichroic filter exciting at 325 nm. Cleaved substrate was detected as blue fluorescence with a 424/44-nm bandpass filter and uncleaved substrate was detected with a 516/20-nm bandpass filter. The results were plotted as the ratio of the blue:green to minimize differential dye loading. The blue:green ratio is calculated in analog mode in the BD LSR hardware before analog-to-digital conversion is done. Viral entry is defined as the percentage of cells showing an increase in blue:green ratio.

gp120 binding assay

293T cells (5×10^5) were transfected with 1 μg of either pc-CCR5 or pc-CD4, or a control plasmid pcDNA3.1 using lipofectamine 2000 (Invitrogen). Two days after transfection, cells were resuspended and washed once with binding buffer [50 mM Hepes, pH 7.5, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 5% bovine serum albumin (BSA), 0.1 mM NaN_3].[20] Finally the cells were resuspended with the binding buffer at 5×10^6/ml. In a 50-μl binding assay, either 0.5 μg soluble CD4 (AIDS Research and Reference Reagent Program) or 0.24 μg gp120 ThaiE (Chiron) was added in the presence or absence of 1 μg RC100 or RC106. After incubation for 1 hr at room temperature, cells were washed twice with PBS and lysed in Triton X-100-containing buffer. Lysates containing 10 μg of protein were separated by SDS–PAGE and transferred to polyvinylidene difluoride (PVDF) filters. Filters were probed with rabbit anti-gp120 serum followed by horseradish peroxidase-conjugated rabbit antihuman antibody and developed with chemiluminescence reagents (Amersham).

Surface plasmon resonance (SPR)

SPR experiments were performed using CM5 sensor chips on a Biacore 2000 system (Biacore, Inc., Piscataway, NJ). HBS-EP running buffer (pH 7.4) contained 10 mM Hepes, 150 mM NaCl, 3 mM EDTA, and 0.005% polysorbate 20. Gp120-LAV (X4 or R5 tropic; AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH) was dissolved at 20 μg/ml in 10 mM sodium acetate, pH 5.0, and immobilized on a CM5 sensor chip using the amine coupling method. The chip was activated by mixing 400 mM EDC [*N*-ethyl-*N*- (3-di-

methylaminopropyl)-carbodiimide hydrochloride] and 100 mM NHS (*N*-hydroxysuccinimide). An immobilization level of approximately 6000 response units (RU) was attained for bound gp 120. Residual reactive groups on the chip surface were blocked using 1.0 M ethanolamine/HCl, pH 8.5.

The flow cell-1 (FC1) chip, which served as a control, lacked immobilized protein but was treated with EDC, NHS, and ethanolamine/HCl. Binding signals were corrected for nonspecific binding by subtracting the FC1 signal. To regenerate chip surfaces, bound ligands were removed with 10 mM HCl. Data were analyzed with BIAevaluation 3.1 software, and curve-fitting was done with the assumption of one-to-one binding.

Confocal microscopy

RC101 was joined to the amine-reactive fluorescent dye BODIPY-FL (Molecular Probes, Eugene, OR) according to the manufacturer's protocol, and the conjugate ($RC101_{BODIPY\text{-}FL}$) was purified by reverse-phase HPLC and resuspended in 0.01% acetic acid. $RC101_{BODIPY\text{-}FL}$ (20 μg/ml) and phycoerythrin (PE)-labeled monoclonal antibodies against either CD4, CXCR4, CCR5, or isotype control (R&D Systems, Minneapolis, MN) were incubated with 2.5×10^5 $CD4^+$-selected PBMC cells for 15 min at room temperature, washed once in fresh R10-50 media. Specimens were imaged on a Leica TCS-SP Confocal Microscope (Heidelberg, Germany) equipped with an argon laser for excitation of BODIPY-FL and PE. Images were collected using Leica Confocal Software. Confocal microscopy was performed in the Carol Moss Spivak Cell Imaging Facility in the UCLA Brain Research Institute.

