RAPID COMMUNICATION

ISOLATION IN CULTURE OF A TYPE C VIRUS FROM A JAPANESE MONKEY SEROPOSITIVE TO ADULT T-CELL LEUKEMIA-ASSOCIATED ANTIGENS

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Co-cultivation of lymphocytes from two Japanese monkeys, one of which was seropositive to adult T-cell leukemia (ATL)-associated antigens (ATLA), gave rise to a lymphoid cell line derived from the anti-ATLA negative monkey. This cell line harbors ATLA and type C virus particles identical in morphology to ATL virus.

Key words: Japanese monkeys — Adult T-cell leukemia virus — Simian type C virus — Lymphoid cell line

A type C retrovirus (ATLV) has been implicated in the etiology of adult T-cell leukemia (ATL) which occurs endemically in southwest Japan.3, 5, 14) A cluster of similar cases has recently been reported among Blacks from the Caribbean.1, 2) We have found that some Japanese monkeys (Macaca fuscata) are seropositive to ATL-associated antigens (ATLA) and type C virus carriers.6, 8) We report here the isolation of a type C virus-producing cell line from lymphocytes of an anti-ATLA negative monkey co-cultivated with lymphocytes from an anti-ATLA positive monkey. The immunological, morphological and biological characteristics of the simian isolate suggest that it is the same virus (or virus group) as ATLV.

Peripheral blood was collected from 2 adult Japanese monkeys. One was an anti-ATLA negative male and the other was an anti-ATLA positive female whose short-term lymphocyte culture expressed ATLA and type C virus particles.6, 8) Both animals were healthy and their peripheral blood smears showed no abnormalities. Mononuclear leukocytes were separated from 10 ml of blood by Ficoll-Hypaque gradient centrifugation and cultured at 10^6/ml in 35 mm Petri dishes (3 ml/dish) with RPMI-1640 medium supplemented with 10% human cord serum, 10% fetal calf serum, and antibiotics. After 2 days, the leukocytes from both donors were combined and co-cultured. The cultures were incubated at 37°C in a humidified 7.5% CO₂ atmosphere and partial medium changes were done twice a week.

Three weeks after the start of co-culture, clumps of cells were formed and slowly increased in size and number. Four weeks later, the first subculture was made and the cells have since been maintained in continuous culture for over 5 months. This cell line, designated Si-2, grows in suspension forming clumps of cells and consists of mostly immature lymphoid cells with occasional giant cells. Si-2 cells were negative for surface immunoglobulin and Epstein-Barr virus nuclear antigen. About 20% of them formed spontaneous rosettes with neuraminidase-treated sheep erythrocytes. The cultured cells did not react with monoclonal antibodies to human T-cells (Leu-1, Leu-2a and Leu-3a). Chromosome analysis of Si-2 showed a normal male macaque karyotype.

When tested for the presence of ATLA by indirect immunofluorescence, almost 100% of Si-2 cells were ATLA-positive as demonstrated by brilliant cytoplasmic fluorescence with anti-ATLA positive sera (Fig. 1A). Moreover, almost all the cells were
fluorescently reactive with antisera to 2 structural proteins, p24 and p19 of a type C retrovirus (HTLV) isolated from an American patient with cutaneous T-cell lymphoma (Fig. 1B). Electron microscopy of Si-2 cells showed many type C virus particles in the extracellular spaces (Fig. 2). The virus particles, which were mostly mature, measured 100-160 nm in diameter and consisted of an electron-dense nucleoid and an outer membrane. Immature virus particles with a doughnut-shape morphology were rarely observed. These virus particles were identical in morphology to ATLV produced by a human cell line (MT-2). Previously, we have found that ATLV-producer cell lines can be established by cocultivation of peripheral lymphocytes from anti-ATLA positive and negative healthy persons. Two of the three cell lines thus established were found, by chromosome analysis, to be derived from the seropositive donors and the other consisted of a mixed population of cells from seropositive and seronegative donors. Consistent with this observation, co-cultivation of peripheral lymphocytes from anti-ATLA positive and negative healthy Japanese monkeys of the opposite sex resulted in the establishment of a type C virus-producer cell line (Si-2) derived from the anti-ATLA negative monkey. This phenomenon, as in the case of human lymphocyte co-culture, was interpreted as implying that during co-culture a type C virus of anti-ATLA positive monkey origin was transmitted to the lymphocytes of the anti-ATLA negative monkey and the
infected lymphocytes gave rise to the transformed cell line. This is the first demonstration of the lymphocyte-transforming capacity of a naturally carried monkey type C virus.

The Si-2 cell line expressed ATLA (ATLV p24 and ATLV-associated cellular proteins) and reacted with antisera to HTLV p24 and p19. These findings indicate that type C virus particles produced by this cell line possess the same structural proteins as ATLV or HTLV. Recently, ATLV has been shown to be identical or closely related to HTLV on the basis of serology and nucleic acid homology.

The present results strongly suggest that the type C virus we have isolated in culture from a Japanese monkey is identical to ATLV on the basis of serology, morphology and biological activity. Further comparative studies on the simian and human isolates may determine their origin. The Si-2 cell line should be useful for studying ATLV infection and immune response in Japanese monkeys.

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REFERENCES


