HIV-1 interaction with human mannose receptor (hMR) induces production of matrix metalloproteinase 2 (MMP-2) through hMR-mediated intracellular signaling in astrocytes

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Abstract

Astrocytes are susceptible to HIV-1 infection. We have recently demonstrated that human mannose receptor (hMR) is directly involved in CD4-independent HIV-1 infection of astrocytes. The apparent paradox between the vivid binding affinity of HIV-1 gp120 protein to hMR and the low efficiency of hMR-mediated HIV-1 infection raises the possibility that HIV-1 binding to hMR alone may negatively affect astrocyte function. In this study, we examined the relationship between HIV-1 interaction with hMR and the production of matrix metalloproteinases (MMPs) in astrocytes. We took advantage of an astroglial cell line U87.MR stably expressing hMR as an in vitro astrocyte model system and human primary astrocytes, and demonstrated that HIV-1 binding to astrocytes induced the production of MMP-2. This induction appeared to be most potent with M-tropic HIV-1 viruses. Increased MMP-2 production was not due to hMR-mediated HIV-1 entry and/or HIV-1 viral gene expression, as the transfection of HIV-1 proviral DNA did not result in MMP-2 production, and the infection of AT-2-treated HIV-1 viruses did not inhibit MMP-2 production. Direct involvement of hMR in HIV-induced MMP-2 production was confirmed by the inhibition of the yeast mannan, an hMR ligand antagonist, and an anti-hMR serum. Furthermore, HIV-induced MMP-2 production in astrocytes was shown to involve hMR-mediated intracellular signaling. Taken together, these results suggest that HIV-1 binding to astrocytes in the absence of HIV-1 viral entry is sufficient to alter astrocyte function through hMR-mediated intracellular signaling. In addition, these results provide new evidence to support the notion that hMR is capable of eliciting intracellular signaling upon ligand binding.

Keywords: HIV-1; Human mannose receptor; Intracellular signaling; Astrocyte; Matrix metalloproteinase 2 production

1. Introduction

A majority of AIDS patients experience HIV-1 infection of the central nervous system (CNS) and exhibit neurological dysfunctions, such as memory loss and motor control deficits [1]. HIV-1 infection-associated neuropathologies include reactive astrogliosis and cerebral atrophy in the early course of infection, and the formation of myelin pallor, multinucleated giant cells, an abnormal blood–brain barrier (BBB), and neuron death at the later stages of the disease [2–9]. Microglia/macrophages are the main target for HIV-1 infection in the brain [10–13].

A number of studies have shown that HIV-1 infects astrocytes [14–17], but in a restricted fashion [18–20]. Astrocytes constitute a major population of cells in the brain and play an indispensable role in maintaining normal brain functions. These functions include secretion of neurotrophic factors [21–23], regulation of the interstitial...
pH [24,25], uptake and metabolism of neurotransmitters [26,27], antioxidant defense via scavenging and transforming oxygen free radicals into nontoxic species [28–30], modulation of neuronal signals [31–33], an essential structural component of the blood–brain barrier (see a review Ref. [34]), and immune responses through producing and secreting a host of cytokines, proteases, protease inhibitors, adhesion molecules, extracellular matrix components that are key mediators of immunity and inflammation [35,36]. Although it is important to note that several above-mentioned functions are still controversial, it is highly conceivable that the dysfunction of astrocytes could potentially contribute to HIV-associated neurological diseases.

Although increased BBB permeability has been regarded as a hallmark of HIV-associated neuropathogenesis, pathogenic mechanisms involved in the loss of the BBB integrity in the course of HIV/AIDS are not well understood. Studies have shown that levels of laminin and type IV collagen, the major protein components of the BBB, are decreased in the HIV-infected brain [37,38]. Corroborating these findings are studies that matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9 that mainly target type IV collagen, are elevated with HIV-1 infection. HIV-1 envelope proteins have been reported to induce MMP-2 overproduction by nerve cells [39], or in the brain of gp120 transgenic mice [40], resulting in the destruction of the extracellular matrix. MMP-9 induction has been reported in the cerebrospinal fluid (CSF) of HIV-infected patients [39,41,42]. MMP-9 has also been linked to neurobehavioral deficits of simian immunodeficiency virus-infected monkeys [43]. HIV-1 infection of microglia and contact between HIV-1 and astrocytes have recently been shown to induce MMP-2 and MMP-9 [44,45]. Although the induction mechanisms are largely unknown, these studies suggest that the release of MMPs from glial cells may represent a critical event in the pathogenesis of HIV-associated neuropathogenesis.