RESULTS AND DISCUSSION

We previously showed that retrocyclin, also termed here RC100, inhibited the replication of HIV-1 on primary lympho-

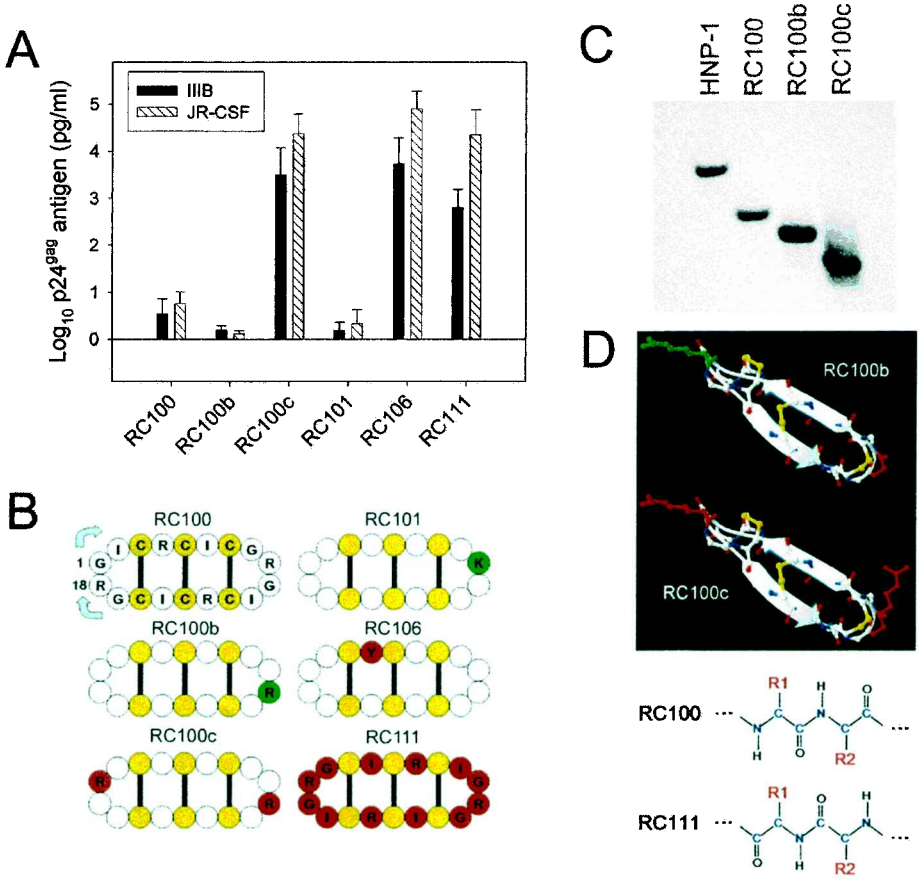

FIG. 1. Changes in primary structure can alter ability of retrocyclin to inhibit HIV-1 infection *in vitro*. (**A**) Two strains of HIV-1 (IIIB and JR-CSF) were utilized in infection assays of $CD4^+$ PBMC in the presence or absence of 20 μg/ml of test peptide. $p24^{gag}$ concentration is shown at 9 days postinfection (n = 3–11 per peptide; error bars indicate SEM). RC101, RC106, and RC111 are retrocyclin congeners based on the RC100 template. (**B**) Sequence and two-dimensional structures of retrocyclin congeners, represented by coin diagrams. Numbers "1" and "18" signify the N- and C-termini of synthetic peptides prior to backbone circularization. Mutations that retain antiviral activity are colored green, while those that ablate activity are colored red. (**C**) Coomassie-stained RC100, RC100b, and RC100c (1 μg each), separated by cationic charge density on an acid-urea (AU)–PAGE gel as previously described.[27] The human α-defensin HNP-1 (1 μg; Bachem, King of Prussia, PA) is shown for comparison. (**D**) Models of RC100b and RC100c made by templating their sequences on the backbone of a homologous peptide from rhesus macaques, RTD-1 (PDB accession code: 1HVZ). (**E**) Backbone structure of two representative amino acids with side chains "R1" and "R2" from RC100 and RC111.

