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INFECTIOUS TRANSMISSION AND REPLICATION OF HUMAN T-CELL LEUKEMIA VIRUS TYPE 1

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1. ABSTRACT

Retrovirus infection proceeds by attachment of the envelope glycoprotein to a cell surface receptor, followed by fusion of the viral and cellular membranes. Once in the cell, the viral enzymes and structural proteins form a replication complex that converts the single-stranded viral genomic RNA into a double-stranded DNA, which is then integrated into the host cell chromosome. For HTLV-1, these events are not well characterized. We have developed cell culture systems, infectious molecular clones, and viral vectors that can be used to characterize the mechanisms of HTLV-1 infection and replication. Infection with cell-free HTLV-1 virions is orders of magnitude less efficient compared with other retroviruses. This inefficiency is the result of a block in the replication process after the virion is bound to the cell surface. We are determining whether this block is conferred by the viral replication enzymes, results from the actions of cellular restriction factors, reflects the need for cell-cell contact, or is caused by a combination of these factors.

2. INTRODUCTION

HTLV-1 belongs to the deltaretrovirus group of retroviruses, whose other members include HTLV-2; primate T-cell lymphotropic viruses (PTLVs or STLVs), STLV-1, STLV-2, PTLV-L; and bovine leukemia virus (BLV). HTLV-1 primarily infects CD4 and CD8 T-cells *in vivo* (1-3), but will infect a wide variety of cell types from a number of different species in vitro (4, 5). The HTLV-1 receptor appears to be expressed ubiquitously and reports over the last 20 years have suggested a number of likely candidates, the most recent being the glucose transporter protein Glut1 (6). HTLV-1 is disseminated in tropical regions worldwide, with endemic foci in southern Japan, Africa, the Caribbean, and western parts of South America. Of the approximately 20 million people infected, about 5% develop either adult T-cell leukemia (ATL) or a chronic inflammatory disease of the central nervous system termed HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (7-9). The provirus load in HTLV-1-infected, asymptomatic carriers ranges from 0.1% to 1% of peripheral blood mononuclear cells (PBMCs), but can be as high as 30% in some HAM/TSP patients. In spite of relatively high numbers of infected cells and evidence of a vigorous humoral and cell-mediated immune response, there is no viremia. Virus gene products are not detected in fresh PBMCs from HTLV-1-infected patients until several hours after the initiation of *ex vivo* culture, suggesting that mechanisms exist to either repress virus gene expression or to eliminate virus-expressing cells *in vivo* (10, 11). For HTLV-1, the number of infected cells (the provirus load) is determined both by infectious spread of the virus and by virus-induced clonal expansion of infected cells (12). The latter is referred to as mitotic replication and is caused by the HTLV-1 Tax protein, which changes the cellular gene expression program and disrupts cell cycle regulation (13, 14). The relative contribution of these modes of replication to the course of infection is not known. In this review, we will focus on infectious spread of the virus.

For reasons that are not yet clear, HTLV-1 infection with cell-free virus is extremely inefficient compared with other retroviruses, including HIV-1. Studies by Fan et al. (15) showed that one in one million HTLV-1 particles produced by a chronically infected cell line were infectious. This specific infectivity is 3 to 4 orders of magnitude lower than HIV-1. Since HTLV-1 and HIV-1 both infect human T lymphocytes *in vivo*, the difference in virion infectivity suggests that the two viruses have evolved different mechanisms to sustain persistent infections. Earlier work did not establish whether the low infectivity of HTLV-1 is due to virion attachment and entry into cells (mediated by Env-receptor interactions) or to defects in reverse transcription (mediated by viral replication enzymes or cellular restriction factors). Using infectious provirus clones, viral vectors, and cell culture infection systems, some of these questions have been addressed and others are currently under investigation.

