CD147 facilitates HIV-1 infection by interacting with virus-associated cyclophilin A

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Cyclophilin A (CyPA) is specifically incorporated into the virions of HIV-1 and has been shown to enhance significantly an early step of cellular HIV-1 infection. Our preliminary studies implicated CD147 as a receptor for extracellular CyPA. Here, we demonstrate a role for CyPA–CD147 interaction during the early steps of HIV-1 infection. Expression of human CD147 increased infection by HIV-1 under one-cycle conditions. However, susceptibility to infection by viruses lacking CyPA (simian immunodeficiency virus or HIV-1 produced in the presence of cyclosporin A) was unaffected by CD147. Virus-associated CyPA communoprecipitated with CD147 from infected cells. Antibody to CD147 inhibited HIV-1 entry as evidenced by the delay in translocation of the HIV-1 core proteins from the membrane and inhibition of viral reverse transcription. Viruses whose replication did not require CyPA (SIV or mutant HIV-1) were resistant to the inhibitory effect of anti-CD147 antibody. These results suggest that HIV-1 entry depends on an interaction between virus-associated CyPA and CD147 on a target cell.

CyPA has been shown to be incorporated into HIV-1 particles during virus morphogenesis through a specific interaction with the CA domain of the Gag precursor polyprotein (11–14) and to function as a chaperone during various cellular processes (2). CyPA also binds with high affinity to immunosuppressive drug cyclosporin A (CsA), and this binding is required for the immunosuppressive effect of CsA (3). In addition to its intracellular functions, CyPA can be secreted into the extracellular environment and has been shown to induce chemotaxis of monocytes, eosinophils, and neutrophils (4, 5). Recent studies in our laboratory (Y.Y., G.Z., M. O’Connor, W. W. Dai, T. Hao, H.G., B.T., B.S., and M.B., unpublished data) prompted us to hypothesize that CD147 might mediate activity of virus-associated CyPA during HIV-1 infection. In this work, we demonstrate that interaction between HIV-1-associated CyPA and CD147 on target cells significantly enhances infection by HIV-1. CD147, therefore, appears to be a cofactor that mediates activity of virus-associated CyPA and is required for efficient infection by HIV-1.

Materials and Methods

Cells. Chinese hamster ovary (CHO)-K1 cell line was purchased from American Type Culture Collection and was cultured in F-12 medium (Life Technologies, Rockville, MD) + 10% bovine fetal serum. Primary nonadherent peripheral blood mononuclear cells (PBMCs) and adherent monocytes were purified from whole blood of healthy donors by Ficoll-Hypaque centrifugation and plastic adherence and were cultured as described (29). PBMC were activated by treating with phytohemagglutinin (5 μg/ml) and IL-2 (20 units/ml) for 3 days. Monocyte-derived macrophages were obtained by allowing plastic-adherent monocytes to differentiate for 7 days in the presence of macrophage colony-stimulating factor (M-CSF) (2 ng/ml).

Transfection and Infection of CHO Cells. Cells grown to 80% confluency in 75-cm² flasks were transfected with 10 μg of CD4,...

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Abbreviations: CyPA, cyclophilin A; CsA, cyclosporin A; A-MLV, amphotropic murine leukemia virus; PBMCs, peripheral blood mononuclear cells; MA, matrix; CA, capsid; RT, reverse transcriptase; CHO, Chinese hamster ovary; SIV, simian immunodeficiency virus; LAV, lymphadenopathy-associated virus.

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viruses, cells were plated at low density (0.01–0.1 x 10^5 cells/well of a 24-well plate) and infected with the virus [1 x 10^6 cpm of reverse transcriptase (RT) activity/well].

**Immunoprecipitation of CD147.** Cells were lysed in a detergent buffer (20 mM triethanolamine, pH 8.0/0.300 mM NaCl/2 mM EDTA/20% glycerol/1% 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate/10 μg/ml leupeptin/10 μg/ml aprotinin/1 mM PMSF) for 30 min at 4°C with continuous rocking and then centrifuged (15,000 g, 15 min). Goat polyclonal anti-CD147 antibody (R & D Systems) was added to the supernatant and incubated for 1 h at 4°C, followed by an overnight incubation at 4°C with Protein G-Sepharose. This antibody did not interfere with CyPA–CD147 interaction, allowing coimmunoprecipitation of these two proteins. Immunoprecipitates were washed three times with 50 mM Tris-HCl, pH 7.5, separated by SDS/PAGE, transferred to poly(vinylidene difluoride) membranes, and analyzed by Western blotting by using rabbit polyclonal anti-CyPA antibody (Affinity Bioreagents, Golden, CO) or anti-EMMPRIN mAb (Leinco Technologies, St. Louis, MO).