cytes.[10] Herein, we identified a second human θ-defensin (pseudo)gene that encodes a nonapeptide precursor segment that differs from the RC100 precursor by the presence of an Gly to Arg substitution (Fig. 1). θ-Defensins are formed by the ligation of identical or different nonapeptide segments. Therefore hominid ancestors with two gene-encoded open reading frames could have combinatorially produced at least three mature θ-defensins: RC100 (mass, 1918.43; net charge, +4), RC100b (mass, 2017.56; net charge, +5), and RC100c (mass, 2116.70; net charge, +6) (sequences and structures shown in Fig. 1). RC100b thus has an additional positive charge as a result of one Gly to Arg substitution, while RC100c has two additional positive charges due to two Gly to Arg substitutions. RC101 differed from RC100 by a single Arg to Lys substitution in one of its β-turns. RC106 contained an Arg to Tyr substitution at position 4. RC111 served as a control in which the amino acid sequence of RC100 was reversed. The structures and electrophoretic properties of these peptides are shown in Figure 1B–D. To investigate the antiviral activity of the θ-defensins, and to define structural features of the peptides that are required for inhibition, we synthesized forms of retrocyclin: RC100b, RC100c, RC101, RC106, and RC111.

The ability of the retrocyclin congeners to inhibit replication of HIV-1 on $CD4^+$ PBMC from seronegative donors was tested

