Novel Assay System Favorable for the Study of Cell-to-Cell Transmission of HIV-1 and Its Application to the Evaluation of Anti-HIV Drugs

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The cell-to-cell transmission of human immunodeficiency virus type 1 (HIV-1) was studied using MOLT-4 cells chronically infected with a variant strain of HIV-1_{SF-2} (MOLT-4/HIV-1_{SF-2H}) and CD4$^+$ human lymphoid MT-4 cells. MOLT-4/HIV-1_{SF-2H} cells produced less than 1 $TCID_{50}$ infectious particles per 10^5 cells per day as determined by the cytopathogenicity in MT-4 cells. However, the expression of envelope glycoproteins gp120 and gp41 on the MOLT-4/HIV-1_{SF-2H} cell membrane was satisfactory for syncytium formation with the uninfected MOLT-4 cells. When MOLT-4/HIV-1_{SF-2H} and MT-4 cells were co-cultured, severe cytopathogenicity was observed in MT-4 cells without being accompanied by the formation of multi-nucleated cells. Thus, the system consisting of MOLT-4/HIV-1_{SF-2H} and MT-4 cells is convenient for exclusive study of the mechanism of cell-to-cell transmission of HIV-1. Using various compounds, it was confirmed that cell-to-cell transmission required both gp120/gp41-CD4 binding and *de novo* DNA synthesis.

Key words human immunodeficiency virus; cell-to-cell transmission; syncytium formation; *de novo* DNA synthesis

It has been generally accepted that the cell-to-cell transmission of human immunodeficiency virus (HIV) is triggered by the binding of HIV-*env*-encoded cell membrane glycoprotein gp120 to CD4 on the membrane of another cell; however, there has been controversy about the involvement of reverse transcription. Recently, we established a novel assay system, suitable for study of the cell-to-cell transmission of HIV, which addresses these issues surrounding the cell-to-cell transmission.

MATERIALS AND METHODS

The following compounds were prepared and purified as described previously: PM-19 $K_7[PTi_2W_{10}O_{40}]\cdot 6H_2O$,[1] PM-48 $K_{13}[Eu(SiW_{11}O_{39})_2]\cdot nH_2O$.[2] HPA-23 was generously provided by Prof. Teze, Université Pierre et Marie Curie. Dextran sulfate (DS, average mol. wt. *ca.* 8000) was purchased from Sigma Chemical. 3′-Azido-2′,3′-dideoxythymidine (AZT) was a product of Burroughs Wellcome. 2′,3′-Dideoxyinosine (ddI) and 2′,3′-dideoxycytidine (ddC) were kindly donated by Nippon Paper, Inc. All other materials used in this study are commercial products of analytical grade.

MT-4 cells and MOLT-4 cells chronically infected with HIV strains (MOLT-4/HIV-1_{IIIB}, MOLT-4/HIV-1_{SF-2H}) were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum; penicillin, 100 units/ml; and streptomycin, 100 μg/ml. Virus stocks of HIV-1_{IIIB} and HIV-1_{SF-2H} were obtained from the culture supernatants of MOLT-4/HIV-1_{IIIB} and MOLT-4/HIV-1_{SF-2H}, respectively. The cell-free virus titer was determined by an end-point titration method using MT-4 cells (5×10^3 cells/200 μl) in 96-well microtiter plates 4 d after infection. The titer of HIV-1_{IIIB} stock was 1.6×10^4 for 50% tissue culture infectious doses ($TCID_{50}$)/ml. However, there was no sign of virus infection in all 8 wells containing 100 μl of the undiluted HIV-1_{SF-2H} stock (the titer was less than 1 $TCID_{50}$/100 μl). The severe cytopathogenicity in MT-4 cells observed by a coculture with MOLT-4/HIV-1_{SF-2H} cells disappeared when both cells were separated by a membrane of 0.45 μm pore size. Furthermore, the supernatant of a mixed culture of MT-4 and MOLT-4/HIV-1_{SF-2H} cells at the ratio of 10 : 1 was not infectious to MT-4 cells.

Various compounds were tested for their anti-HIV activities using both the cell-to-cell transmission and cell-free infection systems. A cell-free infection assay was carried out as follows: MT-4 cells were infected with HIV-1_{IIIB} at a multiplicity of infection (moi) of 0.01 at 37 °C for 1 h. 5×10^3 MT-4 cells were dispensed into each well of 96-well microtiter trays and incubated with individual test compounds in a total volume of 200 μl at 37 °C for 5 d. The 50% effective concentration (EC_{50}) was defined as the concentration at which the cytopathogenicity of HIV-1_{IIIB} in MT-4 cells was inhibited by 50%. The toxicity of each compound was evaluated in parallel with the determination of anti-HIV-1 activity. At the 50% cytotoxic concentration (CC_{50}), the cell viability of mock-infected MT-4 cells was half that of untreated cells. The cell-to-cell transmission of HIV-1_{SF-2H} was assessed according to basically similar procedures to the cell-free infection of HIV-1_{IIIB} except that virus-infected cells were used instead of cell-free viruses.