**Subcellular Fractionation and Western Blot Analysis.** Subcellular fractions of MT-4 cells infected with HIV-1_LAV (lymphadenopathy-associated virus) were prepared by using a previously published protocol (30) with some modifications. Cells were pelleted and incubated on ice in a hypotonic buffer (10 mM Hepes, pH 6.9/10 mM KCl/0.1 mM PMSF/1 μg/ml aprotinin) for 15 min. Cells were disrupted by Dounce homogenization and nuclei were pelleted at 1,500 g for 5 min and discarded. Supernatant was removed and centrifuged at 18,000 g for 45 min. Supernatant from this centrifugation was reserved and referred to as the cytosolic fraction. The pellet containing cytoskeleton and membranes was resuspended in NTENT buffer (150 mM NaCl/10 mM Tris-HCl, pH 7.2/1 mM EDTA/1% Triton X-100/0.1 mM PMSF/1 μg/ml aprotinin) supplemented with 1% N-octyl-β-D-glycopyranoside and was centrifuged at 18,000 g for 30 min. The supernatant from this spin was reserved and referred to as the membrane fraction, whereas the pellet represents the cytoskeleton fraction. Subcellular fractions from the equivalent of 2 x 10^6 cells/lane were fractionated on a 12% SDS/PAGE and analyzed by Western blot assay by using mAbs to matrix (MA), capsid (CA) (both from Advanced Biotechnologies, Columbia, MD), and actin (Sigma).

**Results**

**CD147 Enhances HIV-1 Infection.** Our recent work (16) suggested that the previously described activity of virus-incorporated CyPA during an early step of HIV-1 infection might be mediated by a CyPA-interacting protein. Because of the demonstrated activity of CD147 as a CyPA receptor (V.Y., G.Z., M. O’Connor, W. W. Dai, T. Hao, H.G., B.T., B.S., and M.B., unpublished data), we hypothesized that CD147 might interact with virus-associated CyPA and stimulate virus infection. For quantitative analysis of CD147 activity in HIV-1 infection, we examined one-cycle infection of CHO cells stably expressing human CD147 (CHO.CD147). The CHO cells were selected because flowcytometric analysis revealed that all human cells that we tested expressed high levels of CD147 (Fig. L4). When plated at low density to dilute the anti-retroviral inhibitory factor produced by CHO cells (31), these cells can be infected with recombinant luciferase-expressing HIV-1 pseudotyped with an envelope of amphotropic murine leukemia virus (A-MLV). Such analysis demonstrated a 4- to 5-fold increase of luciferase expression in CD147-transfected cells (Fig. LB). A similar enhancement was observed when three stable CHO.CD147 clones were compared to clones of CHO cells transfected with empty vector pcDNA3.1 (Fig. 1C Bottom). Enhancement of HIV-1 infection correlated with CD147 expression on cells (measured by flow cytometry, Fig. 1C Top). Importantly, the observed increase in HIV-1 infection because of the presence of CD147 was similar to the increase provided by CyPA (ref. 21 and results not shown), suggesting that CD147 accounts for most of CyPA activity (see below). One of these CHO.CD147 clones (CHO.CD147.17) was used in further experiments.

To rule out the possibility that the enhancing effect of CD147 on viral infection had something to do with the inhibitory factor produced by CHO cells (31), two types of experiments were performed. First, we investigated whether the enhancing effect of CD147 correlates with the density at which cells were plated. If CD147 counteracts the activity of the CHO-produced negative factor, than one can expect the CD147-specific enhancing effect to diminish when cell density decreases. However, this phenomenon was not the case; regardless of the plating density, the enhancing effect of CD147 was relatively constant (Fig. 1D). In a second series of experiments, we transiently transfected CHO.CD147 (and control CHO,pcDNA) cells with CD4- and CCR5-expressing vectors and infected them with luciferase-expressing HIV-1 construct pseudotyped with LAV envelope. Because the negative factor produced by CHO cells is specific for the A-MLV envelope (32), it is not expected to influence infection by such virus. Similar to results obtained with the A-MLV envelope, a 3- to 4-fold increase in luciferase expression was observed in cultures of cells expressing CD147 (Fig. 1E).

No increase in HIV-1 infection was observed when the virus was produced in the presence of CsA (Fig. 1F) and thus was deficient in CyPA (Fig. 1F Inset). As a result of CsA treatment, infectivity of this virus in CHO.CD147 (but not in control CHO,pcDNA) cell cultures was significantly attenuated, consistent with a previously demonstrated requirement of CyPA for efficient infection with EmvA-MLV-pseudotyped HIV-1 (15, 33). In contrast, infection with EmvA-MLV-pseudotyped simian immunodeficiency virus (SIV)-based virus, which does not depend on CyPA (34), was unaffected by the presence or absence of CD147 on the target cells (Fig. 1F).

