Human T Lymphotropic Virus Type III Infection of Human Alveolar Macrophages

By Syed Z. Salahuddin, Richard M. Rose, Jerome E. Groopman, Phillip D. Markham, and Robert C. Gallo

The human T-cell lymphotropic virus type III (HTLV-III) has been demonstrated to be the primary causative agent of acquired immunodeficiency syndrome (AIDS) and related disorders. HTLV-III has been recovered from several body tissues and fluids, including peripheral blood lymphocytes, cell-free plasma, lymphocytes in saliva, cerebrospinal fluid, tears, and semen. Preliminary studies suggest that the receptor for HTLV-III infection is located on or closely associated with the surface antigen T4 found on helper/suppressor T cells. In vitro, B lymphoblastoid cell lines, and Epstein-Barr virus (EBV)-transformed fresh lymphocyte have been infected with HTLV-III, and a recent report suggested the possible infection of human macrophages. Pathologic studies of simian brains of animals infected with simian T lymphotropic virus type III (STLV-III), a retrovirus related to HTLV-III, show by electron microscopy viral particles in macrophages. Furthermore, nonlymphoid cells in the brain, possibly glial cells, are infected with HTLV-III in some patients with AIDS and dementia. These studies suggest that a number of cells other than T cells may be infected with HTLV-III. We now report on the infection of human alveolar macrophages in vitro and provide suggestive data that these cells may be infected in vivo as well.

MATERIALS AND METHODS

Pulmonary macrophages were harvested from healthy volunteers and from patients with AIDS by broncholavage using previously described methods. Informed consent was obtained from all participants and the procedures were approved by the Institutional Review Board of the New England Deaconess Hospital. The adherent cell population was obtained from the lung by incubation on plastic and multiple washes with phosphate-buffered saline (PBS) were performed to dislodge any nonadherent cells. The resulting adherent population was judged positive when the cpm using dT<sub>rA</sub>, rA primer template was ≥5,000 and was greater than or equal to threefold higher than cpm using dT<sub>rA</sub>, dA<sub>rA</sub> as primer template. Staining of target pulmonary macrophages with monoclonal antibodies to the HTLV-III correlated with p24 and p15 antigens to determine the percentages of positive cells. Electronmicroscopy and transmission of HTLV-III from supernatant fluids of human alveolar macrophage cultures to normal peripheral blood lymphocytes was performed as previously described.

Characterization of Adherent Cells

Morphology. Standard differential counts were performed on Wright's-Giemsa-stained cytocentrifuge-prepared cells to determine the gross morphology of the cell population and their state of differentiation. Myeloid cells through the metamyelocyte stage were classified as immature.

Cytochemistry. Published procedures were followed for the cytochemical evaluation of fresh and cultured cells. The tests performed included stains for naphthyl ASD chloroacetate esterase, α-naphthyl acetate esterase, acid phosphatase, and myeloperoxidase.

Nitroblue tetrazolium reduction. The nitroblue tetrazolium (NBT) reduction test was performed on suspension and adherent cells from healthy volunteers as well as AIDS patients were infected at days 3 through 5 after plating. These cells were incubated at 37 °C in RPMI-1640 medium after diethylaminoethanol (DEAE) treatment for 20 minutes and then cultured with standard titrated infectious aliquots of HTLV-III harvested from H9 cells. Controls consisted of DEAE-treated macrophages exposed to the supernatants obtained from uninfected H9 cells. Productive infection with HTLV-III was measured by quantitation of reverse transcriptase activity in supernatant fluid under the assay conditions used; a cell culture was judged positive when the cpm using dT<sub>rA</sub>, rA primer template was ≥5,000 and was greater than or equal to threefold higher than cpm using dT<sub>rA</sub>, dA<sub>rA</sub> as primer template. Staining of target pulmonary macrophages with monoclonal antibodies to the HTLV-III correlated with p24 and p15 antigens to determine the percentages of positive cells.

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cells with or without the addition of freshly diluted tetradecamylphoral acetate (TPA), as previously described.3,13 Cells were incubated for 25 minutes at 37 °C in media containing 0.2% NBT and the desired concentration of TPA. Cells were rinsed twice with media, pelleted on microscope slides using Shandon-Elliott cytopsin, and stained with Wright's-Giemsa. Cells with dark formazan deposits were scored positive.