We have recently demonstrated that human mannose receptor (hMR) is required for CD4-independent HIV-1 infection of astrocytes [46]. hMR interaction with some of its ligands has been shown to activate the secretion of lysosomal enzymes, the expression of tumor necrosis factor, interleukin-12, and MMP-9, and signal-regulated protein kinases [47–51]. Therefore, besides the adverse effects of hMR-mediated HIV-1 entry into astrocytes, it is very likely that hMR-mediated intracellular signaling induced by HIV-1 gp120 binding may contribute, to an even more significant extent, to astrocytes dysfunction and eventually to AIDS neuropathogenesis. Thus, we decided to assess the importance of HIV-1 interaction with hMR on astrocytes, with an emphasis on its effects on the production of matrix metalloproteinases (MMPs). These results showed that HIV-1 binding to hMR induced MMP-2 production in astrocytes through hMR-mediated pathway involving the activation of phosphatidylinositol 3-kinase (PI3K) and extracellular signal-regulated kinase (Erk).

2. Materials and methods

2.1. Chemicals and antibodies

Dulbecco’s Modification of Eagle’s Medium (DMEM), penicillin, streptomycin, L-glutamate, and sodium pyruvate were purchased from Invitrogen (Carlsbad, CA). Fetal calf serum (FCS) was from Hyclone (Logan, UT). Gelatin, mannan from *Saccharomyces cerevisiae*, trypsin, and LY-294,002 hydrochloride were purchased from Sigma (St. Louis, MO). Standards for MMP-2 and MMP-9 were obtained from R&D systems (Minneapolis, MN). R-250 Coomassie Brilliant Blue was purchased from Pierce (Rockford, IL). Mouse monoclonal antibodies anti-Erk1, P-Tyr, and PI3-Kinase p110α were purchased from BD Transduction Laboratories (San Diego, CA), and the rabbit anti-PI3K 110 polyclonal antibody and the goat anti-mouse horseradish peroxidase-conjugated secondary antibody were from Santa Cruz Biotechnology (Santa Cruz, CA).

2.2. Cell cultures

U87.MG (human astroglialoma cells) and 293T cells were purchased from American Type Culture Collection (ATCC, Manassas, VA). U87.MR cells stably expressing human mannosene receptor were generated in our laboratory and described elsewhere [46]. U87.CD4.CCR5 cells were obtained from NIH AIDS Reagents Program (donated by Dr. D. Litman). Human primary astrocytes were purchased from Clonetics (East Rutherford, NJ) and were used directly in these experiments without further manipulations and passages. By immunofluorescence staining, using an antibody specific for astrocyte cell marker glial fibrillary acidic protein (GFAP), the percentage of GFAP-positive cells was determined to be more than 92–95%. All cells used in this study were cultured in DMEM supplemented with 10% of fetal calf serum (FCS) was from Hyclone (Logan, UT). L-glutamine, 100 units/ml penicillin, 100 units/ml streptomycin, and 1 mM sodium pyruvate at a humidified, 37 °C, and 5% CO₂ incubator.