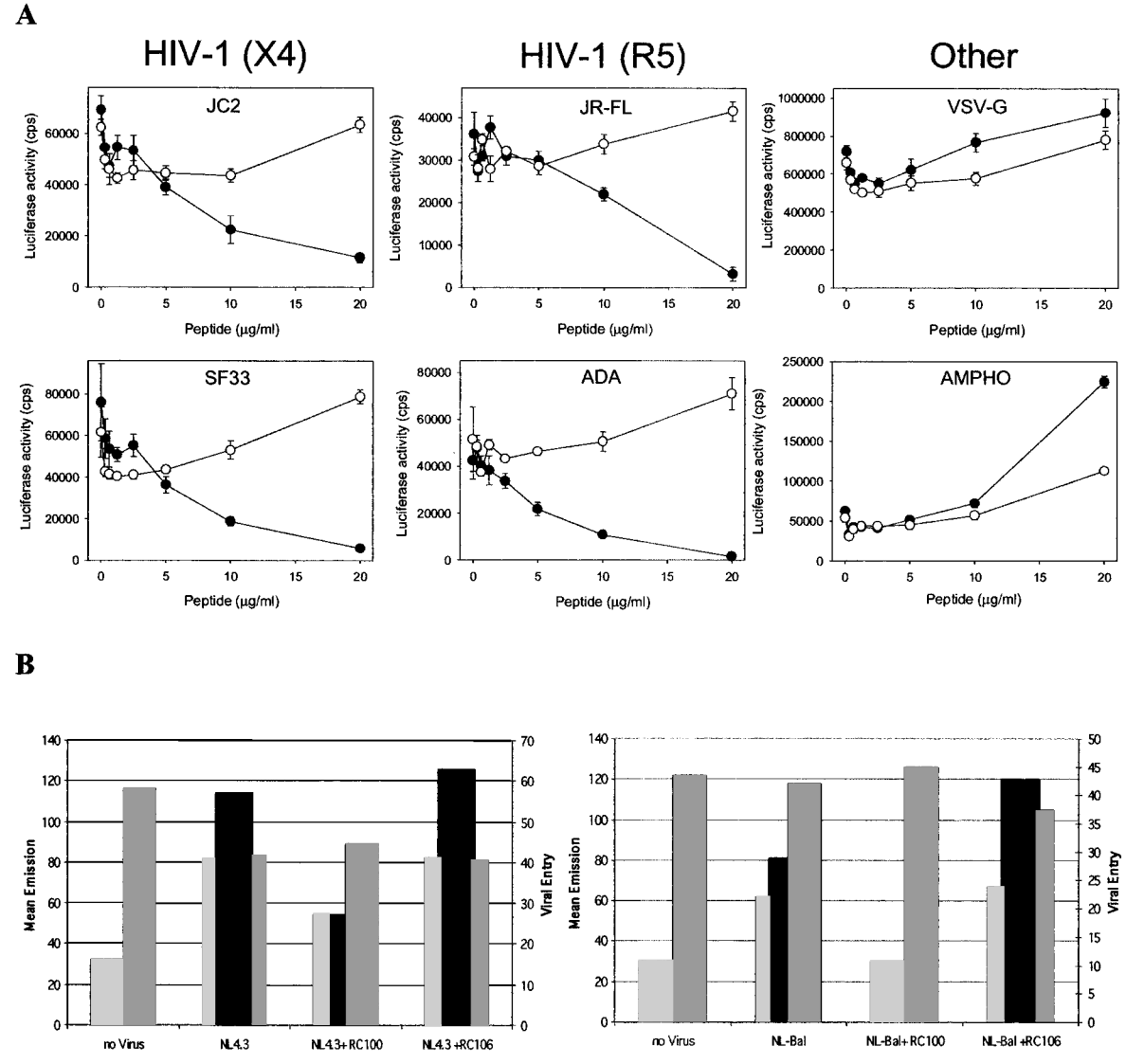

FIG. 2. Retrocyclin blocks HIV-1 infection at the level of viral entry. (**A**) HOS.CD4.CXCR4 or HOS.CD4.CCR5 cells were infected with luciferase reporter viruses pseudotyped by Envs of HIV-1 R5 (ADA and JR-FL) or X4 (JC2 and SF33) viruses or with A-MuLV (AMPHO) Env or VSV-G Env in the presence of RC100 (solid circles) or RC106 (open circles) at the concentrations indicated. Luciferase activity was measured 3 days later ($n = 3$). (**B**) HOS.CD4.CCR5 and HOS.CD4.CXCR4 cells were infected with NL-Bal(BlaM-Vpr) or NL4.3(BlaM-Vpr). After 4–5 hr the virus was removed and the cells were loaded with the BlaM substrate CC2F/AM for 16–18 hr at 25°C. The blue:green ratio was quantitated by flow cytometry. Cleavage of the substrate upon virus entry caused an increase in the blue:green ratio. The blue:green measurement also controls for cell-type differences in dye loading. Center bar = blue mean emission, right bar = green mean emission, and left bar = the percentage of viral entry.

TABLE 1. COMPARISON OF RETROCYCLIN (RC100) IC_{50} AND IC_{90} VALUES IN PRIMARY CELL CULTURE AND HOS.CD4/ENV PSEUDOTYPE SYSTEMS

Assay system	*Virus*	*IC_{50} (μg/ml)*[a]	*IC_{90} (μg/ml)*[a]
Primary $CD4^+$ PBMC	IIIB (X4)	1.0	3.2
	JR-CSF (R5)	1.3	2.6
HOS.CD4	JC2 (X4)	6.1	>20
	SF33 (X4)	5.5	16.0
	JR-FL (R5)	11.9	19.0
	ADA (R5)	4.9	17.8

[a]IC_{50} and IC_{90} values were derived from a four parameter logistic curve using the Fit-curve regression analysis function in SigmaPlot (SPSS, Inc.) of $n = 3$ experiments for each assay system.