3. MEASURING HTLV-1 INFECTION AND REPLICATION *IN VITRO*

HTLV-1 infection and replication have been difficult to examine *in vitro* since the virus displays a very

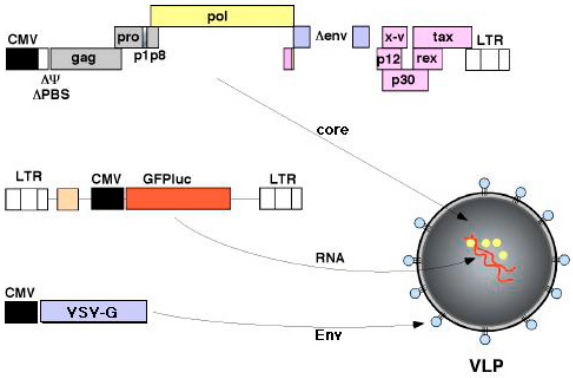

Figure 1. HTLV-1 vectors that together produce virus-like particles in transfected cells. The HTLV-1 packaging plasmid (pCMVHT-Δenv) shown at the top, transfer vector (pHTC-GFPluc) shown beneath the packaging plasmid, and Env expression plasmid are transfected into cells to produce infectious VLPs that carry out a single round of replication.

low infectivity and a tightly regulated gene expression program. In addition, some virus gene products appear to be detrimental to cell growth and proliferation, thus limiting productive infection and detection of virus in infected cells. In contrast to most established T-cell lines, which do not support productive infection, HTLV-1 immortalizes primary human T-cells *in vitro*, and a number of chronically infected cell lines have been established. HTLV-1 infections of primary lymphocytes have been initiated by cell-free infection, but infection is performed more frequently by cocultivating lethally-irradiated HTLV-1 producer cells with target lymphocytes. By either infection method, monoclonal or oligoclonal outgrowth of infected cells is observed several months after infection. This low efficiency probably reflects several levels of selection for T-cells that are susceptible to infection and are able to survive and continuously proliferate in the absence of antigenic stimulation. This experimental system has proved useful for analysis of HTLV-1 mediated T-cell transformation events and for generating immortalized T-cell lines, but it is not suitable for quantitative analysis of infection and replication. These problems have been overcome to a certain extent by monitoring viral DNA generated by reverse transcription of the viral RNA genome in cells infected with cell-free virus (15).

To circumvent some of the problems associated with infection and immortalization of primary T-cells, a spreading infection assay for HTLV-1 was developed using a fetal rhesus lung cell line FRhL-clone B5 (16). Infections can be initiated with cell-free or cell-associated virus from chronically-infected cell lines or from cells transfected with provirus clones; alternatively, direct transfection of the FRhL-B5 cells with provirus DNA can initiate a spreading infection. Virus propagation is easily monitored by measuring the amount of HTLV-1 Gag protein released into the culture medium. The kinetics of virus production is proportional to the titer of input virus and is dependent on the activities of virus gene products. Generally, virus expression in the culture reaches its peak at about 2 to 3 weeks after infection. This system provides a reproducible measure of the replication competence of proviruses through multiple rounds of infection and is a good method to phenotypically analyze virus mutants and to determine the inhibitory effects of antiviral compounds on virus replication. However, the spreading infection assay does not allow one to examine a specific step in the viral infectious cycle.