RESULTS

During a study of the anti-HIV activity of polyoxometalates against various HIV-1 strains,[3] MOLT-4 cells chronically infected with a variant of HIV-1_{SF-2}, HIV-1_{SF-2H}, were established (MOLT-4/HIV-1_{SF-2H}). Cell-free particles were hardly detected by their cytopathogenic effect on MT-4 cells in the culture supernatant of MOLT-4/HIV-1_{SF-2H} cells (less than 10 $TCID_{50}$/ml or approximately 1.5×10^6 cells), in which viral RNA was more easily detected by reverse transcriptase-polymerase chain reactions (RT-PCR) using primer pairs for a *gag* gene

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(data not shown) compared with that of MOLT-4 cells chronically infected with HIV-1_{IIIB} (MOLT-4/HIV-1_{IIIB}; HIV-1 titer, 1.6×10^4 $TCID_{50}$/ml). MOLT-4/HIV-$1_{SF\text{-}2H}$ cells were heavily stained with human anti-HIV-1 polyclonal antibodies plus immunofluorescent anti-human IgG monoclonal antibody. The expression on cell membrane and functional integrity of gp120 and gp41 were confirmed by flow cytometric analysis and the syncytium formation with CD4-positive MOLT-4 cells, respectively (data not shown).

Although the release of infectious particles by MOLT-4/HIV-$1_{SF\text{-}2H}$ cells was not significant, the cytopathogenicity in MT-4 cells co-cultured with MOLT-4/HIV-$1_{SF\text{-}2H}$ cells was as severe as that in MT-4 cells infected with cell-free HIV-1_{IIIB}, probably due to the cell-to-cell transmission of HIV-$1_{SF\text{-}2H}$. A fixed number of MT-4 cells (2.5×10^4/ml) were co-cultured with varying numbers of MOLT-4/HIV-$1_{SF\text{-}2H}$ (2.5, 1.25, 0.625, 0.3125 and 0.15625×10^3/ml) for 5 d, and the relative cell viability of each culture was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) metabolic assay (Fig. 1).[4] As the cell number of MOLT-4/HIV-$1_{SF\text{-}2H}$ increased, the cell viability of the mixed culture decreased. Based on the data in Fig. 1, the ratio in cell number of MOLT-4/HIV-$1_{SF\text{-}2H}$ and MT-4 cells was kept at 1 : 20 in the following experiments.

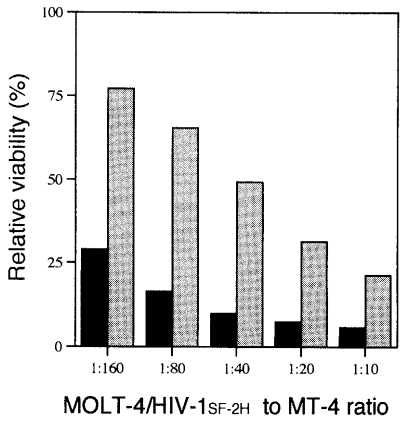

Fig. 1. Relative Viability of Mixed-Cell Cultures

MT-4 cells (2.5×10^4/ml) were co-cultured with varying numbers of untreated (■) or UV-irradiated (▨) MOLT-4/HIV-$1_{SF\text{-}2H}$ cells (2.5, 1.25, 0.625, 0.3125 and 0.15625×10^3/ml) for 5 d. The percent viabilty of each culture relative to that of MT-4-cell monoculture was determined by the MTT metabolic assay.

To study the mode of virus transmission, UV-irradiated MOLT-4/HIV-$1_{SF\text{-}2H}$ cells (MOLT-4/HIV-$1_{SF\text{-}2H}$-UV) with a viability of less than 1% in 24 h were used instead of MOLT-4/HIV-$1_{SF\text{-}2H}$ cells. The cytopathogenicity in MT-4 cells was far lower when co-cultured with MOLT-4/HIV-$1_{SF\text{-}2H}$-UV than with MOLT-4/HIV-$1_{SF\text{-}2H}$ cells, suggesting that the virus transmission required the existence of viable MOLT-4/HIV-$1_{SF\text{-}2H}$ cells. In contrast, MT-4 cells infected with HIV-1 could not release progeny viruses infectious to the surrounding uninfected MT-4 cells.

In Table 1, polyoxometalates PM-19 and PM-48 and DS with an average molecular weight of 8000 (DS8000) are known to block virus–cell fusion, while the nucleoside analogues AZT, ddI and ddC inhibit reverse transcriptase and *N*-methyl-1-deoxynojirimycin is reportedly a glycosidation inhibitor of HIV-*env* glycoproteins. The cell-to-cell transmission of HIV-$1_{SF\text{-}2H}$ is susceptible to the same compounds as the cell-free infection of HIV-1_{IIIB}. The susceptibilities show some difference between these two assays in terms of selectivity index (SI); the ratios of the SI values for HIV-$1_{SF\text{-}2H}$ to HIV-1_{IIIB} range from approximately 100 for PM-19 and PM-48 to 0.03 for AZT.

