The Role of Dynamin in HIV Type 1 Env-Mediated Cell–Cell Fusion

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Abstract

HIV-1 envelope glycoproteins are the key viral proteins that mediate HIV-1 entry and cell–cell fusion. In contrast to HIV-1 entry, the mechanism of HIV-1 Env-mediated cell–cell fusion is relatively unclear. This study demonstrated that dynasore, a dynamin inhibitor, suppressed HIV-1 Env-mediated cell–cell fusion. Dynasore sensitivity of HIV-1 Env-mediated cell–cell fusion varied depending on the viral strains. Results from testing a panel of gp41 cytoplasmic tail truncation mutants suggested that the gp41 cytoplasmic tail might play a role in dynasore sensitivity. HIV-1 Env-mediated cell–cell fusion could also be suppressed by a dynamin dominant-negative mutant DNM2(K44A). In summary, these results suggested that dynamin 2 might play a role in HIV-1 Env-mediated cell–cell fusion.

HIV-1 INFECTION CAN RESULT IN fusion between susceptible CD4⁺ cells to form multinucleated syncytia. Similar to HIV-1 infection, syncytia formation involves the interaction between HIV-1 envelope glycoproteins and cellular receptor CD4 and chemokine receptors. It has been proposed that cell–cell fusion is a pathogenic mechanism that leads to CD4⁺ T cell depletion.^{1–3} In addition to its potential role in CD4 depletion, syncytia formation has also been implicated as an efficient mechanism of HIV-1 spread between infected and uninfected susceptible cells,^{4,5} although formation of virological synapses, nanotubes, and filopodial bridges might also mediate HIV-1 cell–cell spread.^{6–9}

It is clear that HIV-1 gp120 and gp41 are the viral proteins that mediate both HIV-1 infection and HIV-1 Env-mediated cell–cell fusion. However, other than CD4 and chemokine receptors, cellular factors involved in the HIV-1 entry and HIV-1 Env-mediated cell–cell fusion remain to be further studied. The cellular protein dynamin 2 was implicated in HIV-1 entry through endocytosis.¹⁰ Dynamin 2 is a large cellular GTPase involved in the formation of endocytic vesicles through fission.^{11,12} It is interesting that dynamin could also mediate a seemingly reverse activity by being involved in membrane fusion.^{13,14} Although dynamin 2 was implicated in cell-free HIV-1 infection through endocytosis,¹⁰ it is not clear whether dynamin 2 might be involved in HIV-1 Env-mediated cell–cell fusion. Here, we used a cell–cell fusion model to show that dynamin 2 might play a role in HIV-1 Env-mediated cell–cell fusion.

To determine whether dynamin plays a role in HIV-1 Env-mediated cell–cell fusion, COS cells expressing HIV-1 Env

from various HIV-1 strains were used to fuse with TZM-bl cells, a reporter cell line that expresses luciferase upon fusion^{15,16} in the presence of the dynamin GTPase inhibitor dynasore (Tocris Bioscience, Ellisville, MO). The HIV-1 sequence spanning *env* and *tat* genes were cloned into the expression vector pSHRS. The viral Envs used in this study were derived from the R5 viruses ADA, AC10.0.29, and YU-2; the X4 viruses NL4-3, HIV-1 8x, and Wtt; and the dual tropic virus DH012. ADA, NL4-3, and YU-2 were obtained from the NIH AIDS Research and Reference Reagent Program. AC10.0.29 (AC10) is a molecular clone derived from an R5 primary isolate.¹⁷ The DH012 Env used in this study contains gp120 from the dual tropic DH012 and gp41 from NL4-3.¹⁸ HIV-1 8x is an HIV-1 IIIB variant lacking the cytoplasmic tail of HIV-1 gp41, whereas Wtt is an HIV-1 8x revertant with the full gp41 cytoplasmic tail restored.^{19–21} Electroporation was used to transfect COS cells with the expression vector pSRHS containing HIV-1 Env genes.¹⁶ COS cells (1×10^6 cells/ml) were transfected with HIV-1 Env-expressing vectors (0.5 μ g) for 1 day before mixing with TZM-bl cells for fusion.

The sensitivities of the Env-mediated cell–cell fusions to dynasore varied among the tested HIV-1 Envs in the order of DH012 > NL4-3 = Wtt > YU-2 > AC10.0.29 > 8x > ADA (Fig. 1a). The dynasore concentration required to inhibit DH012-Env mediated cell–cell fusion by 50% (IC₅₀) is 34 μ M, whereas the IC₅₀ for HIV-1 ADA Env-mediated cell–cell fusion is > 230 μ M. As shown in Fig. 1b, the effect of dynasore on HIV-1 Env-mediated cell–cell fusion was not due to cytotoxicity. Under the experimental conditions, dynasore had minimal cytotoxicity against both COS and TZM-bl cells at 230 μ M.