Retroviral vectors are powerful tools that can be used to quantitatively analyze virus infection during a single round of replication. Because virus-like particles (VLPs) deliver a surrogate virus genome that can be designed to exclusively express reporter gene products in the infected cell, vectors can overcome the problems associated with monitoring wild-type virus infections. We developed HTLV-1 vectors and single-cycle replication assays that can be used to dissect the steps in the HTLV-1 infectious cycle. As shown in Figure 1, VLPs are generated by cotransfecting cells with a packaging plasmid, a transfer vector, and an envelope expression plasmid. We constructed an HTLV-1 packaging plasmid (pCMV-HT1Δenv) that expresses viral structural, enzymatic, and regulatory proteins under the control of a cytomegalovirus (CMV) promoter (17, 18). The vector lacks the viral RNA packaging signal and primer-binding site (PBS) so that its RNA cannot be incorporated into virions and replicated. The packaging plasmid also lacks a functional *env* gene, so that we can pseudotype VLPs with other envelope proteins expressed from a different plasmid. The HTLV-1 transfer vectors express a surrogate HTLV-1 genomic mRNA containing an internal CMV promoter and a reporter gene; this RNA is packaged into virions and is reverse transcribed, integrated, and expressed in the infected cell. Various transfer vectors were created that contain genes for firefly luciferase, enhanced yellow fluorescent protein, green fluorescent protein-luciferase fusion protein, neomycin resistance, and hygromycin resistance to make it simple to follow virus replication in different experimental settings. VLPs provide a rapid and sensitive way to measure HTLV-1 infection, which is quantified by enzyme assay, flow cytometry, or colony formation. For comparative analyses of HTLV-1 and HIV-1 infectivity, we cloned the reporter genes described above into HIV-1 vectors (obtained from Didier Trono, University of Geneva, Switzerland) (19). The HIV-1 and HTLV-1 vectors are very similar in construction, organization, and transcriptional activity.

4. HTLV-1 INFECTION IS RESTRICTED AT A POST-ENTRY STEP

One of the long-standing questions that we wanted to address is why infections with HTLV-1 virions are less efficient than with HIV-1. We tested the possibility that the difference in infectivity between HTLV-1 and HIV-1 is due to differences in virion binding and entry into target cells. The retroviral vectors allow us to pseudotype HTLV-1 and HIV-1 VLPs with the same envelope glycoprotein; in most of these experiments, we have used vesicular stomatitis virus G protein (VSV-G). We found that when both viruses were pseudotyped with

VSV-G protein, transduction with HTLV-1 VLPs was at least 3 orders of magnitude lower than with HIV-1 VLPs. The large difference in HTLV-1 versus HIV-1 VLP infectivity was observed with other Env pseudotypes as well, and in a variety of cell lines. Care was taken to insure that equal amounts of HTLV-1 and HIV-1 VLPs were used for infection and that VLPs incorporated equal amounts of Env. The specific infectivity of the HTLV-1 and HIV-1 VLPs that we observed in single-cycle infections is in good agreement with the specific infectivity reported for wild-type viruses (15).

We also examined the possibility that HTLV-1 virions rapidly lose the ability to infect cells after their release into the culture medium. The infectivity of HTLV-1 VLPs incubated at 37˚C prior to infection, decreased with a half-life of approximately 4 hours. In parallel experiments, HIV-1 VLPs had a half-life comparable to HTLV-1, arguing against an unusual instability of HTLV-1 virions. The half-life of VLPs was independent of the Env protein used to pseudotype HTLV-1 and HIV-1 VLPs. Because the large difference in HTLV-1 and HIV-1 infectivity is observed when VLPs are pseudotyped with the same Env and because HTLV-1 VLPs are not unusually labile, we think that the block to efficient HTLV-1 infection is at a step after virion binding and entry into the target cell.

5. HTLV-1 REVERSE TRANSCRIPTASE

After the viral core enters the cytoplasm of an infected cell, the next step is reverse transcription of the virion RNA. Because reverse transcriptase (RT) is the key enzyme in this process, it is quite possible that inefficient reverse transcription contributes to the low infectivity of HTLV-1. Retroviral *pol* genes encode RT (composed of polymerase and RNaseH domains) and integrase (IN). The *pol* gene is translated as a Gag-Pol precursor protein, which is cleaved by the viral protease in the virion to produce RT and IN subunits. In some retroviruses, RT is cleaved between the polymerase and RNaseH domains. For example, in HIV-1 a fraction of RT is cleaved further to produce the smaller subunit; a similar cleavage does not occur in murine leukemia virus RT. Cleavage of the HTLV-1 Pol polyprotein and the subunit composition of the active RT have not been defined. Furthermore, the amount of Pol protein incorporated into HTLV-1 virions has not been determined. One would expect that HTLV-1 virions contain less RT than HIV-1, since production of the HTLV-1 Pol precursor requires two ribosomal frameshifting events whereas the HIV-1 Pol precursor requires only one.

