High Temperature Condition Enhances Retrovirus Infection to Cell

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The amount of retrovirus infection depended on the temperature during the virus entry period. Higher amount of retrovirus [human immunodeficiency virus type-1 (HIV-1) and murine leukemia virus] infected to cell at 40°C than at 37°C. One-hour adsorption at 40°C marked more than 10 times large amount of infection than that at 37°C. This phenomenon is observed in both X4 and R5 type HIV-1 strains. At 40°C condition, more than four times much provirus DNA was accumulated in cell than that at 37°C condition, while same amount of virus adsorption was observed in both at 40 and 37°C adsorption. Similar enhancement was observed in murine leukemia virus infection, while little effect was monitored in rhabdovirus (VSV) infection. This result said that high temperature condition prompted the retrovirus penetration (membrane fusion mediated way) to cell. This observation would applicable to improve the gene transduction efficiency using retrovirus vector.

Key words —— retrovirus, infectivity, temperature

INTRODUCTION

Realizing the mechanism of human immunodeficiency virus type-1 (HIV-1) infection to cell is important to develop the clinical strategy against the progression of acquired immunodeficiency syndrome (AIDS). Replication mechanism of HIV-1 in human cell has been cleared for last two decades. Significant interactions of viral components and host cellular factors have identified.[1–3] In contrast, physiological condition like fever has not been focused as a disorder-regulating factor. However, inflammatory response including fever has important roles to the progress of infectious diseases.

Temperature condition affects to cell morphology and enzymatic activity to keep homeostasis. Viral reverse transcriptase, viral integrase, cellular RNA polymerase and viral protease are essential to HIV-1 replication. These enzymes have been identified there adequate working temperature.[4–8] Morphological change of cell is due to the reassortment of cytoskeleton and modulation of cell membrane. Fusion mediated entry of HIV-1 is influenced by the cell membrane mobility. From biochemical analysis, membrane fluidity is controlled by membrane constitution and temperature condition.[9,10] This study aimed to clear the effect of fever (high temperature condition) on HIV-1 proliferation *in vitro*. The influence of temperature on the first step of HIV-1 replication (entry) was mainly focused. Whether high temperature condition prompts to the virus-cell fusion have monitored. To clear the dynamics of virus-cell interaction, another kind of retrovirus (A-MuLV) and rabdovirus (VSV) were used in parallel. The comparison of retroviruses and rabdovirus infectivity cleared the specific effect of heat on the retrovirus infectivity. Further, different effect of heat presumed that fusion mediated penetration machinery might be well affected by body temperature.

MATERIALS AND METHODS

Cells and Pseudotyped Virus —— GHOST/CXCR4 and GHOST/CCR5 cells were maintained in Dulbecco's modified Eargle's medium (ICN, Costa Mesa, CA, U.S.A.) supplied with 10% of heat inactivated fetal calf serum (PAA, Linz, Austria), 100 μg/ml of streptomycin (Meiji, Tokyo, Japan), 100 IU/ml of penicillin (Banyu, Tokyo, Japan), 500 μg/ml of geneticin (PAA), 100 μg/ml of

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Hygromycin B (Wako, Osaka, Japan) and 50 μg/ml of puromycin (Invitrogen, Carlsbad, CA, U.S.A.). Luciferase reporter gene carrying pseudotyped virus was made by transfection of pNL4-3-luc[11] construct with NL4-3 (pCXN), JR-FL (pCXN), A-MuLV (pJD1) or VSV-G (pCXN) envelope expression vector into 293T cell by calcium phosphate precipitation method. Three days after transfection, pseudotyped viruses were harvested and passed through 0.45-μm filters (Millipore).

Virus Adsorption —— NL4-3 virus (275 ng in 1 ml solution) adsorbed to GHOST/CXCR4 cells (5×10^5 cells in 6-wells plate) at 40 or 37°C for 75 min. Then, cell-free virus and nonspecifically attached virus was removed by washing with magnesium and calcium ions free phosphate buffered saline (PBS). Finally, cell was lysed with 1% TritonX-100. Virus antigen (p24) amount was measured by HIV antigen EIAII Abbott (Dainabot, Tokyo, Japan) HIV antigen detection system.

Virus Infection —— Pseudotyped virus (NL4-3, A-MuLV and VSV-G) infected to GHOST/CXCR4 cells (5×10^4 cells in 48-wells plate) at 40 or 37°C. JR-FL virus infected to GHOST/CCR5 cell (5×10^4 cells in 48-wells plate) at 40 or 37°C. After adsorption for 1 hr, cell was washed to remove free virus by medium twice. Then culture continued with fresh medium under 5% CO_2 conditions at 37°C. Virus adsorption was performed in medium containing 50 mM HEPES pH 8.0 (Sigma, Deisenhofen, Germany).