FIG. 1. Inhibition of HIV-1 Env-mediated cell-cell fusion by dynasore. **(a)** HIV-1 Env expressing COS cells were fused with TZM-bl cells for 24 h. Fusion of each Env in the presence of dynasore is defined as percent of fusion with dynasore/fusion without dynasore. **(b)** Effect of dynasore on WTT and 8X Env-mediated cell-cell fusion: Fusion of each Env in the presence of dynasore is expressed as relative light units (RLU) to show the absolute fusion efficiency of the two envelopes. Cell surface expression of the Envs was determined by flow cytometry and is shown in the inset. The detailed protocol for the flow cytometry analysis was previously described.³⁷ A sheep anti-HIV-1 gp120 antiserum (NIH AIDS Research and Reference Reagent Program) was used in the FACS analysis. The control in the inset is the FACS analysis in the absence of the anti-HIV-1 gp120 antibodies. **(c)** The viability of COS and TZM-bl cells was determined in the presence of dynasore for 24 h. A CellTiter-Glo luminescent cell viability assay (Promega, San Luis Obispo, CA) was used to determine the cytotoxicity of dynasore. Each data point represents the mean $\pm$ SD of three independent experiments. Color images available online at www.liebertonline.com/aids

The differential sensitivity of various HIV-1 Envs to dynasore suggested that dynamin played a role in HIV-1 envelope-mediated cell-cell fusion. The differential dynasore sensitivity also supported the notion that inhibition of the HIV-1 Env-mediated cell-cell fusion was not due to cytotoxicity.

The IC₅₀ of dynasore against Wtt-Env-mediated cell-cell fusion was approximately 57 μ M, whereas the IC₅₀ for the 8x-Env was greater than 230 μ M (Fig. 1a). This differential sensitivity of Wtt and 8x to dynasore was not due to differential surface expression or the fusion efficiency of the Envs (Fig. 1b). The two Envs had similar fusion efficiency in the presence of low concentrations of dynasore. FACS analysis also indicated that the two envelopes had a similar level of surface expression (Fig. 1b, inset). The only difference between the two viruses is that 8x lacks the cytoplasmic tail of gp41. The differential sensitivities between Wtt and 8x to dynasore suggested that the cytoplasmic tail of HIV-1 gp41 might be a key determinant for the dynasore inhibitory activity. HIV-1 gp41 contains a long cytoplasmic tail (CT) of approximately 150 amino acids. The CT is highly conserved in length and sequence across diverse strains of HIV-1, suggesting functional significance for this region of the HIV Env. The CT contains an antibody-reactive putative beta turn region^{22,23} and three conserved alpha helical domains. The conserved alpha helical domains are also termed lentivirus lytic peptides (LLP).^{24,25}

To determine the gp41 cytoplasmic motifs that are critical for dynasore sensitivity, a series of Wtt gp41 cytoplasmic tail truncation mutants were used in the Env-mediated cell-cell fusion assay (Table 1a). Wtt-Env mutants, Wtt.802, Wtt.769, and Wtt.723, remained quite sensitive to dynasore when most of the cytoplasmic tail was truncated from the C-terminus (Table 1b). In contrast, Wtt.707, Wtt.714, and Wtt.718, completely lacking or with a short cytoplasmic tail (0–12 amino acids), were much less sensitive to dynasore (Table 1b). It is interesting that Wtt.723 was at least 5-fold more sensitive to dynasore when compared to Wtt.718, even though the Env surface expression level of Wtt.723 and Wtt.718 was comparable (data not shown). Substitution of amino acids 719–723 of Wtt.723 with five alanines resulted in a construct, Wtt.7235A, that was much less sensitive to dynasore. In contrast to dynasore, the HIV-1 entry inhibitor IC9564 was equally potent against Wtt.723 and Wtt.718. IC9564 is a betulinic acid derivative that targets HIV-1 gp120.²⁶ This result further supported the notion that there was a differential interaction between dynamin and the two HIV-1 Env variants, which might be responsible for their differential sensitivity to dynasore.

Endocytosis was implicated as a route for HIV-1 infection.^{10,27–30} An endocytic motif YXX Φ (amino acid residues 712–715) resides in the N-terminus of the gp41 cytoplasmic tail.^{31–33} To determine whether the YXX Φ motif affects dynasore sensitivity of HIV-1 Env-mediated cell-cell fusion, the YXX Φ motif of Wtt.718 was mutated to GXX Φ in Wtt.718(Y712G). The data in Table 1b show that knocking out the endocytic motif did not affect dynasore sensitivity.

To further determine the role of dynamin 2 in HIV-1 Env-mediated cell-cell fusion, a dominant negative dynamin 2 mutant, DNM2(K44A), was tested for its effect on HIV-1 Env-mediated cell-cell fusion. AC10.0.29, YU2, 8x, ADA, or DH012 Env expression vectors were cotransfected into COS cells with either wild-type dynamin 2, the dominant negative DNM2(K44A) mutant, or an empty expression vector as a

TABLE 1. DIFFERENTIAL SENSITIVITY OF HIV-1 WTT VARIANTS TO DYNASORE

(a)

(b)

