Spread of HTLV-I Between Lymphocytes by Virus-Induced Polarization of the Cytoskeleton

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Cell contact is required for efficient transmission of human T-lymphotropic virus type 1 (HTLV-I) between cells and between individuals, because naturally infected lymphocytes produce virtually no cell-free infectious HTLV-I particles. However, the mechanism of cell-to-cell spread of HTLV-I is not understood. We show here that cell contact rapidly induces polarization of the cytoskeleton of the infected cell to the cell-cell junction. HTLV-I core (Gag protein) complexes and the HTLV-I genome accumulate at the cell-cell junction and are then transferred to the uninfected cell. Other lymphotropic viruses, such as HIV-1, may similarly subvert normal T-cell physiology to allow efficient propagation between cells.

The human T cell lymphotropic virus type 1 (HTLV-I) is an oncogenic exogenous retrovirus that infects between 10 and 20 million people world-wide. Two to 3% of infected individuals develop adult T-cell leukemia/lymphoma (1), and a further 2-3% develop a variety of chronic inflammatory syndromes, most notably HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (2, 3).

HTLV-I is transmitted between individuals by transfer of infected lymphocytes, either in breast milk, semen or blood (4). Transfusion with cell-free blood products appears to carry a negligible risk of HTLV-I infection (5). In vitro, efficient spread of HTLV-I infection also requires cell contact (6, 7). Cell contact is required because lymphocytes naturally infected with HTLV-I produce very few cell-free HTLV-I virions and because, of the virions that are released, only 1 in 107 to 108 is infectious (8, 9).

The mechanism of cell-to-cell spread of HTLV-I is not understood. HTLV-I expresses a surface glycoprotein, the envelope (Env) protein, which is required for infectivity (9) and for cell-cell fusion and syncytium formation (10–12). Env is presumed to bind to a cellular receptor for HTLV-I, but the receptor has not yet been identified (13, 14). Certain integrins, including ICAM-1, ICAM-3 and VCAM, act as cofactors for HTLV-I-induced cell fusion (15, 16).

In this study we tested the hypothesis that HTLV-I is transmitted directly across the cell-cell junction. We used confocal microscopy to examine the distribution of HTLV-I Gag and Env proteins and the HTLV-I genome in fresh, unstimulated peripheral blood mononuclear cells (PBMCs) isolated directly from HTLV-I-infected individuals and fixed and stained within 24 hours. We conclude that HTLV-I subverts normal T-cell physiology to spread efficiently between host cells, without the need to release cell-free virus particles.

Polarization of virus proteins and nucleic acids. In isolated lymphocytes, we observed clusters of Gag p19-and p15-staining material, predominantly near the cell membrane, as previously described (17) (Fig. 1, a, b; fig. S1a, S1d). Env gp46 staining appeared uniformly on the cell surface (Fig. 1c). When T cells were allowed to form conjugates with neighbouring cells, within 40 minutes there was strong polarization to the area of cell-cell contact of both Gag protein (Fig. 1, d, e) and Env protein (Fig. 1f).

The adhesion molecule talin accumulated at the cell-cell junction in conjugates containing HTLV-I-infected T cells, forming a ring in about 35% of cases (Fig. 2, b, c; fig. S2a-c), or as a single patch (about 60% of cases) or multiple patches (5%). HTLV-I Gag protein accumulated either in the central talin-free domain (Fig. 2, b, c; fig. S2, a, b) or as clusters overlying or adjacent to talin patches (data not shown). This accumulation of Gag protein in the center of the cell to cell junction was observed in conjugates between CD4+ and CD4+ T cells (Fig. 2, b, c), CD4+ and CD8+ T cells (fig. S2a) and CD8+ and CD8+ T cells (data not shown). The presence in these accumulations of nucleocapsid staining (p15) (Figs. 1d, 2a) is significant because the nucleocapsid binds the retroviral genome and incorporates it into the virion (18).

