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Enhanced production of p24 Gag protein in HIV-1-infected rat cells fused with uninfected human cells

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Abstract

Although many human molecules have been suggested to affect replication of human immunodeficiency virus type 1 (HIV-1), the distribution of such cofactors in human cell types is not well understood. Rat W31/D4R4 fibroblasts expressing human CD4 and CXCR4 receptors were infected with HIV-1. The provirus was integrated in the host genome, but only a limited amount of p24 Gag protein was produced in the cells and culture supernatants. Here we found that p24 production was significantly increased by fusing HIV-1-infected W31/D4R4 cells with uninfected human cell lines of T-cell, B-cell, or macrophage lineages. These findings suggest that human cellular factors supporting HIV-1 replication are distributed widely in cells of lymphocyte and macrophage lineages. We also examined whether the amount of p24 produced by rat–human hybrid cells was correlated with expression levels of specific human genes. The results suggested that HP68 and MHC class II transactivator (CIITA) might up- and down-regulate p24 production, respectively. It was also suggested that HIV-1 replication is affected by molecules other than those examined in this study, namely, cyclin T1, cyclin-dependent kinase 9, CRM1, HP68, and CIITA.

Keywords: HIV-1; Rat model; Cell fusion; Cyclin T1; CDK9; CRM1; HP68; CIITA

Introduction

Replication of human immunodeficiency virus type 1 (HIV-1) is initiated by binding of the viral envelope to the specific surface receptors on target cells. The viral envelope gp120 glycoprotein binds to a human CD4 molecule expressed on T cells and macrophages. A chemokine receptor CXCR4 on T cells or CCR5 on macrophages is also required for virus entry into cells (Kozak et al., 1997). CD4 molecules and chemokine receptors of rodents, which are naturally resistant to HIV-1 infection, do not bind to gp120; therefore, a major barrier to HIV-infection exists at the level of virus entry (Pleskoff et al., 1997). Recent studies showed that rat-derived cells expressing human CD4 and CXCR4 or CD4 and CCR5 became susceptible to HIV-1 viruses (Keppler et al., 2001). However, the rat cell lines produced infectious virus particles still at much lower levels than in human cells, suggesting the existence of additional human factors important for HIV-1 replication.

Over the past couple of decades, several critical steps in HIV-1 replication have been identified. HIV-1 gene expression relies upon complex machinery controlled by two viral regulatory proteins, Tat and Rev. Tat activates the transcription of the viral genome and requires the cellular protein kinase activity termed TAK/P-TEFb, composed of cyclin T1 and cyclin-dependent kinase 9 (CDK9), for its transactivation function (Herrmann and Rice, 1995; Chen et al., 1999). It is reported that the host MHC class II transactivator (CIITA) is recruited instead of Tat during an early phase of

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infection (Saifuddin et al., 2000). Rev is necessary for the accumulation of incompletely spliced HIV-1 RNAs in the nucleus and exports them to the cytoplasm cooperating with the cellular exportin 1/CRM1 molecule (Cmarko et al., 2002). During HIV-1 assembly, Gag polypeptides multimerize into immature HIV-1 capsids. The cellular ATP-binding protein, HP68, is required for this process (Zimmerman et al., 2002; Lingappa et al., 2006).

Although many human molecules have been suggested to affect HIV-1 infection and replication, the distribution of such cofactors in human cell types is not well understood. Here we infected rat fibroblasts coexpressing human CD4 and CXCR4 with HIV-1, fused them with uninfected human cell lines of T-cell, B-cell, or macrophage lineages, and then examined virus production and expression profiles of human genes in the fused cells.

Materials and methods

Cells

Rat W31 fibroblasts (Kanki et al., 2000) were transfected with plasmids carrying the human CD4 gene and those carrying the CXCR4 gene. The expression plasmid of the human CD4 gene (Yamamura et al., 1991) was kindly provided by Dr. Karasuyama (Tokyo Medical and Dental University, Tokyo, Japan). The CXCR4 cDNA was amplified using total RNAs extracted from human peripheral blood mononuclear cells and then subcloned into the pcDNA3.1/Zeo vector (Invitrogen, Carlsbad, CA). Transfection was carried out using Lipofectamine (Invitrogen) according to the manufacturer's protocol. The transfectant, designated as W31/D4R4 cells, was maintained in DMEM supplemented with 10% fetal calf serum (FCS), 400 μg/ml of G418 (GIBCO-BRL, Rockville, MD), and 40 μg/ml of Zeocine (Invitrogen). Several weeks later, cloned W31/D4R4 cells were obtained with limiting dilution.

Human cell lines, including Hut78 and Jurkat (T-cell lymphoma), U937 (macrophage-like cell line), and GI, Raji, Swei, and WT46 (B-cell lymphoma), were cultured in RPMI 1640 medium supplemented with 10% FCS.