2.3. Transfection and HIV-1 virus preparation

293T cells were seeded in a 10 cm² culture dish at a density of 2×10⁶ cells per dish and allowed to grow overnight. Next day, the cells were transfected with HIVGFP reporter DNA [52] (16 μg) and each of envelope expressing plasmids (4 μg) using the standard calcium precipitation method. The envelope plasmids include M-tropic YU-2.6 env, T-tropic HXB2.env, and vesicular stomatitis virus glycoprotein (VSV-G) [52]. The transfection medium was replaced with fresh complete medium 12–16 h thereafter, and the cells were continued to culture for 48 h. The culture supernatants were then collected, removed of cell debris by a brief centrifugation, and stored at −80 °C as the virus stock. For the U87.MR cells, the transfection was performed using Lipofectamine 2000 (Invitrogen) according
to the manufacturer’s instructions. Using the pcDNA3.GFP as an indicator, the transfection efficiency was determined to be at least 30% or higher in U87.MR cells. 2',3'-Dithiodipiridine (aldithiol-2) (AT-2) inactivation of HIV-1 viruses was performed as described [53]. Briefly, virus preparations were treated with 1 mM AT-2 for 1 h at 37 °C and then kept on ice for 2 h. At the end of the inactivating procedure, the treatment agent was removed by ultracentrifugation at 17,000×g for 1 h at 4 °C. Viral pellets were resuspended in regular cell culture medium.

### 2.4. Reverse transcriptase (RTase) assay

To determine the RTase activity of the viruses, an aliquot of virus stock was pelleted at 18,200×g and 4 °C for 1 h to recover the viruses. The viruses were suspended in 10 μl of the dissociation buffer containing 50 mM Tris–HCl, pH 7.5, 0.25% Triton X-100, 20% glycerol, 1 mM DTT, and 0.25 M KCl and were inactivated by three cycles of frozen-thawing. To the suspension was then added 35 μl of the reaction buffer containing 50 mM Tris–HCl, pH 7.5, 1 mM DTT, 10 mM MgCl2, and 0.25% Triton X-100, and 5 μl of 1 mg/ml poly (A),(dT)15 (Roche, Indianapolis, IN) and 1 μl of [3H]-thymidine 5'-triphosphate tetrasodium salt (ICN, Irvine, CA). The mixture was incubated at 37 °C for 1 h and then spotted on the DE81 ion exchange chromatographic disk (Whatman, Clifton, NJ). After an extensive wash with 2× SSC (0.3 M NaCl, 30 mM Na citrate, pH 7.0) and dehydration with 100% ethanol, the disks were counted for 3H-incorporation using a scintillation counter. The counts per minute were used to express the RTase activity.

### 2.5. HIV-1 infection

U87.MG or its derivatives were seeded in a 6-well plate at a density of 4×10⁶ cells per well and allowed to grow in a serum-free medium overnight. Next day, the cells were washed with phosphate-buffered saline (PBS) and then infected with HIV-1 viruses equivalent to 10 K cpm RTase per minute were used to express the RTase activity. Infected with HIV-1 viruses equivalent to 10 K cpm RTase serum-free medium overnight. Next day, the cells were harvested using a cell harvester, and stored at 4 °C until the harvested cell debris is removed by ultracentrifugation at 17,000×g for 1 h at 4 °C. Viral pellets were resuspended in regular cell culture medium.

### 2.6. Gelatin zymography

The total protein of the cell lysates was used to normalize the amount of cell culture supernatants for MMP production assay. The supernatants were mixed with an equal volume of the zymography sample loading buffer containing 0.5 M Tris–HCl, pH 6.8, 4% SDS, 20% glycerol, and 0.01% bromophenol blue, and were incubated at room temperature (RT) for 10 min. The mixtures were then electrophoretically separated on 10% polyacrylamide gel containing 2 μg/ml of gelatin. For gelatin zymography development, the gel was first incubated in the renaturing buffer containing 2.5% Triton X-100 at RT for 1 h. The gel was then incubated in the developing buffer containing 50 mM Tris–HCl, pH 6.8, 0.2 M NaCl, 5 mM CaCl2, and 0.02% Brij 35 at RT for 30 min, replaced with the fresh developing buffer, and continued at 37 °C for 20 h with constant agitation. Finally, the gel was stained in 0.5% Coomassie blue R-250 for 3 h and destained in methanol:acetic acid:water (50:10:40) until clear bands appeared in a blue background gel. The bands are areas in which MMPs degrade the substrate gelatin. The bands were quantified using a densitometer (Alpha InnoTech, San Leandro, CA).