Luciferase Assay —— Forty-eight hours post virus infection, cell was washed by PBS twice and lysed with 100 μl of 1 × lysis buffer (Promega, Madison, WI, U.S.A.). Luciferase activity was measured after mixing 10 μl of lysate with 50 μl of luciferase substrate solution (Promega) by counting the light intensity by luminometer (Lumat LB 9501/16; EG&G Berthold, Bad Wildbad, Germany). Each test was performed in triplicate.

Polymerase Chain Reaction (PCR) —— NL4-3 virus (100 ng) infected to GHOST/CXCR4 cell (5×10^5 cells in 6-wells plate) at 37 or 40°C for 1 hr. Cell was washed and supplied medium. Twenty-four hours post virus infection, cell was lysed with urea lysis buffer (4.7 M urea, 1.3% SDS, 0.23 M NaCl, 0.67 mM EDTA, 6.7 mM Tris pH 8.0). DNA was isolated by phenol chloroform extraction method followed by ethanol precipitation. PCR was performed in 1 × PCR buffer (20 mM Tris; pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of dNTPs and 0.05 U/μl of recombinant Taq DNA polymerase; Invitrogen) with 0.5 mM of primers [M667 (5′-GGCTAACTAGGGAACCCACTG-3′) and M661 (5′-CCTGCGTCGAGAGAGCTCTGGTTT-3′) primers to amplify provirus DNA (R/gag region), or glo-A (5′-ACACAACTGTGTTCACTAGC-3′) and glo-B (5′-CAACTTCATCCACGTTCACC-3′) primers to amplify β-globin coding DNA].[12]

RESULTS AND DISCUSSION

Enhanced HIV-1 Infection under High Temperature Condition

Infectivity of NL4-3 (X4 type HIV-1 using CXCR4 molecule as a coreceptor), A-MuLV (amphotropic murine leukemia virus using PiT-2 molecule as receptor) and VSV-G (derived from VSV, which enter cell by endocytosis) pseudotyped viruses to GHOST/CXCR4 cell and JR-FL (R5 type HIV-1 using CCR5 molecule as a coreceptor) pseudotyped virus to GHOST/CCR5 cell were measured. The infectivity was up-regulated under high temperature (40°C) condition than that under normal temperature (37°C) in NL4-3, JR-FL and A-MuLV infection cases (Fig. 1). NL4-3 virus infection to GHOST/CXCR4 cell at 40°C was enhanced 14.8 times higher than that at 37°C. JR-FL virus infection to GHOST/CCR5 cell at 40°C was enhanced 8.9 times higher than that at 37°C. Similar to HIV-1, infection of pseudotyped virus with murine leukemia virus (amphotropic) envelope to GHOST/CXCR4 cell at 40°C showed up-regulation of infectivity (3.5 times) from that at 37°C (Fig. 1). These results said high temperature condition prompted retrovirus infection. Virus (NL4-3) adsorption (to GHOST/CXCR4 cell) amount at 40°C (3.71 ng) was as same as one at 37°C (4.20 ng) (Fig. 2). One of recent paper[13] showed slightly enhanced adsorption by high temperature condition. The previous observation might be obtained by the result of trypsin treatment of cell before the cell lysis, which measured already fused virus amount, only. In contrast, this experiment measured the whole amount of virus, which ligates cellular receptors with or without fusion to cell, by removing virus that attached on cell surface nonspecifically. High temperature condition never affected to the capacity of virus adsorption to receptor. Affinity of virus ligand (glycoprotein 120, gp120) to cellular receptor (CD4 and CXCR4) was not influenced by changed temperature. Instead of equal amount of virus adsorbed, the total of completed infection event was different by temperature

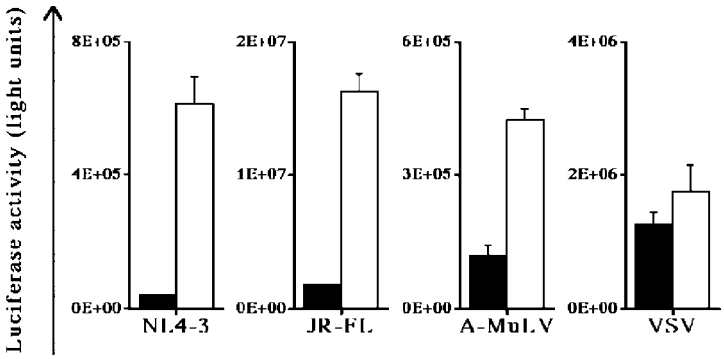

Fig. 1. Infectivity of NL4-3, A-MuLV or VSV-G to GHOST/CXCR4 Cell and JR-FL to GHOST/CCR5 Cell at 37 or 40°C
Solid bar means infectivity at 37°C. Open bar means infectivity at 40°C. Average count of triplicate tests was aligned.