HIV-1 infection

W31/D4R4 cells (5×10^5) were pretreated with 2 μg/ml of polybrene for 30 min and then the T-tropic HIV-1 strain, SF33 (Tateno and Levy, 1988), was applied to the cells (equivalent to 200 ng of p24 Gag protein) followed by incubation for 3 h at 37 °C. The supernatants were then removed, and cells were washed 3 times with PBS and digested by trypsin to remove viruses that had not entered the cells. The cells were resuspended in the selection medium and cultured at 37 °C.

PCR and RT-PCR for HIV-1 genes

Genomic DNAs were extracted from the HIV-1-infected W31/D4R4 cells by the standard method. Total RNAs were extracted from the cells with TRIzol Reagent (Invitrogen). The RNAs were subjected to DNase I treatment to remove contaminating DNAs. cDNAs were synthesized with 4 μg of the DNase-treated RNAs using the SuperScript III kit (Invitrogen). PCR for HIV-1 genes was performed using primer sets described previously (York-Higgins et al., 1990; Brandt et al., 1992).

ELISA for p24 Gag protein

HIV-1 p24 Gag protein was quantified using the p24 assay ELISA kit (Zeptometrix, Buffalo, NY). Culture supernatants and cell lysates were subjected to this assay. Tissue culture medium was changed with a fresh one 24 h prior to the assay. The supernatants were centrifuged to remove cell debris; 450 μl of the solution was taken and then mixed with 50 μl of the lysis buffer appended to the kit. The resultant mixture served as culture supernatant samples. For cell lysate samples, 1×10^7 cells were resuspended in 450 μl of fresh medium and then mixed well with 50 μl of the lysis buffer. After centrifugation for removal of the pellets, the supernatants were used as cell lysate samples.

Cell fusion

HIV-1-infected W31/D4R4 cells were maintained for 3 months. Cells (5×10^6) were then washed extensively with PBS, digested by trypsin, and mixed with an equal number of human cells. The mixed cell pellets were overlaid with 1 ml of a 50% solution of polyethylene glycol and stirred gently. After incubation at 37 °C for 1 min, PBS was added slowly followed by centrifugation (500×*g* for 5 min) to remove supernatants. The pellets were resuspended in the selection medium and incubated at 37 °C. The medium was changed with a fresh one every 3 or 4 days. Three weeks later, p24 concentrations were determined in the fused cells and culture supernatants, and the expression of human genes in the rat–human hybrid cells was examined by RT-PCR (see RT-PCR for human genes).

To evaluate the efficiency of cell fusion, human cells were labeled with PKH26 Red Fluorescent Cell Linker Kit (Sigma-Aldrich, St. Louis, MO), according to the manufacturer's protocol, prior to cell fusion. The labeled cells were then fused with uninfected W31/D4R4 cells as described above. Fused cells were incubated in the selection medium at 37 °C for 1 week. The percentage of fused cells was calculated by dividing the number of fluorescence-labeled cells by that of living cells.

RT-PCR for human genes

Expression of human genes, including cyclin T1, CDK9, CRM1, HP68, and CIITA, was examined by RT-PCR. Hybrid cells were harvested 21 days after fusion, and total RNAs were extracted and then subjected to cDNA synthesis. cDNAs were also prepared from parental human cells that were not subjected to fusion with W31/D4R4 cells. The first round of PCR was run for 15 cycles (95 °C for 30 s, annealing for 30 s, 72 °C for 30 s) with sense 1 and antisense 1 primers. The second round of PCR was run for 35 cycles (95 °C for 30 s, annealing for 30 s, 72° for 30 s) with sense 2 and antisense 2 primers. Primer sequences and the annealing temperature are shown in Table 1. These primer sets were specific for the human genes and did not amplify the corresponding rat genes.

Table 1
Primers used for RT-PCR

Name of genes		Sequences (5′ to 3′)	Annealing temperature
Cyclin T1	Sense 1 (2)[a]	agctggaaaatagcccatcc	60 °C
	Antisense 1	aggaggttctgatggcagag	
	Antisense 2	ctgctggagccacagaattt	
CDK9	Sense 1	gccaagatcggccaaggcaccttcgg	56 °C
	Sense 2	ggtgttcaaggccaggcaccgca	
	Antisense 1 (2)[a]	cccatcacgagtgataagcacatta	
CRM1	Sense 1	tgttggagcaagtaggaccag	55 °C
	Sense 2	gcaatgcatgaagaggacga	
	Antisense 1 (2)[a]	cctgaacctgaacgaaatgc	
HP68	Sense 1	gagttgtcctgtagttcgaatc	55 °C
	Sense 2	gtacgatgatcctcctgactggc	
	Antisense 1	aactctcctcctgaaagatcttca	
	Antisense 2	tcgttcttttaggtgggttaaatca	
CIITA	Sense 1 (2)[a]	ctgggattcctacacaatgcg	60 °C
	Antisense 1	ctgggatcatctcaggctga	
	Antisense 2	tcagcatcgctgttaagaagctc	

[a] The same primer was used for the first and second rounds of PCR.