### 2.7. Immunoprecipitation (IP) and Western blot

For IP, cell lysates (500 μg protein) were incubated with 1 μg appropriate antibodies in a total of 1 ml IP binding buffer containing 0.5% NP40, 2 mM EDTA 137, mM NaCl, 10% glycerol, 50 mM Tris–HCl, pH 8.0, at 4 °C for 1 h with constant agitation. 25 μl of protein A sepharose CL-4B (Amersham, Piscatway, NJ) was added to the mixture, and the mixture was incubated at 4 °C for 1 h. The sepharose beads were recovered by a brief centrifugation and washed in the IP washing buffer containing 0.5% NP-40, 2 mM EDTA, 0.4 M NaCl, 10% glycerol, 50 mM Tris–HCl, pH 8.0, for three times. Finally, the beads were suspended in 25 μl SDS sample loading buffer, heated at 100 °C for 5 min, and subjected to electrophoretic fractionation on 10% SDS-PAGE. For Western blot, the proteins on the SDS-PAGE was electrotransferred to the HyBond-P membrane (Amersham). After the transfer, the membranes were blocked in 5% dry non-fat milk (for Erk1, PI3K 110, and hMR) or 3% of BSA (for p-Tyr) for 1 h, then incubated with appropriate primary antibodies and horseradish peroxidase-conjugated secondary
antibodies. Finally, the membranes were developed with a chemiluminescence ECL kit (Amersham).

3. Results

3.1. Induction of MMP-2 in HIV-1-infected astrocytes

To determine whether HIV-1 infection would alter MMP production in astrocytes, we took advantage of U87.MR cells, which was derived from astroglial U87.MG cells and engineered to stably express hMR, the astrocyte receptor for CD4-independent HIV-1 infection [46]. We infected U87.MR cells with HIV-GFP reporter viruses pseudotyped with M-tropic YU-2 envelope protein (YU2.env) and determined the production of MMP-2 and MMP-9 in the culture supernatants of these cells 48 h after infection. The infection efficiency, as determined by GFP expression, was about 6% [46]. Gelatin zymography showed that HIV-GFP viruses pseudotyped with YU2.env increased MMP-2 production by about 3-fold over the HIV-GFP viruses containing no envelope protein (No env) (Fig. 1A). In contrast, HIV-GFP viruses pseudotyped with YU-2 env only gave rise to a slight induction of MMP-2 in the parental U87.MG cells, which do not express hMR [46]. The higher basal level of MMP-2 in the supernatants of the U87.MR cells are likely due to other mannosylated ligands such as mannos-binding protein (MBP) in the fetal calf sera that were used as a supplement in the cell culture medium and their bindings to hMR, and as a result, leading to intracellular signaling and MMP-2 production. To determine whether the MMP-2 induction was a result of HIV-1 infection, we heat-inactivated the viruses and determined its effects on the MMP-2 production. Surprisingly, heat inactivation at 100 °C for 10 min did not considerably alter the MMP-2 induction (Fig. 1A), suggesting that viral entry may not be required for this induction. To determine the kinetics of MMP-2 induction, we infected U87.MR cells with HIV-1 viruses pseudotyped with YU-2 envelope, collected culture supernatants at 0, 12, 24, 36, and 48 h, and determined the MMP-2 level. We also included U87.MG cells as the control. The results showed that MMP-2 induction occurred as early as 12 h after infection and maximized at 48 h (Fig. 1B). In contrast, there was only a basal level of MMP-2 in the supernatants of U87.MG cells infected with HIV-1 viruses. Thus, 48 h was used as the time point for MMP-2 determination throughout the study.

In addition, we also infected the cells with various amounts of viruses. We found that at low levels of input viruses, MMP-2 production showed a dose-dependent increase, whereas at higher levels of the input viruses, MMP-2 induction was inhibited (Fig. 2). This inhibition at higher levels of input viruses may likely be due to the competition of other hMR ligands in the virus stock (serum-containing culture supernatants), such as soluble mannos-binding proteins with HIV-1 gp120 for hMR. Nevertheless, there was no MMP-9 induction in the U87.MR cells upon HIV-1 infection (data not shown).