DISCUSSION

According to previous publications,[5] the cell-to-cell transmission of HIV-1_{IIIB} from HIV-infected H9 cells (H3B) to uninfected H9 or HUT78 cells was accounted for by cell–cell fusion (synsytium formation), which was rarely observed in our assay system consisting of MOLT-4/HIV-$1_{SF\text{-}2H}$ and MT-4 cells. To minimize the involvement of cell–cell fusion in the cell-to-cell transmission, St Luce *et al.* treated U-937 promonocytic cells chronically infected with HIV-1_{IIIB} (U-$937_{HIV\text{-}IIIB}$) with mitomycin C in advance of the 24-h cultivation with the granulocyte-macrophage colony-stimulating factor (GM-CSF)-stimulated human peripheral monocyte-derived macrophages (MDM).[6] After removal of U-$937_{HIV\text{-}IIIB}$ cells, the production of HIV-1_{IIIB} by the HIV-1-harboring MDM cells was monitored by reverse transcriptase activity, which became evident at 10 d, reaching a peak value at around 30 d of incubation. The cytopathogenicity in MT-4 cells in our

Table 1. Comparison of Inhibitory Activities of Various Compounds against Cytopathogenicity in MT-4 Cells Induced by Cell-to-Cell Transmission of HIV-$1_{SF\text{-}2H}$ and Cell-Free Infection of HIV-1_{IIIB}

Compound	HIV-$1_{SF\text{-}2H}$			HIV-1_{IIIB}		
	CC_{50} (μg/ml)	EC_{50} (μg/ml)	SI	CC_{50} (μg/ml)	EC_{50} (μg/ml)	SI
PM-19	280	0.045	6200	270	4.0	68
PM-48	290	0.081	3600	160	6.1	26
HPA23	7.3	—	—	12	—	—
DS8000	>800	1.4	>570	>800	1.7	>470
AZT	25	0.090	280	19	0.0022	8600
ddI	59	8.5	6.9	50	4.3	12
ddC	17	2.4	7.1	8.5	1.4	6.1
MDNM	>1000	17	>59	>1000	22	>45

Abbreviations: PM-19, $K_7[PTi_2W_{10}O_{40}] \cdot 6H_2O$; PM-48, $K_{13}[Eu(SiW_{11}O_{39})_2] \cdot 30H_2O$; HPA23, $(NH_4)_{17}Na[NaSb_9W_{21}O_{86}] \cdot 14H_2O$; DS8000, dextran sulfate 8000; AZT, 3′-azido-2′,3′-dideoxythymidine; ddI, 2′,3′-dideoxyinosine; ddC, 2′,3′-dideoxycytidine; MDNM, *N*-methyl-1-deoxynojirimycin.

assay system progressed at a rate basically similar to that in MT-4 cells infected with cell-free HIV-1_{IIIB}, the assay being completed within 5 d. In conclusion, the advantages of using our assay system are summarized as follows: (1) the release of infectious particles is almost negligible, (2) cell–cell fusion is rarely observed and (3) the assay can be completed within 5 d as in the case of cell-free infection.

There have been two controversial observations with regard to the role of DNA synthesis in a cell-to-cell transmission; HIV-infected CD4-positive cell lines need the DNA synthesis,[5] while DNA *per se* is transmitted in the cases of HIV-infected peripheral blood lymphocytes and U937 cells,[6,7] probably reflecting a high level of extrachromosomal HIV-1 DNA.[8] Furthermore, these proviral DNA molecules may be capable of independent replication and transcription of viral genes.[8] As shown in Table 1, the nucleoside reverse transcriptase inhibitors suppressed the cytopathogenicity in MT-4 cells induced by the cell-to-cell transmission of HIV-1_{SF-2H}, similarly to the cell-free infection of HIV-1_{IIIB}, supporting the crucial role of reverse transcriptase in this system. The decreased SI value of AZT against the cell-to-cell transmission compared with the cell-free infection could be accounted for by an AZT-resistant phenotype of HIV-1_{SF-2H}, though this remains to be elucidated.

The gp120-CD4 interaction should be a pivotal step in the cell-to-cell transmission. The cytopathogenicity of HIV-1_{SF-2H} in MT-4 cells is susceptible to inhibitors of virus–cell fusion such as DS and polyoxometalates PM-19 and PM-48 to the same extent as in the case of the cell-free infection of HIV-1_{IIIB}. The specificity of polyoxometalates for HIV-1_{SF-2H} over HIV-1_{IIIB} might not result from the difference in the modes of virus transmission, cell-free infection and cell-to-cell transmission, because the same was true in the case of syncytium formation between MOLT-4 cells uninfected and chronically infected with respective HIV-1 strains (data will be published elsewhere).

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