We have recently begun to determine how the HTLV-1 Pol precursor is processed and how the cleavage products are assembled into the active RT enzyme. These analyses are a prerequisite for correctly expressing a recombinant RT for physical and biochemical analyses. We have constructed a variety of provirus expression plasmids that express higher levels of the Pol precursor compared to wild-type virus and encode epitope-tagged Pol proteins. We have also generated antibodies that recognize the amino-terminus and middle of HTLV-1 RT. Immunoblotting of HTLV-1 virion extracts revealed that the Pol precursor is cleaved to produce RT and IN proteins. We have determined the amino acid sequences of the protease cleavage sites that are used to generate the amino- and carboxy-termini of HTLV-1 RT. In the course of these studies, we identified a previously unknown cleavage site that defines the amino-terminus of RT, which is different from what was predicted from earlier studies (20). We also showed that the addition of 8 amino acids to the amino-terminus of RT abolished polymerase activity. The carboxy-terminus of RT is identical to that predicted from amino acid sequence alignments, reported previously (21). In addition to RT and IN, we detect smaller Pol cleavage products that contain either polymerase or RNaseH domains. Affinity purification of RT from HTLV-1 virions revealed that HTLV-1 RT is a heterodimer, composed of p65 and p49 subunits, analogous to the p66 and p51 subunits of HIV-1 RT. The p49 subunit of HTLV-1 RT is derived from p65 by cleavage between the polymerase and RNaseH domains. We are currently determining the amino acid sequence of the cleavage site between the polymerase and RNaseH domains by mutagenesis and by amino acid sequencing of affinity-purified RT in virions. The combined molecular weights of the p65 and p49 subunits is consistent with the molecular weight of HTLV-1 RT determined by gel filtration chromatography (22).

The amount of HTLV-1 RT protein per virion is the subject of some debate with little if any experimental data. HTLV-1 virions are known to exhibit lower RT activity compared to other retroviruses; whether this is due to lower amounts of RT protein per virion or to a lower specific activity of HTLV-1 RT is unknown, but should be resolved in the near future. To more accurately quantify differences in RT activity between HTLV-1 and HIV-1 virions, we performed RT assays of virion extracts using bacteriophage MS2 RNA template and measured cDNA products by real-time PCR. We observed that the level of RT activity per virion is approximately 1000-fold lower for HTLV-1 compared with HIV-1. It is interesting to note that the magnitude of the difference in HTLV-1 and HIV-1 virion-associated RT activities approximates the difference in their cell-free infectivity. Why HTLV-1 would have evolved to express low levels of RT activity and to have a low infectivity seems counter-intuitive. However, these attributes may reflect mechanisms by which the virus has adapted to evade innate cellular defense mechanisms or may suggest that virus replication is enhanced when virus is transmitted by cell-cell contact.

6. INHIBITORS OF HTLV-1 REPLICATION

Earlier studies of inhibitors of HTLV-1 replication from several groups used labor-intensive methods that were difficult to quantify (23-26). In contrast, the single-cycle replication assay provides a rapid, sensitive, and quantitative method to address important problems related to the development of antiviral agents directed against HTLV-1. This is demonstrated in analyses of HTLV-1 susceptibility to nucleoside reverse transcriptase inhibitors (NRTIs), which not only provide