When CD4+ or CD8+ T cells isolated from a HAM/TSP patient were allowed to form conjugates with T cells from a healthy uninfected donor for 120 minutes, in addition to accumulation of Gag p19 staining at the cell-cell junction there was frequent Gag p19 staining in the cells derived from the uninfected donor (Fig. 1, g, h). We observed transfer of Gag p19 staining from CD4+ T cells and CD8+ T cells to both CD4+ and CD8+ allogeneic T cells. This process may represent the initial establishment of HTLV-I infection in a newly infected individual, which involves contact between allogeneic lymphocytes.

Polarization of Gag complexes to the cell-cell junction and transfer to the uninfected cell were also observed in conjugates between CD4+ T cells and both B cells (fig. S1b, S1c) and NK cells (fig. S1e, S1f).

We used an antisense peptide nucleic acid (PNA) probe in fluorescence in situ hybridization (FISH) to detect the (plus...
HTLV-I, we counted the orientation patterns triggered by antigen recognition. Associated with HTLV-I infection of the T cell, and was not raised the possibility that the MTOC polarization was triggered by TCR-mediated recognition of HTLV-I antigens (Fig. 1d, 1e). After 120 min incubation, HTLV-I RNA was transferred from HTLV-I infected cell to uninfected cell (Fig. 3, e, f), like the Gag protein (Fig. 1g, h).

Reorientation of microtubule organizing center. We observed frequent reorientation of the microtubule organizing center (MTOC) to the area of cell-cell contact in lymphocyte conjugates (Fig. 2d; Fig. S2, d, e): in each case the MTOC lay immediately adjacent to the accumulation of HTLV-I Gag protein. This close apposition of polarized Gag molecules to the MTOC suggested that the microtubule cytoskeleton affected the polarization of Gag. In CD4⁺-CD4⁰ T cell conjugates, treatment with 33N M nocodazole for 90 min blocked the polarization and transfer of Gag protein, both in the absence (Fig. 2e, f) and the presence (Fig. 2g) of the T-cell activator phytohemagglutinin-L (leucoagglutinin, PHA-L). These results suggest that microtubules are involved in transporting Gag-containing material towards the cell-cell junction before transfer into the recipient cell.

Binding of the T-cell receptor (TCR) to MHC/antigen complexes on the surface of another cell causes reorientation of the responding T-cell’s MTOC to the cell-cell junction. Furthermore, Yamamoto et al. (20) found that ligation of ICAM-1 on the cell surface induces expression of HTLV-I genes, which suggests the existence of a positive feedback loop between cell-cell adhesion and HTLV-I gene expression (Fig. S3).

HTLV-I Gag protein, in complex with the HTLV-I genome, appears to be transported to the MTOC by a microtubule-dependent process. Microtubules have been shown to be involved in the intracellular transport of other viruses, e.g. adenovirus and herpesvirus (21–23). The junction formed between an HTLV-I-infected T cell and another T cell shared two similarities—ordered talin domains and MTOC polarization—with the “immunological synapse” (IS) (24). However, in the present study the MTOC polarization occurred within the HTLV-I-infected cell, not towards the infected cell. Therefore, MTOC polarization was not triggered by recognition of HTLV-I antigens presented by a neighboring T cell, and the structures we report here cannot be considered an “immunological” synapse. The term “virological synapse” may be more appropriate.

HTLV-I can infect almost any mammalian cell in vitro, but in vivo it is almost confined to T cells, for unknown reasons (25–27). It is possible that T cell-specific factors are required either for efficient HTLV-I replication or for the process of cell-to-cell transfer reported here.

We conclude that HTLV-I exploits the normal physiology of the T cell to enable efficient cell to cell transmission by forming a close contact with the recipient cell and using the cytoskeleton to propel viral material into the recipient cell (fig. S3). Although HTLV-I has a peculiarly strong dependence on cell contact for efficient transmission of the virus between cells, it is possible that other lymphotropic viruses, such as HIV-1 (28, 29), use a similar mechanism to spread between lymphocytes.