as previously described.[10] $CD4^+$-enriched PBMC were infected with HIV-IIIB or JR-CSF HIV-1 isolates in the presence or absence of 20 μg/ml peptide.[10] The cultures were maintained in medium with or without the peptide and supernatants were sampled for $p24^{gag}$ antigen quantitation. Data are shown for Day 9 at the peak of replication (Fig. 1A). The results showed that RC100, RC100b, and RC101 reduced replication of both viruses by at least three orders of magnitude. Thus the addition of a single positive charge in RC100b did not reduce activity. The inclusion of two additional positive charges in RC100c removed activity. RC106 was inactive, showing that Arg_4 was required for activity. RC111 was inactive (Fig. 1A) demonstrating the requirement for the correct order of the amino acid sequence as opposed to content (Fig. 1E).

To determine the step in virus replication that is blocked by retrocyclin, we infected HOS.CD4.CXCR4 or HOS.CD4.CCR5 cells with HIV-1-based luciferase reporter viruses.[18,21] These viruses infect cells in a single cycle but do not replicate due to a frameshift in *env*. Reporter viruses were generated as pseudotypes bearing the glycoprotein of the X4 isolates JC2 and SF33, the R5 isolates JR-FL and ADA, amphotropic murine leukemia virus (A-MuLV), or vesicular stomatitis virus (VSV-G).[22] The last two pseudotypes served to control for postentry or nonspecific effects of the peptides. Cells were incubated with increasing concentrations of RC100 or RC106, the inactive congener containing an Arg to Tyr substitution, and then infected separately with the six reporter viruses. RC100 inhibited entry of the X4- and R5-specific HIV-1 pseudotyped reporter viruses and did not inhibit entry of the A-MuLV or VSV-G Env pseudotypes (Fig. 2A). RC106, which differs from RC100 by only a single amino acid and had no activity on HIV-1 replication in PBMC, had no inhibitory activity on the luciferase reporter viruses. These findings demonstrated that RC100 blocked R5 and X4 pseudotypes at virus entry. Furthermore, the block was not at the level of reverse transcription, integration, transcription, or any nonspecific effects on cell viability or cellular function, because this would have also reduced luciferase activity of the VSV-G and A-MuLV pseudotypes. Thus these findings suggest that retrocyclin inhibits at a step prior to reverse transcription.

IC_{50} and IC_{90} values for retrocyclin (RC100) in these studies and experiments with primary $CD4^+$ PBMC are presented