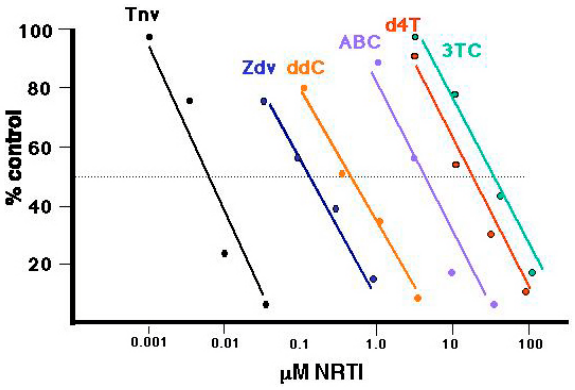

Figure 2. Inhibition of HTLV-1 replication by NRTIs in single-cycle infection assays. A) Filtered supernatants from transiently transfected 293T cells (recombinant virus producers) were used to infect FRhL-B5 cells (virus targets). Recombinant virus infection and replication in the target cells was determined by performing luciferase assays on cell extracts 72 hr after infection. Cells were treated with varied concentrations of nucleoside analogs for 15 hr prior to infection and maintained with drugs during and after infection. Luciferase activities are expressed as the percent activity relative to no-drug controls and are plotted against the Log10 of the drug concentration (µM). Best line fitting was performed by linear regression analysis. Results for the NRTIs, tenofovir (tfv), AZT (Zdv), ddC, abacavir (ABC), d4T, and 3TC are shown.

clinically important data but also reveal information about HTLV-1 RT (27). We determined IC50 values for the nucleoside analogs lamivudine (3TC), abacavir, tenofovir (bis-POC-PMPA), stavudine (d4T), zidovudine (AZT), and zalcitabine (ddC) (Figure 2). HTLV-1 was susceptible to all six NRTIs tested. Tenofovir was the most potent inhibitor of HTLV-1 replication (IC50, 5 nM), followed in order by AZT (IC50, 0.11 µM), ddC (IC50, 0.27 µM), abacavir (IC50, 4.6 µM), d4T (IC50, 14.5 µM), and 3TC (IC50, 22.0 µM). The potencies of the compounds against HTLV-1 had a different order than for HIV-1, suggesting differences in the catalytic properties of the two RT enzymes. We were particularly interested in determining how efficiently 3TC inhibits HTLV-1 replication, since 3TCTP was reported to be inactive against HTLV-1 RT *in vitro* (28). To establish that HTLV-1 RT was the target of 3TC action, we constructed a mutant HTLV-1 containing a methionine-to-valine substitution in the YMDD motif of the active site of RT. This mutation was chosen because the analogous methionine-to-valine mutation in HIV-1 RT confers resistance to 3TC (29). We showed that 3TC weakly inhibits the replication of wild-type HTLV-1 and the YVDD mutant is resistant to 3TC (27). These results are in good agreement with those of Balestrieri et al., who examined 3TC inhibition of wild-type HTLV-1 replication in primary lymphocytes (26). In summary, these studies demonstrated the applicability of the single-cycle assay for HTLV-1 drug testing and phenotypic analyses of viral mutants.

7. PERSPECTIVE

HTLV-1 and HIV-1 both infect T-cells *in vivo*, yet the infectivity of HTLV-1 virions is several orders of magnitude lower than HIV-1. This observation suggests that HTLV-1 has evolved different strategies for propagation and persistence compared to HIV-1. Although there are no conclusive quantitative data, it is widely believed that HTLV-1 infection by cell-cell contact is more efficient than with cell-free virus. Igakura et al. provided visual evidence of HTLV-1-infected T-cells in contact with, and transmitting virus to, uninfected T-cells (30). The next important step that needs to be taken is to determine whether transmission of HTLV-1 between T-cells that have formed a "virological synapse" improves virus replication. It is possible that the cell-to-cell route of transmission either allows a larger number of virions to be delivered to the target cell or that cell contact-dependent signals are required to enhance virus replication. The HTLV-1 vectors that we have described here, combined with new cell culture systems that mimic HTLV-1 transmission via cell-cell contacts, should provide quantitative data to better define the molecular mechanisms of HTLV-1 infection and replication.

