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) is the etiologic agent of the acquired immunodeficiency syndrome (AIDS) and preferentially infects T4 lymphocytes. Other cell types, notably B lymphocytes and other nonlymphoid cells, also have been reported to be infected in vitro by HTLV-III. We now report on the susceptibility of human pulmonary macrophages to infection with HTLV-III in vitro. Alveolar macrophages infected with HTLV-III produced low levels of virus that could be transferred to autologous human peripheral blood mononuclear leukocytes as long as 2 weeks after initiation of infection. Unlike HTLV-III infection of T lymphocytes, macrophages appeared more resistant to viral-mediated cytopathic effects. Primary cultures of pulmonary macrophages from two of four patients with AIDS spontaneously produced low levels of virus detected as precipitable reverse transcriptase activity, suggesting that these cells were infected in vivo. Because tissue macrophages are long-lived cells, they may act as a reservoir of HTLV-III, capable of transmitting the virus to other susceptible cells such as T lymphocytes, causing periodic low-level viremia. Macrophage infection with HTLV-III may be one mechanism for the establishment of viral persistence in infected hosts.

© 1986 by Grune & Stratton, Inc.

MATERIALS AND METHODS

Pulmonary macrophages were harvested from healthy volunteers and from patients with AIDS by bronchoalveolar lavage using previously described methods.14 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 studied morphologically with inverted and phase contrast microscopy and tested for phagocytic capacity for 1-μm latex beads.15,16 The adherent cells were tested for nonspecific esterase (naphthol acetate esterase).17 Pulmonary macrophages were also assayed for the expression of cell surface antigens OKT1, OKT3, OKT4, OKT8, and OKT11 (Ortho Pharmaceuticals, Raritan, NJ; Leu 1, Leu 3a, Leu4, and Leu 12 (Becton Dickinson, Mountain View, Calif); and B1 and B4 (Coulter Immunology, Fla) as previously described.18 To minimize the possible nonspecific staining due to the use of murine monoclonal antibodies, antimouse immunoglobulin conjugate was included in immunoassays. 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 alligots of HTLV-III harvested from H9 cells.1 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 fluid15 under the assay conditions used; a cell culture was judged positive when the cpm using dT15, dA, primer template was ≥5,000 and was greater than or equal to threefold higher than cpm using dT15, dA, 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.14 Electronmicroscopy and transmission of HTLV-III from supernatant fluids of human alveolar macrophage cultures to normal peripheral blood lymphocytes was performed as previously described.15

Characterization of Adherent Cells

Morphology. Standard differential counts were performed on Wright's-Giemsa-stained cytospin-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 chloracetate esterase,14 α-naphthyl acetate esterase,14 acid phosphatase,11 and myeloperoxidase.16

Nitroblue tetrazolium reduction. The nitroblue tetrazolium (NBT) reduction test was performed on suspension and adherent

From the Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Md; and the Division of Pulmonary Medicine and Hematology/Oncology, Department of Medicine, New England Deaconess Hospital and Harvard Medical School, Boston.

Address reprint requests and correspondence to Dr Robert C. Gallo, Laboratory of Tumor Cell Biology, Bldg 37, Room 6A09, National Cancer Institute, Bethesda, MD 20892.

Supported in part by grants from the American Cancer Society, The Massachusetts AIDS Research Council, and a contract from the National Cancer Institute.

Submitted Nov 8, 1985; accepted Feb 14, 1986.

© 1986 by Grune & Stratton, Inc.

0006-4971/86/6801-0007$03.00/0
cells with or without the addition of freshly diluted tetradecamylphoral acetate (TPA), as previously described. 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-Elliot cytopin, 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 cytoscifuge 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 the 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.

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 C3-deficient mouse complement to the EA preparation (EAC rosettes) by published procedures.

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 antisera reactive with peripheral blood monocytes 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 bronchoalveolar lavage were tested following three to five days in cell cultures. At this time they were >98% monocyteic, (>94% phagocytic for latex beads, and >99% positive for nonspecific esterase, indicating a nearly uniform population of alveolar macrophages). Furthermore, these cells were negative for T and B cell-related antigens OKT1, OKT3, OKB8, and OKT11; Leu1, Leu3a, Leu4, and Leu12; 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 MO2 (Coulter Immunology) monoclonal antibodies directed against monocyteid/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 electron microscopy, 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. 
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 (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.

REFERENCES


3. Popovic M, Sarnagadharan MG, Reed E, Gallo RC: Detection, isolation, and continuous production of cytopathic human T lymphotropic retrovirus (HTLV-III) from patients with AIDS and pre-AIDS. Science 224:497, 1984


6. Cheingsong-Popov R, Weiss RA, Dalgleish A, Tedder RS,

7. Popovic M, Read-Connole E, Gallo RC: T4 positive human neoplastic cell lines susceptible to and permissive for HTLV-III. Lancet 2:1472, 1984


Human T lymphotropic virus type III infection of human alveolar macrophages

SZ Salahuddin, RM Rose, JE Groopman, PD Markham and RC Gallo