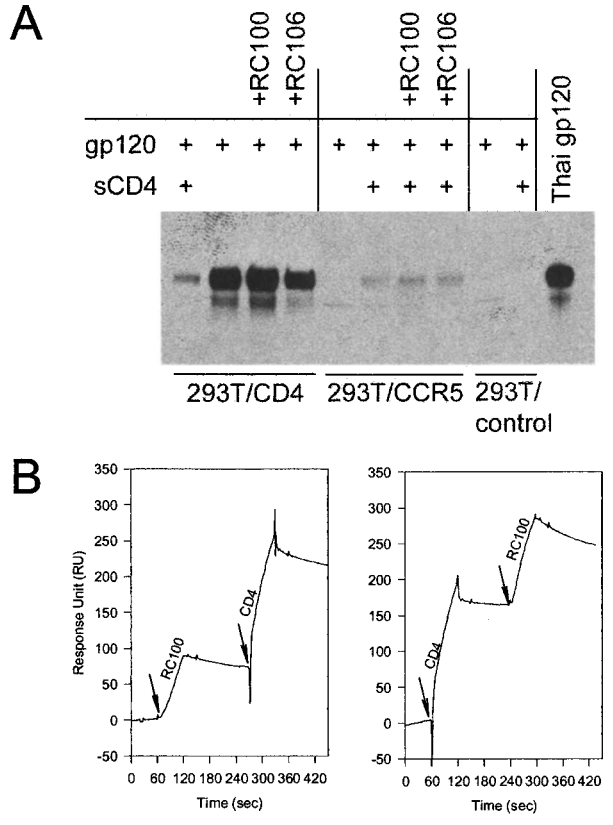

FIG. 3. Retrocyclin does not prevent gp120 from binding CD4 or CCR5. (**A**) 293T cells, transfected with plasmids encoding CD4, CCR5, or control DNA, were incubated in the presence or absence of soluble CD4 (sCD4), ThaiE gp120, RC100, and RC106, and then washed of unbound protein. Western analysis for gp120 was performed on cell lysates subjected to SDS–PAGE. ThaiE gp120 (10 ng) was used as a standard. (**B**) Surface plasmon resonance binding isotherms are shown for RC100 and CD4 binding to immobilized gp120. Arrows indicate the introduction of either 500 nM RC100 or 500 nM CD4 to the flow cell. The left panel shows that the binding of CD4 is not affected by RC100 bound to immobilized gp120, and the right panel reveals that the binding of RC100 is not affected by the presence of CD4 bound to immobilized gp120.

in Table 1. Note that retrocyclin was generally more active in infections utilizing primary PBMC, and that the activity differences are strain dependent. Not surprisingly, the IC_{50} and IC_{90} values for the inactive congener RC106 are >20 μg/ml (data not shown).

To further localize the effect of retrocyclin on HIV-1 replication, we used the Vpr β-lactamase assay that we recently described.[23] In this assay, HIV-1 virions are prepared that are loaded with an enzymatically active β-lactamase-Vpr fusion protein (BlaM-Vpr). When this virus infects a cell, BlaM-Vpr is introduced into the cytoplasm. The target cells are then loaded with the membrane-permeable BlaM substrate dye, CCF2/AM. The ratio of cleaved to uncleaved substrate was then measured by flow cytometry as the ratio of blue:green fluorescence (Fig. 2B). This assay specifically measured release of virion contents into the cytosol.[23] HOS.CD4.CCR5 and HOS.CD4.CXCR4 cells were incubated with RC100 or control peptide RC106 at 20 μg/ml and then exposed to X4 or R5 Vpr-BlaM-loaded virions. FACS analysis of the cells showed that RC100 prevented NL4.3 and NL-Bal HIV-1 entry. The RC106 control peptide was inactive. In the aggregate, these studies suggest that retrocyclin specifically blocks virus entry.

To investigate the mechanism by which retrocyclin blocks HIV-1 entry, we used an *in vitro* assay to test whether retrocyclin blocked the interaction between gp120 and CD4 or CCR5 (Fig. 3A). 293T cells transfected with either CD4 or CCR5 expression vectors were incubated with 20 μg/ml retrocyclin. Recombinant gp120 was added and the cells were washed after 1 hr to remove the free gp 120. The cells were lysed and then bound gp120 was detected on immunoblots probed with anti-gp120 antisera. These experiments showed that retrocyclin did not fully inhibit the binding of gp120 to CD4 or CCR5. SPR was also used to investigate whether RC100 might compete with gp120 for binding to CD4 or CCR5. In this analysis, RC100 did not inhibit the binding of soluble CD4 (sCD4) to gp120 nor did sCD4 inhibit the binding of RC100 to gp120 (Fig. 3B). These results suggest that retrocyclin does not directly interfere with gp120 interactions with CD4 or chemokine receptors on the target cells. However, it should be noted that this does not preclude the possibility that retrocyclin interferes with the binding of virions to, for example, glycosaminoglycans or glycolipids.