References and Notes

27. E. Hanon et al., Immunity 13, 657 (2000).
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Supporting Online Material
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Materials and Methods
Figs. S1 to S3
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References

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Fig. 1. HTLV-I Gag and Env proteins are unpolarized in an isolated T cell, but accumulate at the cell-cell junction within 40 min of cell contact; Gag protein is transferred from HTLV-I-infected T-cells to uninfected T-cells within 120 min. a, b, c: Single confocal sections showing isolated CD4+ T cells from a patient with HAM/TSP. a, CD4+ T cell, tubulin-alpha (green) and Gag p19 (red). b, CD4+ T cell, tubulin-alpha (green), Gag p15 (red). c, CD4+ T cell, Env gp46 (red). d, e, f: Confocal images showing polarization of HTLV-I Gag and Env proteins to the cell-cell junction. Conjugates were allowed to form for 40 min between fresh CD4+ T cells from a patient with HAM/TSP. d, CD4+ T cell, Gag p15 (red). e, CD4+ T cell, Gag p19 (red). f, CD4+ T cell, Env gp46 (red). g, h: Confocal images showing transfer of Gag p19 protein from HTLV-I-infected T-cells to uninfected T-cells. Conjugates were allowed to form for 40 min. g: HTLV-I-infected CD4+ and normal CD4+ T cell, Gag p19 (red). h: HTLV-I-infected CD8+ and normal CD4+ T cell, Gag p19 (red). HTLV-I-infected T-cells were marked with carboxyfluorescein succinimidyl ester (CFSE) (green). The transmission picture (blue; b-h) is superimposed on a 0.4 micrometer confocal fluorescence single section (red; c-f, red and green; b, g, h). Scale bar represents 5 micrometers.

Fig. 2. a-c. HTLV-I Gag protein and talin accumulate in distinct domains at the cell-cell junction. Conjugates were allowed to form for 40 min between fresh cells from a HAM/TSP patient. a, CD4+ T cell conjugate, talin (green), Gag p19 (red). b, c, CD4+ T cell conjugate, talin (green) and Gag p19 (red). c, Z-axis image reconstruction from b, talin (green) and Gag p19 (red). d-g; The microtubule organizing center lies adjacent to the polarized HTLV-I Gag protein at the cell-cell junction; however, treatment with nocodazole, an inhibitor of tubulin polymerization, blocks both the polarization to the cell-cell junction and cell-cell transfer of Gag protein. d, CD4+ T cell conjugate, tubulin-alpha (green), HTLV-I Gag p19 (red). e, conjugate between HTLV-I-infected CD4+ T cell labeled with CFSE (green) and normal CD4+ T cell, HTLV-I Gag p19 (red). f, autologous CD4+ T cell conjugate, tubulin-alpha (green), HTLV-I Gag p19 (red). g, autologous CD4+ T cell conjugate, formed in the presence of phytohemagglutinin-L (leucoagglutinin; PHA-L), talin (green), HTLV-I Gag p19 (red). e-g; treatment with nocodazole. The transmission picture (blue; a, e) is superimposed on a 0.4 micrometer confocal fluorescence single section (red and green). Scale bars represent 5 micrometers.

Fig. 3. HTLV-I genome accumulates at the cell-cell junction and is then transferred to the uninfected cell. a-d; Conventional fluorescence images showing plus-strand HTLV-I nucleic acid by PNA-FISH and transmission picture (blue), e, f: Confocal images showing plus-strand HTLV-I nucleic acid by PNA-FISH and transmission picture (blue). a, b, d-f: HTLV-I nucleic acid (red). a, b; CD4+ T cell from HAM/TSP patient, conjugation time 40 min. c; Jurkat cell (negative control), conjugation time 40 min. d; MT2 cell (positive control). e, f; CD4+ T cell from HAM/TSP patient (marked green with CFSE) and control (uninfected) CD4+ T cell, conjugation time 120 min. Scale bars represent 5 micrometers.