HIV-1 <i>Env</i> s	Dynasore IC_{50} (μ M)	IC9564 IC_{50} (μ M)
Wtt	57 $\pm$ 15 (9)	0.14 $\pm$ 0.01 (7)
Wtt.802	53 (1)	ND
Wtt.769	27 (1)	ND
Wtt.723	40 $\pm$ 15 (3)	0.16 $\pm$ 0.01 (3)
Wtt.723.5A ^a	136 $\pm$ 23 (5)	ND
Wtt.718	>230 (9)	0.22 $\pm$ 0.02 (6)
Wtt.718(Y712G)	>230 (8)	1.53 $\pm$ 0.15 (6)
Wtt.714	>230 (1)	ND
Wtt.707	>230 (4)	0.73 $\pm$ 0.06 (3)
8x	>230 (10)	2.25 $\pm$ 0.16 (5)

(a) HIV-1 Env constructs of HIV-1 Wtt variants. The number at the C-terminus indicates the last amino acid residue of each construct. The numbering system is based on the HIV-1 Wtt Env sequence previously published.^{19–21} TR and FS in the figure denote the transmembrane region and frame shift, respectively.

(b) Inhibition of HIV-1 Env-mediated cell–cell fusion. IC_{50} is the dynasore concentration that inhibited fusion by 50%. The numbers in parentheses indicate the number of independent experiments. ND, not determined.

^aWtt.723.5A is a Wtt.723 variant with five alanines replacing the last five amino acids of Wtt.723.

control. Env expressing COS cells were mixed with TZM-bl cells and Env-mediated cell–cell fusion was measured at various time points. The dominant negative mutant DNM2(K44A) inhibited YU-2 and AC10 Env-mediated cell–cell fusions by more than 75%, 9 h after initiation of cell–cell fusion (Fig. 2). The inhibitory effect was not as strong at 24 h but fusion was still inhibited at this time point by approximately 50%. Similar to the lack of sensitivity to dynasore, ADA, and 8x Env-mediated cell–cell fusion were also resistant to DNM2(K44A). On the other hand, DH012 Env-mediated cell–cell fusion was very sensitive to dynasore inhibition when compared to YU-2 and AC10 (Fig. 1a). However, DH012 Env-mediated cell–cell fusions did not exhibit such a differential sensitivity to DNM2(K44A). The lack of correlation between dynasore and DNM2(K44A) sensitivity suggested that the mechanism of inhibition by the two dynamin inhibitors might not be the same.

Dynamin 2 was implicated in HIV-1 virus entry by regulating the fusion between virus and endosome.¹⁰ Results from

this study showed that Env from various HIV-1 strains including R5, X4, and dual-tropic viruses exhibited a range of sensitivity to dynasore. The fact that a greater than 5-fold difference in dynasore sensitivity between Wtt.723 and Wtt.718 suggests that the cytoplasmic tail of gp41 might be an important determinant for dynasore sensitivity.

Dynamin was linked to vacuole fusion mediated by SNARE. The SNARE proteins mediate membrane fusion through conformational changes and formation of helix bundles.^{34,35} This is reminiscent of HIV-1 gp41-mediated membrane fusion that involves both conformational changes and formation of 6-helix bundles. It is possible that there might be a direct or indirect interaction between dynamin 2 and gp41. However, it is not clear why some HIV-1 Env-mediated cell–cell fusion, such as that of ADA and 8x, are not sensitive to dynasore. One possible explanation for the lack of dynasore sensitivity is that these HIV-1 Envs might be able to mediate cell–cell fusion without dynamin. It is well documented that HIV-1 might enter susceptible cells through different

FIG. 2. Inhibition of HIV-1 Env-mediated cell-cell fusion by dynamin dominant negative mutant DNM2(K44A). Plasmids expressing dynamin 2 and a mutant, DNM2 and DNM2(K44A), were kindly provided by Drs. Pizzato and Göttinger.³⁸ DNM2, DNM2(K44A), or empty vector (pBJ5) was cotransfected with HIV-1 Env (AC10, YU-2, DH012, ADA, or 8x) into COS cells at a ratio of 8:1 (2 μ g:0.25 μ g) for 1 day. The Env-expressing cells were incubated with the TZM-bl cell for various times as indicated. The Env-mediated fusion with the control empty vector transfected COS cells and TZM-bl cells was defined as 100% control fusion. Each data point represents the average of two independent experiments.

mechanisms including transcytosis, endocytosis, and fusion of virus and cell membrane.³⁶ Dynamin 2 was implicated in free HIV-1 entry through endocytosis. On the other hand, it is not clear whether dynamin 2 plays a role in the fusion of virus and cell membrane. Many viral and cellular factors, such as variation in Env sequence, level of receptor expression, or cell type, may affect the Env-mediated cell-cell fusion. Thus, it is possible that dynamin 2 is not required for ADA and 8x Env-mediated cell-cell fusion. In summary, the results of this study suggested that dynamin 2 might play a role in HIV-1 Env-mediated cell-cell fusion.^{37,38} By regulating HIV-1 Env-mediated cell-cell fusion, dynamin 2 might play a role in viral spread from infected cells to uninfected cells and HIV-1 pathogenesis.

Acknowledgments

This study was supported by the Collaboration for AIDS Vaccine Discovery (Bill and Melinda Gates Foundation) and by the U.S. National Institutes of Health (AI065310).

Author Disclosure Statement

No competing financial interests exist.

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