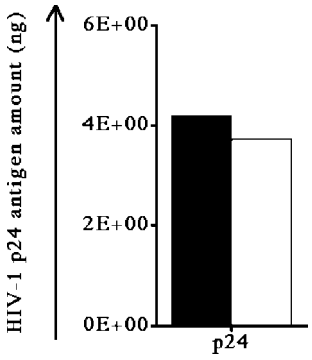

Fig. 2. Adsorption of NL4-3 Virus on GHOST/CXCR4 Cell at 37 or 40°C Condition
Solid bar shows the p24 amount adsorbed at 37°C. Open bar shows the p24 amount adsorbed at 40°C.

during adsorption period (Fig. 1). Virus penetration efficiency was enhanced by high temperature condition. By contrast, VSV-G enveloped pseudotyped virus had slight enhancement (1.4 times) of infection at 40°C from that at 37°C (Fig. 1). This gap was too small to conclude that heat modulated the infectivity. These observations meant the effect of high temperature during adsorption to virus penetration was typical in retrovirus infection. Recent report insisted that the enhancement of HIV-1 infection under high temperature condition was due to the increment of multiple-site binding of virus ligands and cellular receptors.[13] However, almost no up-regulation of VSV infection to cell (Fig. 1) said that multiple-site binding is not the main reason for enhanced infection of HIV-1 at 40°C. Figure 1 result meant the enhancive effect of high temperature on virus infectivity is retrovirus specific phenomenon. No modulation of VSV infectivity should be brought from the difference of penetration way. Otherwise, we could not explain why VSV infection got no influence by heating, since VSV (enveloped virus) also needs multiple-site binding for its penetration to cell. Retrovirus penetrates cell by fusion mediated way while VSV enters cell by endocytosis. The different modulation of infectivity by high temperature condition among virus types was due to the mechanism of virus penetration. Fusion of both retrovirus and host cell membranes needs high mobility of phospholipids in lipid bilayers, which is well obtained by high temperature condition. In contrast, up-taking large molecules by endocytosis needs rearrangement of cytoskeletons, which isn't influenced by temperature. The different effect of temperature brought the diverse results among infection of retrovirus (significant enhancement) and VSV (little enhancement).

Increment of Virus Penetration by High Temperature Infection

Virus entry sum was evaluated by semi-quantitative PCR on provirus DNA, which reflects the quantity of virus penetration. GHOST/CXCR4 cell infected with NL4-3 virus for 1 hr at 40°C carried 4 to 5 times higher amount of provirus DNA than that at 37°C one (Fig. 3). The gap of virus infectivity by temperature was due to the efficiency of virus penetration to cell. Because of equal amount of p24 (HIV-1) adsorption was observed (Fig. 2), the discrepancy of synthesized provirus DNA amount should be obtained from the different efficiency of virus-cell fusion step.

Figure 3 result could not exclude another hypothesis that high temperature condition enhanced reverse transcription step in cytoplasm. However, increasing temperature up to 40°C makes a slight enhancement on activity of retrovirus RT.[4,5] These findings indicate the effect of heat on promoting virus

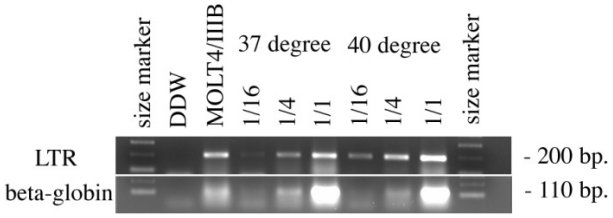

Fig. 3. Provirus DNA in GHOST/CXCR4 Cell Infected with NL4-3 Virus at 37°C (37 degree) or 40°C (40 degree)
Provirus amount was measured by semi-quantitative PCR (1/16, 1/4 and 1/1 dilution). Upper panel (LTR) means provirus DNA (R/gag region) quantification. Lower panel (beta-globin) means β-globin coding DNA quantification.

infection is independent to modulate RT activity. Significant increment of provirus formation depends on the up-regulation of virus penetration to cell.