Based on results presented in Fig. 1, we conclude that CD147 enhances HIV-1 infection. This effect is independent of the viral envelope but requires the presence of virus-associated CyPA.

**Virus-Associated CyPA Interacts with CD147.** To directly demonstrate interaction between viral CyPA and cellular CD147, we performed coimmunoprecipitation analysis. Primary PBMC cultures were infected with equal amounts (by RT activity) of HIV-1 (produced in the presence or absence of CsA) pseudotyped with the A-MLV envelope (to increase the efficiency of infection). After a 30-min incubation at 4°C, cells were either left at 4°C (to allow virus attachment but not fusion) or were transferred to 37°C for 1 h (to allow attachment followed by virus-cell fusion) and lysed, and CD147 was immunoprecipitated by using an anti-CD147 polyclonal antibody. Immunoprecipitated material was analyzed by Western blotting with antibodies to CyPA or CD147 (immunoprecipitation control). As shown in Fig. 2, no CyPA was detected in the immunoprecipitates from mock-infected cells (lane 1) or cells incubated with the virus at 4°C (lane 2), indicating that cellular CyPA does not interact with CD147 under such experimental conditions and that virus attachment is not sufficient for interaction between viral CyPA and CD147. However, CyPA was coprecipitated with CD147 from cell lysates after the 1-h incubation of virus and cells
at 37°C (lane 3). Under similar conditions, no CyPA coprecipitated with CD147 if infection was performed with HIV-1 grown in the presence of CsA (lane 4), indicating that CyPA detected in this assay is derived from infecting virions. We conclude that virus-associated CyPA interacts with CD147. This interaction appears to be temperature-dependent, suggesting that rearrangement of viral and/or cell membrane proteins is required for stable binding of CyPA to CD147.

**Anti-CD147 mAb Inhibits Infection by CyPA-Dependent HIV-1 Variants.** To investigate the mechanism of CD147 activity in more detail in the context of primary cells, we used an anti-CD147 antibody to block interaction between CyPA and CD147. As shown in Fig. 3A, anti-CD147 antibody, but not isotype-matched control mAb, inhibited infection by both CCR5- (ADA) and CXCR4-dependent (LAV) HIV-1 strains in primary PBMC cultures. The incomplete suppression of infection by this anti-CD147 mAb
(~50%) might be because of its poor CD147-neutralizing activity. Indeed, a much more potent inhibitory effect (~80% inhibition of infection, not shown) was observed with a different anti-CD147 antibody, E11F4 (35). Unfortunately, this antibody was unavailable in the amounts necessary to perform all of the experiments described in this report. Therefore, we used a less effective commercially available antibody. Nevertheless, this antibody allowed us to obtain valuable information about the mechanism of CD147 activity (see below).

To prove that the effect of the anti-CD147 mAb was due to inhibition of interaction between viral CyPA and CD147, we took advantage of SIV constructs, which differ in the ability to incorporate CyPA. The SIVmac239 construct does not incorporate CyPA and its replication is resistant to CsA, whereas SIVmac239(HIV-CA), which carries a chimeric Gag containing a small fragment of HIV-1 CA, incorporates CyPA and is sensitive to CsA (34). As shown in Fig. 3B, replication in human PBMC of SIVmac239(HIV-CA) was inhibited...
CD147 Regulates HIV-1 Entry. Using a semiquantitative PCR assay, we analyzed the amount of HIV-1-specific RT products in anti-CD147 mAb-treated PBMC cultures at different times after infection. Using long terminal repeat R/U5 primer pair (29), we amplified a short DNA fragment that is produced both by endogenous intravirion RT (36) and intracellularly early after the virus entry (37) and can be used as a measurement for the efficiency of entry/initiation of RT. Results presented in Fig. 4 demonstrate a reduction in the amount of this RT product in anti-CD147 mAb-treated cells compared to control (untreated) or isotype-treated cultures. The difference between anti-CD147-treated and -untreated cells was greatest at early time points (1.5 and 4 h) after inoculation and decreased thereafter, suggesting that the antibody delayed virus entry. The anti-CD4 mAb, which inhibits HIV-1 attachment, reduced the amount of long terminal repeat R/U5-amplified fragment at all time points with similar efficiency (Fig. 4A).