Phagocytosis. Suspension cells were mixed with either sterile carbonyl iron (Technicon Instruments Corp. Tarrytown, NY) at a concentration of 10% (vol/vol) or with formalin-fixed Candida albicans at a concentration of 4 x 10^5/mL with or without the addition of freshly diluted TPA, and incubated at 37 °C for 25 minutes. After incubation, cells were rinsed twice with media, and cyt centrifuge smears were prepared; these were stained with Wright's-Giemsa before evaluation. Adherent cells on the plastic surface of culture flasks were treated with either carbonyl iron or C albicans, rinsed, and evaluated directly under an inverted microscope after Wright's-Giemsa staining.

Detection of EBV antigens and antibodies. Serum from donors were tested for the presence of antibodies reactive against Epstein-Barr virus nuclear antigen (EBNA) and EBV-virus capsid antigen (VCA), and cells were tested directly for these antigens by published procedures.16

Rosette formation. Leukocytes were tested for their ability to form rosettes with either sheep or bovine erythrocytes either directly (E rosette) after treatment of erythrocytes with anti-sheep or bovine erythrocyte serum (EA rosettes) or addition of C_r-deficient mouse complement to the EA preparation (EAC rosettes) by published procedures.20

Surface-bound immunoglobulin. The presence of cell surface-bound Ig (Slg) was determined by direct procedures using fluorescence-labeled F(ab')_2 fragments of anti-human IgG, A, and M.

Monoclonal antibody profile. Murine monoclonal antiserum reactive with peripheral blood monocytes22 was used to detect monocyte-macrophages. Reacting cells were identified using FITC-labeled anti-murine IgG, F(ab')_2-specific serum.

RESULTS

The adherent cell cultures obtained by broncholavage were tested following three to five days in cell cultures. At this time they were >98% monocytic, (>94% phagocytic for latex beads, and >99% positive for nonspecific esterase, indicating a nearly uniform population of human pulmonary macrophages). Furthermore, these cells were negative for T and B cell-related antigens OKT1, OKT3, OKT8, and OKT11; Leu 1, Leu 3a, Leu 4, and Leu 12; and B1 and B4. A low percentage of these adherent cells (2% to 3%) expressed the T4 antigen. This did not appear to be the result of low-level contamination of macrophage cultures with lymphocytes since these studies were performed on rigorously prepared and characterized adherent cells after 3 to 5 days in culture. One hundred percent of these adherent cells were positive for OKM1, M5 (Ortho Diagnostic), Leu M1, and M3 (Beckton Dickinson), and MO1 and MO3 (Coulter Immunology) monoclonal antibodies directed against myeloid/macrophage cells. This further indicates lack of contamination of the cultures with lymphocytes.

The results of infecting pulmonary macrophages from normal donors and AIDS patients in vitro are shown in Table 1 and Fig 1. Primary macrophage cultures from AIDS patients demonstrated low levels of reverse transcriptase activity (threefold to fivefold background) observed in two of four cases, suggesting the presence of cellular infection in vivo. Despite this suggestion of viral infection, HTLV-III (or other viruses) could not be visualized by electromicroscopy, and attempts to transmit HTLV-III from these cells to normal T cells were unsuccessful.

Pulmonary macrophages from both AIDS patients and normal donors were susceptible to HTLV-III infection in vitro. As shown in Fig 1, 3 to 5 days after introduction to cell culture, adherent pulmonary macrophages from normal donors were exposed to HTLV-III, and levels of reverse transcriptase activity were detectable within 7 days. This production of virus was then amplified by addition to the culture of lectin-stimulated peripheral blood mononuclear cells from allogeneic donors on day 22 of culture. A marked increase in HTLV-III production was then obtained as detected by both release of precipitable reverse transcriptase activity and increase in the percentage of suspension cells expressing HTLV-III core-related proteins p15 and p24 (Table 1). The adherent cell population did not demonstrate gross cytopathic changes morphologically postinfection and was viable by trypan-blue exclusion for >65 days. Following HTLV-III infection of the adherent macrophage population, ~18% to 30% of the cells expressed HTLV-III core-related antigens, a much higher proportion of the macrophages than the T4 antigen-expressing population (2% to 4%).