To further investigate the mechanism by which RC100 blocked HIV-1 entry, we visualized its binding to the cell surface by fluorescence microscopy. RC100 could not be directly fluoresceinated because it lacks a free amino-terminus or amine side chains. Instead, we utilized RC101, which is biologically active and contains a lysine that provides a free ε amino group for conjugation of BODIPY-FL as previously described.[10] Activated primary lymphocytes were incubated with $RC101_{BODIPY\text{-}FL}$, stained with antibody against CXCR4, CCR5, or CD4, and then visualized by confocal microscopy. This analysis revealed that $RC101_{BODIPY\text{-}FL}$ colocalized with CXCR4, CCR5, and CD4 (Fig. 4). The fluorescent peptide was also present in patches devoid of CXCR4, CCR5, and CD4 suggesting that retrocyclin may interact with additional molecular targets. Indeed other studies by our group have shown that retrocyclin is a lectin that binds to glycolipids with high affinity.[24]

In summary, our data indicate that retrocyclin prevents entry of HIV-1. The peptide blocks entry of virus independent of coreceptor usage but is specific for the HIV-1 glycoprotein. The peptide did not interfere with gp120/CD4 or gp120/CCR5 interaction (Fig. 3A) yet binds with high affinity to gp120[24] (Fig. 3B); however, retrocyclin added directly to virions did not prevent infection of $CD4^+$ T cells.[10] Taken together this suggests that retrocyclin either acts after virion binding or possibly at alternative sites of virion binding including (glyco)lipids.

Potential mechanisms whereby retrocyclin blocks entry are as follows. Retrocyclin could block a postbinding gp120 or gp41 conformational change that occurs on the pathway to fusion. It could prevent the insertion of the gp41 amino terminal fusion peptide into the target cell membrane or, by analogy to the fusion inhibitory peptide T20, prevent prehairpin to hairpin conversion.[25] However, the latter is less likely as our studies have shown that retrocyclin does not bind gp41.[24] Therefore, the most likely scenario is that retrocyclin inhibits the fusion of viral and cellular membranes through interactions with lipids on the cell surface. Indeed RTD-1, the rhesus monkey homolog of retrocyclin, has been shown to bind parallel to lipid membrane surfaces,[26] and similar binding by retrocyclin may alter cell surface properties in a manner that prevents the fusion of cellular and viral membranes. Retrocyclins and retrocyclin-like molecules represent a new class of uptake inhibitors, and pro-

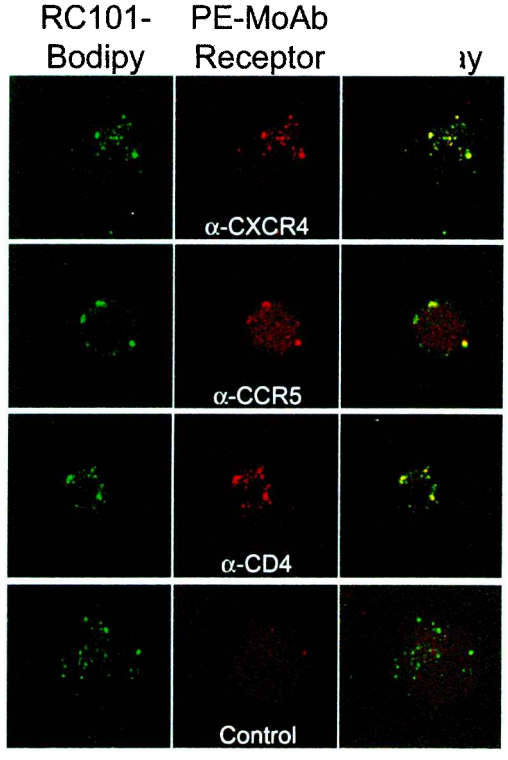

FIG. 4. Retrocyclin colocalizes with CD4, CXCR4, and CCR5 in patches on the surface of T cells. $RC1O1_{BODIPY\text{-}FL}$ (green) and PE-labeled monoclonal antibodies against CD4, CXCR4, and CCR5 (red) were detected by confocal fluorescence microscopy in patches on the surface of activated $CD4^+$-selected PBMC. RC101 colocalized with antibodies against all three cofactors in patches on the surface of PBMC (yellow), but not with PE-labeled isotype control antibodies. Cell diameters are ~15 μm.

vide new templates for the development of antiviral therapeutics and preventatives.

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