As a different way to look at early steps of infection, we analyzed the intracellular distribution of HIV-1 MA and CA proteins early after de novo infection. Previous studies (30) demonstrated that soon after infection, MA relocates from the membrane to the cytoskeleton, whereas CA migrates into the cytosol. This observation is consistent with earlier demonstration that virus uncoating involves dissociation of CA from the nucleoprotein complex (23, 24). Consistent with these results, we observed a characteristic translocation of the MA protein from the membrane into the cytoskeleton fraction 1.5 h after infection (Fig. 4B, isotype panels, lane CS), whereas CA was found predominantly in the cytosol at this time point (isotype panels, lane C). In contrast, protein rearrangement was significantly delayed in cells treated with the anti-CD147 antibody (Fig. 4B, αCD147 panels). This result indicates that in the presence of anti-CD147 mAb rearrangement of CA and MA, presumably associated with formation of the RT complex (30), is delayed and supports the idea that CD147 is involved in the regulation of HIV-1 entry.

Discussion

Taken together, results presented in this report indicate that CD147 interacts with virus-associated CyPA and regulates an early step in HIV-1 replication. A recent study by Saphire et al. (28) suggested a role for CyPA in virus attachment through binding to sulfated proteoglycans (heparans). It seems plausible that CyPA interaction with CD147 is downstream of CyPA–heparan interaction. Indeed, initial interaction of CyPA with heparans might facilitate its subsequent binding to CD147, similar to the situation with chemokines whose binding to glycosaminoglycans enhances subsequent interaction with their receptors (38, 39). Similarly, binding of CyPB to heparans presumably presents the immunophilin for interaction with its functional receptor (type I site) (40). Involvement of a CyPA-interacting receptor in an early step of HIV-1 infection is consistent with our earlier finding (16) that exogenously added CyPA inhibits HIV-1 infection, presumably by competition for CD147 with virus-associated CyPA.

Several mechanisms may account for the activity of CD147 during the early phase of the HIV-1 life cycle. Intracellular signaling events initiated by CyPA–CD147 interaction (V.Y., G.Z., M. O’Connor, W. W. Dai, T. Hao, H.G., B.T., B.S., and M.B, unpublished data) might induce MA phosphorylation, which was suggested to regulate detachment of the RT complex from the membrane (41). Signaling from CD147 might also induce cytoskeleton rearrangements, facilitating virus entry. It is also conceivable that CyPA binding to CD147 might affect conformation of another CyPA-binding partner, CA, leading to destabilization of the capsid shell. This latter mechanism is similar to the one proposed in earlier studies (15). Finally, CyPA binding to CD147 might promote transition from the step of hemifusion to complete fusion (42), allowing liberation of the RT complex into the cytoplasm. All these mechanisms are not mutually exclusive and may well function simultaneously or sequentially.

Interaction between virus-associated CyPA and CD147 during HIV-1 entry suggests that different binding sites on CyPA are engaged by CA and CD147. This notion is supported indirectly by our finding that CyPA-CD147 binding is resistant to CsA (V.Y., G.Z., M. O’Connor, W. W. Dai, T. Hao, H.G., B.T., B.S., and M.B, unpublished data). It should be noted that the mechanisms of interaction between free CyPA and CD147 do not necessarily apply to the interaction between CD147 and HIV-1-associated CyPA. Indeed, in contrast to free CyPA studied there, CyPA in the virus particle is bound to the capsid protein (11). The activities and interactions of free vs. bound CyPA may be very different. As an example, a complex between CyPA and CsA binds and inhibits calcineurin, whereas neither CsA nor CyPA alone are capable of binding calcineurin (3, 43). More studies will be needed to define the binding interactions between CD147 and CyPA during HIV-1 infection.

Expression of the human CD147 on CHO cells does not appear to be absolutely necessary for HIV-1 infection (in contrast to CD4, CCR5 or CXCR4), as low level infection occurs without it. This is illustrated by only a 6- to 8-fold difference between CyPA-positive and CyPA-deficient HIV-1 in one-cycle infection (21). During long-term culture, this difference evolved to enhance the virus can achieve. It appears that HIV-1 evolved to enhance its infection process by using not only a cellular receptor but also its ligand, thus subverting the target cell’s ligand–receptor interaction pathway for the virus’s own purpose.

The use of CyPA and CD147 for infection sets HIV-1 apart from other primate lentiviruses that do not rely on these factors. The ability to incorporate CyPA and use CD147 might complement the viral strategies acquired at the early stages of HIV evolution, during adaptation of the virus to the human host, and might contribute to high replication capacity and pathogenicity of HIV-1. Further detailed analysis of CD147 activity during HIV-1 infection is likely to identify the exact step regulated by CD147 and might define novel targets for anti-HIV interventions.

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