3.2. hMR-dependent MMP-2 production in astrocytes

Next, we determined the role of hMR in MMP-2 production in astrocytes. We first took advantage of U87.CD4.CCR5 cells, the other derivative of U87.MG cells. These cells stably express HIV-1 receptors CD4 and CCR5 and are susceptible to M-tropic HIV-1 infection [54]. We compared MMP-2 production in HIV-infected U87.MR and U87.CD4.CCR5 cells. As shown above, HIV-1 infection induced MMP-2 production in U87.MR cells by about 3-fold and little in the U87.MG cells (Fig. 3). Although the same amount of HIV-GFP viruses pseudotyped with YU2.env infected U87.CD4.CCR5 cells more efficiently, i.e., over 50%, as assessed by GFP expression, the MMP-2 production in these cells was...
barely detectable. These results again suggest that HIV-1 entry is not related to the MMP-2 production in astrocytes. To further test the hypothesis, we transfected U87.MR cells with HIV-GFP proviral DNA, which allows to bypass the interaction of HIV-1 viruses and hMR at the cell surface, and determined MMP-2 production in these cells. Consistent with our previous findings, HIV-1 infection induced MMP-2 production in U87.MR cells (Fig. 4). In contrast, the transfection of HIV-1 proviral DNA did not result in an increased MMP-2 production in these cells. Nevertheless, it is interesting to note that a more active MMP-2 (the lower band) was produced in these transfected cells. In addition, to further ascertain whether the hMR-mediated viral entry, followed by HIV-1 gene expression, is also involved in MMP-2 production, we determined MMP-2 induction using HIV-1 viruses inactivated by AT-2 treatment, which has been shown to inactivate HIV-1 viruses but retain the conformational and functional integrity of their surface gp120 glycoproteins [53]. The results showed that AT-2 treatment only had a very slight effect on MMP-2 production (Fig. 4), suggesting that MMP-2 induction is primarily a result of HIV-1 binding to the hMR on the cell surface. Taken together, these results demonstrate that hMR expression and its interaction with HIV-1 are directly involved in MMP-2 induction in astrocytes.

3.3. MMP-2 production in astrocytes is specific to M-tropics HIV-1 viruses

We have recently shown that both M-tropic and T-tropic HIV-1 viruses are equally capable of gaining entry into astrocytes through the hMR [46]. Thus, we then determined whether T-tropic HIV-1 viruses were also able to induce MMP-2 production in these cells. We infected U87.MR cells with HIV-1 viruses pseudotyped with the HIV-1 HXB2 envelope protein (HXB2.env), representative of T-tropic HIV-1 envelope proteins. In addition, we also included, as a control, vesicular stomatitis virus glycoprotein (VSV-G), which confers the indiscriminative ability of HIV-GFP viruses to enter almost any types of cells. Compared to HIV-GFP viruses containing no env, HIV-GFP viruses pseudotyped with HXB2.env showed little effects on MMP-2 production in U87.MR cells, as well as in U87.MG cells (Fig. 5). On the other hand, as shown before,
HIV-GFP viruses pseudotyped with YU2.env, which is representative of M-tropic HIV-1 envelope proteins, induced MMP-2 production in U87.MR cells and not much in the U87.MG cells. In agreement with our previous results, although HIV-GFP viruses pseudotyped with VSV-G entered both U87.MG and U87.MR cells, no MMP-2 induction was detected in these cells.

3.4. Inhibition of MMP-2 production in astrocytes by the hMR ligand antagonist yeast mannan

Yeast mannan has been shown to be the potent antagonist for hMR ligands, including HIV-1 viruses and gp120 protein [46]. Thus, we next determined whether yeast mannan would abrogate MMP-2 production in astrocytes upon HIV-1 infection. We pre-treated U87.MR cells with 3 mg/ml yeast mannan and then infected them with HIV-GFP viruses pseudotyped with YU2.env. The yeast mannan was also present during the infection and after infection. The cell culture supernatants were serially diluted to allow the accurate determination of the effects of the yeast mannan on MMP-2 production. The MMP-2 level in the original supernatants collected from the cells treated with the yeast mannan and then infected with HIV-GFP viruses pseudotyped with YU2.env was similar to that in the 1:8 diluted supernatant collected from the cells infected with the same HIV-GFP viruses (Fig. 6). Hence, the inhibition of the yeast mannan was over 80%. These results further ascertain that MMP-2 production results from the interaction of hMR and HIV-1 viruses or gp120 and raise the possibility of hMR-mediated intracellular signaling.