DISCUSSION

These studies demonstrate that human pulmonary macrophages obtained from both normal volunteers and patients with AIDS are permissive for infection with HTLV-III.
INFECTION OF MACROPHAGES

Table 1. Infection of Alveolar Macrophages From Normal Donors and AIDS Patients HTLV-III, on Day 12 Postinfection

<table>
<thead>
<tr>
<th>Specimen* Source</th>
<th>Patient No.</th>
<th>Uninfected Cells</th>
<th>HTLV-III Infected Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT† Activity (cpm x 10^-3)</td>
<td>IFA‡ OKT4 (% Positive)</td>
<td>RT† Activity (cpm x 10^-3)</td>
</tr>
<tr>
<td>AIDS</td>
<td>1</td>
<td>&lt;2</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>&lt;4</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Healthy heterosexual donors</td>
<td>5</td>
<td>&lt;4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>&lt;3</td>
<td>11</td>
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<tr>
<td></td>
<td>7</td>
<td>&lt;4</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>&lt;4</td>
<td>5</td>
</tr>
</tbody>
</table>

ND, not done.
*Adherent cell cultures were established as described. These cells were >97% positive for nonspecific esterase (naphthyl acetate esterase) and ability to phagocytize latex beads; they were negative for T cell (OKT1, or T3, OKT4, OKT8, Leu 1, Leu 3, and Leu 4) and B cell (Leu 12, B1, and B4) antigens.
†Reverse transcriptase activity released into supernatant fluids of cell culture was determined as previously described: + positive, − negative, ± low levels of activity. These determinations were made every 5 days.
‡HTLV-III specific protein (p15, p24), T cell, and B cell markers were determined by immunofluorescence assays (IFA) as described.

Therefore, like retroviruses of other mammalian species, particularly visna and STLV-III, HTLV-III is capable of productively infecting mononuclear phagocytes. This infection appears to differ from that of lymphocytic cells in that the viability of the infected macrophages in vitro does not appear to be as adversely affected by HTLV-III. It is reasonable to speculate that mononuclear phagocytes may be infected in vivo in patients with HTLV-III–related diseases (HRD). The data of Shaw and colleagues demonstrating HTLV-III proviral DNA in nonlymphoid cells in brain tissues obtained from patients with AIDS-related dementia further support the concept that there are nonlymphoid targets of this retrovirus. Because macrophages are relatively long-lived cells capable of close interaction with lymphocytes, and do not appear in vitro to be as susceptible to HTLV-III–induced cytopathic effects as do lymphocytes, it is possible that infected macrophages in vivo could propagate viral infection in the hosts by transfer of virus to lymphocytes. HTLV-III infection of mononuclear phagocytes could thereby be one mechanism for the establishment of viral persistence.

It is still undetermined whether in vitro HTLV-III enters the macrophages by specific interaction with a cell surface receptor, possibly related or unrelated to the T4 antigen, or whether the virus is simply phagocytosed and maintained in a viable state within vacuoles without integration as proviral DNA. Studies are underway to address these questions.

Because macrophages play an important role in the defense against certain neoplastic and infectious processes, alterations in these activities could contribute to host immunosuppression and further predispose to the development of malignancy and opportunistic infections. This is particularly clinically evident with respect to pulmonary macrophages in that patients with AIDS often manifest opportunistic infections of the lung. In this regard, it has been demonstrated that certain monocyte-macrophage functions such as antigen presentation are deficient in cells obtained from individuals with AIDS and related disorders.

Strategies for anti-HTLV-III viral therapy, as well as immunomodulatory therapy, in patients with AIDS requires full definition of the target cells of this virus and possible reservoirs in vivo of viral infection. Our observations that in vitro pulmonary macrophage can be infected with HTLV-III and appear to be less susceptible to the cytopathic effects of this retrovirus suggest that tissue macrophages should be considered as potential reservoirs of HTLV-III in vivo.

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