Future Aspects

Polybrene is applying to enhance the gene transduction into human cell using retrovirus vector.[14] However, cytotoxicity is not negligible. This report teaches us the effective gene transduction way by retrovirus (including lentivirus) vector, without chemical substances. Facilitative strategy for vector virus infection is strongly desired in clinical gene therapy field. Slightly increased temperature condition (until 40°C) during exposure of cell to vector virus up-regulates the gene transduction efficiency. This research cleared not only HIV-1 but also MuLV, which is adopted to gene carrying vehicle, is easily expedited the infection to cell by high temperature condition. Furthermore, human cell sustains little damage up to 40°C. This observation is applicable in clinical, where murine derived retrovirus vector is used.

Increased infection of HIV-1 (NL4-3 and JR-FL) under high temperature condition informs that fever might prompt the replication of HIV-1 and progress of AIDS state. We have no clear case study about body temperature's effect on HIV-1 replication *in vivo*. Statistical analysis of the relationship of body temperature between HIV-1 carriers and AIDS patients in clinical field remained for future study.

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REFERENCES

1) Tasara, T., Hottiger, M. O. and Hubscher, U. (2001) Functional genomics in HIV-1 virus replication: protein-protein interactions as a basis for recruiting the host cell machinery for viral propagation. *Biol. Chem.*, **382**, 993–999.
2) Wahl, S. M., Greenwell-Wild, T., Peng, G., Ma, G., Orenstein, J. M. and Vazquez, N. (2003) Viral and host cofactors facilitate HIV-1 replication in macrophages. *J. Leukoc. Biol.*, **74**, 726–735.
3) Wang, W. K., Chen, M. Y., Chuang, C. Y., Jeang, K. T. and Huang, L. M. (2000) Molecular biology of human immunodeficiency virus type 1. *J. Microbiol. Immunol. Infect.*, **33**, 131–140.
4) Gerard, G. F., Potter, R. J., Smith, M. D., Rosenthal, K., Dhariwal, G., Lee, J. and Chatterjee, D. K. (2002) The role of template-primer in protection of reverse transcriptase from thermal inactivation. *Nucleic Acids Res.*, **30**, 3118–3129.
5) Huang, M., Zensen, R., Cho, M. and Martin, M. A. (1998) Construction and characterization of a temperature-sensitive human immunodeficiency virus type 1 reverse transcriptase mutant. *J. Virol.*, **72**, 2047–2054.
6) Ingles, C. J. (1978) Temperature-sensitive RNA polymerase II mutations in Chinese hamster ovary cells. *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 405–409.
7) Manchester, M., Loeb, D. D., Everitt, L., Moody, M., Hutchison, C. A., 3rd and Swanstrom, R. (1991) Analysis of temperature-sensitive mutants of the HIV-1 protease. *Adv. Exp. Med. Biol.*, **306**, 493–497.
8) Wiskerchen, M. and Muesing, M. A. (1995) Identification and characterization of a temperature-sensitive mutant of human immunodeficiency virus type 1 by alanine scanning mutagenesis of the integrase gene. *J. Virol.*, **69**, 597–601.
9) Dynlacht, J. R. and Fox, M. H. (1992) Heat-induced changes in the membrane fluidity of Chinese hamster ovary cells measured by flow cytometry. *Radiat. Res.*, **130**, 48–54.
10) Dynlacht, J. R. and Fox, M. H. (1992) The effect of 45 degrees C hyperthermia on the membrane fluidity of cells of several lines. *Radiat. Res.*, **130**, 55–60.
11) Connor, R. I., Chen, B. K., Choe, S. and Landau, N. R. (1995) Vpr is required for efficient replication of human immunodeficiency virus type-1 in mononuclear phagocytes. *Virology*, **206**, 935–944.
12) Zack, J. A., Arrigo, S. J., Weitsman, S. R., Go, A. S., Haislip, A. and Chen, I. S. (1990) HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. *Cell*, **61**, 213–222.
13) Harada, S., Akaike, T., Yusa, K. and Maeda, Y. (2004)

Adsorption and infectivity of human immunodeficiency virus type 1 are modified by the fluidity of the plasma membrane for multiple-site binding. *Microbiol. Immunol.*, **48**, 347–355.
14) Davis, H. E., Morgan, J. R. and Yarmush, M. L. (2002) Polybrene increases retrovirus gene transfer efficiency by enhancing receptor-independent virus adsorption on target cell membranes. *Biophys. Chem.*, **97**, 159–172.