3.5. hMR-mediated intracellular signaling in astrocytes upon HIV-1 binding

To determine the possible roles of hMR-mediated intracellular signaling in astrocytes upon HIV-1 infection, we pre-treated U87.MR cells in a buffer containing 1 mM sodium orthovanadate, which is often used to inhibit the enzymatic activity of a number of phosphatases. We then incubated the cells with HIV-1 viruses and collected the cells at different times and analyzed for protein phosphorylation by Western blot using anti-P-Tyr antibody. Our preliminary results indicated significant changes of tyrosine phosphorylation of three proteins, approximately at the molecular weights of 168 kDa, 110 kDa, and 42 kDa, in U87.MR cells upon HIV-1 stimulation, but no changes of any proteins were detected in the U87.MG cells (data not shown). Based on the molecular weights of these proteins, we stipulated these three proteins to be tyrosine-phosphorylated hMR (p-MR), PI3K p110α (p-PI3K), and Erk1 (p-Erk), respectively. To confirm the identities of these three proteins, we performed immunoprecipitation using the antibodies specific for each of these proteins and then analyzed the immunoprecipitates by Western blot using anti-P-Tyr antibody and each of these three antibodies. The results showed that HIV-1 stimulation induced tyrosine phosphorylation of hMR, PI3K p110α, and Erk1 within 5 min of the stimulation, which gradually returned to or close to the basal levels (Fig. 7).
3.6. Inhibition of MMP-2 production in astrocytes by a PI3K inhibitor, yeast mannan, and anti-hMR sera

Then, we determined whether HIV-1 infection also resulted in MMP-2 reduction in human primary astrocytes and whether hMR-mediated intracellular signaling was involved in HIV-1 infection-induced MMP-2 production. We pre-treated human primary astrocytes with 20 μM LY-294,002, a specific inhibitor of PI3-K p110α, and then infected the cells with HIV-1 viruses. The same concentration of LY-294,002 was maintained during and after the viral infection. We then determined MMP-2 production. We also included DMSO, the solvent for LY-294,002, as a control. Compared to the untreated cells, LY-294,002-treated cells reduced MMP-2 production in HIV-infected cells close to that of the cells infected with HIV-GFP containing no env (Fig. 8). Nevertheless, a slight decrease of MMP-2 production in the cells treated with LY-294,002 and then infected with HIV-1 containing no env was likely due to the effects of DMSO, since DMSO treatment alone showed a similar reduction of MMP-2 production in these cells infected with HIV-1 viruses containing YU2.env or no env. In addition, we also treated the cells with anti-hMR sera and hMR antagonist yeast mannan and determined their effects on MMP-2 production. The results showed that treatment of these cells with yeast mannan and anti-hMR sera both abrogated MMP-2 production, while the pre-immune sera did not show any effects. Conversely, we found that yeast mannan and anti-hMR sera blocked the tyrosine phosphorylation of hMR, PI3K p110α, and Erk1 induced by HIV-1 viruses (data not shown). These results indicate that hMR-mediated intracellular signaling plays an important role in HIV-1 infection-induced MMP-2 production in astrocytes.