REFERENCES

1. Cho I, M. Sugimoto, S. Mita, M. Tokunaga, F. Imamura & M. Ando: In vivo proviral burden and viral RNA expression in T cell subsets of patients with human T lymphotropic virus type-1-associated myelopathy/tropical spastic paraparesis. *Am J Trop Med Hyg* 53, 412-418 (1995)
2. Hanon E, J. C. Stinchcombe, M. Saito, B.E. Asquith, G. P. Taylor, Y. Tanaka, J. N. Weber, G. M. Griffiths & C. R. Bangham: Fratricide among CD8(+) T lymphocytes naturally infected with human T cell lymphotropic virus type I. *Immunity* 13, 657-664 (2000)
3. Richardson JH, A. J. Edwards, J. K. Cruickshank, P. Rudge & A. G. Dalgleish: In vivo cellular tropism of human T-cell leukemia virus type 1. *J Virol* 64, 5682-5687 (1990)
4. Clapham P, K. Nagy, R. Cheingsong-Popov, M. Exley & R. A. Weiss: Productive infection and cell-free transmission of human T-cell leukemia virus in a nonlymphoid cell line. *Science* 222, 1125-1127 (1983)
5. Ho DD, T. R. Rota & M. S. Hirsch: Infection of human endothelial cells by human T-lymphotropic virus type I. *Proc Natl Acad Sci U S A* 81, 7588-7590 (1984)
6. Manel N, F. J. Kim , S. Kinet, N. Taylor, M. Sitbon & J.L. Battini: The ubiquitous glucose transporter GLUT-1 is a receptor for HTLV. *Cell* 115, 449-459 (2003)
7. Uchiyama, T: Human T cell leukemia virus type I (HTLV-I) and human diseases. *Annu Rev Immunol* 15, 15-37 (1997)
8. Manns A, M. Hisada & L. La Grenade: Human T-lymphotropic virus type I infection. *Lancet* 353, 1951-1958 (1999)
9. Bangham CR: HTLV-1 infections. *J Clin Pathol* 53, 581-586 (2000)
10. Hanon E, P. K. Goon, G. P. Taylor, H. Hasegawa, Y. Tanaka, J. N. Weber & C. R. Bangham CR: High production of interferon gamma but not interleukin-2 by human T- lymphotropic virus type I-infected peripheral blood mononuclear cells. *Blood* 98, 721-726 (2001)
11. Chung HK, H. A. Young, P. K. Goon, G. Heidecker, G. L. Princler, O. Shimozato, G. P. Taylor, C. R. Bangham & D. Derse: Activation of interleukin-13 expression in T

