Entry of Adult T-Cell Leukemia-associated Virus into Human Peripheral Blood Leukocytes

Brief Report

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With 3 Figures

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Summary

Within 10 minutes after tritium-labeled adult T-cell leukemia-associated virus (ATLV) inoculation, silver grains were found over human lymphocytes. At the time of entry of ATLV, the viral envelope was observed to fuse with the cell membrane.

Recently a human leukemia virus called adult T-cell leukemia (ATL)-associated virus (ATLV) was found in ATL cultured cells (6, 7). This retrovirus (14) is thought to be involved in the etiology of ATL on the basis of sero-epidemiological, biological and biochemical evidence. The entry mechanism of retrovirus infection has been investigated for Rauscher leukemia virus (8), avian sarcoma and leukemia viruses (2) and murine leukemia virus (4, 12), but there is no direct evidence concerning ATLV infection. The present study examined with the entry mechanism of ATLV as the first step to understanding the early events in ATLV infection.

ATLV was produced extracellularly from an ATLV-producing cell line (MT-2) which was established by MIYOSHI et al. (9). To obtain 3H-uridine labeled viruses MT-2 cells were incubated in RPMI-1640 medium containing 5 μCi/ml of tritiated uridine ([5-3H] uridine, specific activity 28 Ci/mmol, Radiochemical Centre Amersham, England) for 4 days and then centrifuged for separation. The labeled cells were further incubated without hot uridine for 4 days and then centrifuged. The virus-containing medium was collected from the supernatant fluid. The labeled virus particles were then partially
purified by differential centrifugation and filtration by our modified method (3). Cellular debris was removed by low-speed centrifugation. The virus-containing supernatant fluid was then filtered through a 0.45 μm membrane filter, centrifuged at 48,000×g for 90 minutes and washed twice with phosphate-buffer saline (PBS, pH 7.2). The resulting virus pellet was resuspended in RPMI-1640 solution. This virus suspension was used immediately as the inoculum virus (50 to 150-fold concentrate: there is no quantitative method for counting the virus).

Human peripheral blood leukocytes (PBL) from healthy anti-ATLA ATL-associated antigen)-negative adults were prepared by Ficoll-Conray gradient centrifugation. The cells (3.5×10⁷ cells per 25 ml culture bottle) were treated with medium containing 1 per cent phytohemagglutinin-M (PHA) for one day at 37°C. After washing with PBS, the cultures were inoculated with 1.0 ml of concentrated virus suspension. Viral adsorption was carried out at 37°C with occasional swirling. The inoculated cells were incubated with RPMI-1640 medium at 37°C, and were harvested at various intervals after the inoculation.

At the end of each incubation period, the cultures were prefixed in situ for 60 minutes at 4°C with cold 0.25 per cent glutaraldehyde buffered at pH 7.2. Glutaraldehyde-fixed peripheral blood leukocytes were treated with 0.2 M glycine in PBS for 1 hour to block the remaining free aldehyde residues. After washing, the cells were further treated with appropriately diluted human sera from anti-ATLA-positive patients or anti-ATLA-negative healthy adults at room temperature for 60 minutes (11). Then they were washed several times with PBS and treated with the ferritin-conjugated IgG F(ab’)2 fragment (Cappel Laboratories, Inc., Cochranville, Pa., U.S.A.) at room temperature for 60 minutes. Thereafter, the cells were washed several times, fixed with both 2 per cent glutaraldehyde and 1 per cent OsO₄ and then dehydrated with ethanol and embedded in Epon 812.

For light microscopic autoradiography sections 1 μm thick were mounted on glass slides. These were dipped in warmed emulsion (Sakura nuclear research emulsion NR-M2) and exposed for one week. The specimens were developed with D-19 at 20°C, and fixed, and stained with toluidine blue. Ultrathin sections were stained with uranyl acetate followed by lead citrate and were examined with a Hitachi H-500 or H-800 electron microscope.

First we determined when the virus particle either entered the cell, or was incorporated into the cell after virus inoculation. Fig. 1 showed a light microscopic autoradiograph of peripheral blood leukocytes 10 minutes after inoculation of tritium-labeled ATL virus particles. The silver grains in PBL appeared round and black. They were also observed in PBL 30 minutes after the inoculation. Electron microscopic autoradiography confirmed the presence of silver grains in the cytoplasm of human peripheral blood leukocytes 30 minutes after the inoculation (data not shown). The presence