4. Discussion

In the normal adult brain, there is little MMP expression. Increased MMP expressions have been asso-
associated with several CNS diseases, including viral infection, possibly through their ability to promote breakdown in the BBB integrity and cell death [55]. In this study, we showed that HIV-1 infection was able to induce MMP-2 production in astrocytes (Figs. 1 and 2). This induction appeared to result from the HIV-1 binding to the hMR expressed on the astrocytes. This was supported by (1) HIV-1 binding to CD4 and CCR5 did not lead to MMP-2 production (Fig. 3); (2) the transfection of HIV-1 proviral DNA (to bypass the hMR-mediated entry pathway) did not induce MMP-2 production (Fig. 4); (3) the treatment of cells with the yeast mannan abrogated MMP-2 production (Fig. 6); and (4) the heat inactivation of HIV-1 viruses did not affect MMP-2 induction (Fig. 1A), as only the mannosylated carbohydrate moiety is needed for hMR binding. In addition, our time-course studies showed that MMP-2 induction occurred as early as 12 h within HIV-1 infection (Fig. 1B). Although MMP-2 and MMP-9 both belong to gelatinases with the same substrate specificity, their expression and induction have been found to differ in distinct cell types. We showed astrocytes to be the another example. Unlike MMP-2, MMP-9 production was very low and was not induced in HIV-infected astrocytes. Nevertheless, MMP-9 has been noted at elevated levels in the CSF or the brain of HIV-infected individuals [41,42]. In addition, another study has shown that exposure of human or simian primary astrocytes to HIV-1 viruses leads to the induction of both MMP-2 and MMP-9 [45]. The discrepancy is very likely due to that primary astrocytes may express additional receptor(s) for HIV-1 gp120 that are responsible for MMP-9 induction. Studies have shown that MMP-9 is mainly produced by activated T cells and monocytes [50,56]. Considering the fact that astrocytes are abundantly located at the BBB, our results raise the possibility that MMP-2 induction upon HIV-1 binding causes the initial BBB leakage and subsequent infiltration by activated or infected T cells and monocytes, which, conversely, produce more MMP-9 and further cause the BBB damage and neuron death.

Direct contact between HIV-1 particles and astrocytes has been shown to result in the transduction of activation signals and the modulation of astrocytic functions [57–59]. Although the production of MMP-2 is regulated in both physiological and pathological conditions and at the transcriptional level, surprisingly, little is known about the signaling pathways that control this process. A recent study has shown that MMP-2 production requires the activity of PI3K [60]. Consistent with this finding, we showed that HIV-1 infection led to the tyrosine phosphorylation of hMR itself, PI3K p110α, and Erk (Fig. 7). Importantly, the treatment of cells with the PI3K inhibitor LY-294-002 completely inhibited MMP-2 induction in HIV-1-infected astrocytes (Fig. 8). Biochemical characterization of HIV-1 surface glycoprotein gp120 has shown that 11 of 24 glycan side chains of gp120 are either completely mannosylated or highly mannosylated [61–63]. Interestingly, although hMR has been shown to support the viral entry of both T-tropic and M-tropic HIV-1 viruses through the mannosylated carbohydrate moieties [46], to our surprise, only M-tropic HIV-1 viruses were able to induce MMP-2 production in astrocytes (Fig. 5) and not in CD4- and CCR5-expressing astrocytes (Fig. 3). This may suggest that the mannosylated carbohydrate moieties of HIV-1 gp120 involved in hMR-mediated viral entry and signaling may be different. However, whether the gp120 proteins between M-tropic and T-tropic HIV-1 viruses are glycosylated differently is currently unknown. Previous studies have shown that HIV-1 strains derived from AIDS patients with dementia differ from viruses derived from non-demented patients, primarily in the V3 sequences of the gp120 envelope protein [64,65]. Thus, it is conceivable that both the primary amino sequence within the V3 region of gp120 and the mannosylation (or glycosylation) of gp120 are important for HIV-associated neuropathogenesis. The molecular pathways leading to MMP-2 production by M-tropic HIV-1 viruses merits further investigation.

In addition, of note is that direct HIV gene expression (transfection) appeared to increase the active form of MMP-2 protein without any changes in the total level of MMP-2, while HIV-1 binding to hMR (infection) increased the latent form of MMP-2 by about 4-fold (Fig. 4). These results raise a possibility that HIV-1 binding to hMR, followed by productive HIV-1 gene expression (replication), in cells other than astrocytes would lead to increased production of active MMP-2.

In sum, our results showed that HIV-1 binding to hMR resulted in MMP-2 production in astrocytes through a pathway involving hMR-mediated intracellular signaling pathway. MMP-2 degrades type IV collagen of the extracellular matrix within the BBB and leads to the alteration of the BBB integrity. Thus, these findings suggest that MMP-2 may be one of the mechanisms by which astrocytes contribute to HIV-associated neuropathogenesis.

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References