cells from HTLV-1-infected individuals and in chronically infected cell lines. *Blood* 102, 4130-4136 (2003)
12. Wattel E, J. P. Vartanian, C. Pannetier & S. Wain-Hobson: Clonal expansion of human T-cell leukemia virus type I-infected cells in asymptomatic and symptomatic carriers without malignancy. *J Virol* 69, 2863-2868 (1995)
13. Yoshida M: Multiple viral strategies of HTLV-1 for dysregulation of cell growth control. *Annu Rev Immunol* 19, 475-496 (2001)
14. Yoshida M: HTLV-1 Tax: regulation of gene expression and disease. *Trends Microbiol* 1, 131-135 (1993)
15. Fan N, J. Gavalchin, B. Paul, K. H. Wells, M. J. Lane & B. J. Poiesz BJ: Infection of peripheral blood mononuclear cells and cell lines by cell- free human T-cell lymphoma/leukemia virus type I. *J Clin Microbiol* 30, 905-910 (1992)
16. Derse D, J. Mikovits, D. Waters, S. Brining & F. Ruscetti: Examining the molecular genetics of HTLV-I with an infectious molecular clone of the virus and permissive cell culture systems. *J Acquir Immune Defic Syndr Hum Retrovirol* 12, 1-5 (1996)
17. Derse D, J. Mikovits, M. Polianova, B. K. Felber & F. Ruscetti: Virions released from cells transfected with a molecular clone of human T-cell leukemia virus type I give rise to primary and secondary infections of T cells. *J Virol* 69, 1907-1912 (1995)
18. Derse D, S. A. Hill, P. A. Lloyd, H. K. Chung & B. A. Morse: Examining human T-lymphotropic virus type 1 infection and replication by cell-free infection with recombinant virus vectors. *J. Virol.* 75, 8461-8468 (2001)
19. Naldini L, U. Blomer, P. Gallay, D. Ory, R. Mulligan, F. H. Gage, I. M. Verma & D. Trono: In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science* 272, 263-267 (1996)
20. Heidecker G, S. A. Hill, P. A. Lloyd & D. Derse: A novel protease processing site in the transframe protein of human T-cell leukemia virus type 1 PR76(gag-pro) defines the N terminus of RT. *J Virol* 76, 13101-13105 (2002)
21. Trentin B, N. Rebeyrotte & R. Z. Mamoun: Human T-cell leukemia virus type 1 reverse transcriptase (RT) originates from the pro and pol open reading frames and requires the presence of RT-RNase H (RH) and RT-RH-integrase proteins for its activity. *J Virol* 72, 6504-6510 (1998)
22. Rho HM, B. Poiesz, F. W. Ruscetti & R. C. Gallo: Characterization of the reverse transcriptase from a new retrovirus (HTLV) produced by a human cutaneous T-cell lymphoma cell line. *Virology* 112, 355-360 (1981)
23. Macchi B, I. Faraoni, J. Zhang, S. Grelli, C. Favalli, A. Mastino & E. Bonmassar: AZT inhibits the transmission of human T cell leukaemia/lymphoma virus type I to adult peripheral blood mononuclear cells in vitro. *J Gen Virol* 78, 1007-1016 (1997)
24. Matsushita S, H. Mitsuya, M. S. Reitz & S. Broder: Pharmacological inhibition of in vitro infectivity of human T lymphotropic virus type I. *J Clin Invest* 80, 394-400 (1987)
25. Zhang J, E. Balestrieri, S. Grelli, C. Matteucci, V. Pagnini, C. D'Agostini, A. Mastino & B. Macchi: Efficacy of 3'-azido 3'deoxythymidine (AZT) in preventing HTLV-1 transmission to human cord blood mononuclear cells. *Virus Res* 78, 67-78 (2001)
26. Balestrieri E, G. Forte, C. Matteucci, A. Mastino & B. Macchi: Effect of lamivudine on transmission of human T-cell lymphotropic virus type 1 to adult peripheral blood mononuclear cells in vitro. *Antimicrob Agents Chemother* 46, 3080-3083 (2002)
27. Hill SA, P. A. Lloyd, S. McDonald, J. Wykoff & D. Derse: Susceptibility of human T cell leukemia virus type I to nucleoside reverse transcriptase inhibitors. *J Infect Dis* 188, 424-427 (2003)
28. Garcia-Lerma JG, S. Nidtha & W. Heneine: Susceptibility of human T cell leukemia virus type 1 to reverse- transcriptase inhibitors: evidence for resistance to lamivudine. *J Infect Dis* 184, 507-510 (2001)
29. Tisdale M, S. D. Kemp, N. R. Parry & B. A. Larder: Rapid in vitro selection of human immunodeficiency virus type 1 resistant to 3'-thiacytidine inhibitors due to a mutation in the YMDD region of reverse transcriptase. *Proc Natl Acad Sci U S A* 90, 5653-5656 (1993)
30. Igakura T, J. C. Stinchcombe, P. K. Goon, G. P. Taylor, J. N. Weber, G. M. Griffiths, Y. Tanaka, M. Osame & C. R. Bangham: Spread of HTLV-I between lymphocytes by virus-induced polarization of the cytoskeleton. *Science* 299, 1713-1716 (2003)

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