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Transformation of Animal Cells with Human T-Cell Leukemia Virus Type II

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Human T-cell leukemia virus type II (HTLV-II) was tested for its ability to transform normal animal cells. The HTLV-II-infected human T-cell line, HTLV-IIA was lethally X-irradiated and cocultivated with normal leukocytes of rabbit and crab-eating monkey and spleen cells of hamster. The transformed cell lines, designated Ra-IIA, Si-IIA and Ham-IIA, were established. These cell lines were shown to be infected with HTLV-II by the polymerase chain reaction method combined with the digoxigenin-enzyme-linked immunosorbent assay method. These cell lines were examined for viral antigens by the indirect immunofluorescence method. Although the cytoplasma of over 90% of the cells of Si-IIA cell line was brilliantly stained, Ra-IIA and Ham-IIA cells were not stained. Electron microscopy of cells of the Si-IIA line revealed C-type virus particles in the extracellular spaces.

Key words: HTLV-II — Transformation — Animal cell — PCR-Dig-ELISA

Human T-cell leukemia virus type II (HTLV-II) has been associated with only two patients having unusual T-cell malignancies resembling hairy-cell leukemia.[1,2] However, recently, a high rate of HTLV-II infection was found in seropositive intravenous (IV) drug abusers in New Orleans.[3] To identify HTLV-II's association with or causative role in specific diseases, an experimental model in animals may be a useful tool. In the present study, we investigated whether HTLV-II could transform animal cells. Here, we report that blood cells of normal rabbit and monkey and spleen cells of the hamster can be rapidly transformed by cocultivation with an HTLV-II infected human T-cell line.

Blood was obtained from a young-adult male rabbit and by venous puncture from a young-adult male crab-eating monkey. The sera reacted negatively to anti-human T-cell leukemia virus type I (HTLV-I) antibody. Leukocytes were separated from 10 ml of the blood by "Ficoll-Hypaque" gradient centrifugation and cultured at 10^6/ml in 35 mm Petri dishes with RPMI 1640 medium supplemented with 10% human cord serum, 10% fetal calf serum, and antibiotics. Hamster spleen cells were aseptically removed from a young-adult male hamster, squeezed onto a 150 mesh platinum screen with a spatula and flushed through the screen with culture medium. They were cultivated at 10^6/ml in each dish with culture medium. Simultaneously, 10^6 lethally irradiated (9,000 R) HTLV-II producer human T-cell line cells (HTLV-IIA)[4] were added to each dish.

One week after coculturing, scattered foci of the cell aggregates were noted on macrophage sheets. These cell aggregates increased in size and number, and the first subculture was made three weeks later. The transformed cells were morphologically lymphoid and grew in suspension (Fig. 1). The transformed cell lines, designated as Ra-IIA, Si-IIA and Ham-IIA, have male normal karyotypes of the rabbit, monkey and hamster, respectively. These cell lines were examined for the expression of HTLV-II antigen by indirect immunofluorescence (IF) using sera of an HTLV-II seropositive drug abuser. Although the cytoplasma of over 90% of the cells of Si-IIA cell line was brilliantly stained, Ra-IIA and Ham-IIa cells were not stained. These cell lines were negative for Epstein-Barr virus nuclear antigen. Electron microscopy of cells of the Si-IIA line revealed C-type virus particles of mature, immature and non-cored types in the extracellular spaces (Fig. 2). Control rabbit and monkey lymphocytes, and hamster spleen cells and irradiated HTLV-IIA cells cultured alone degenerated within two months. To detect the HTLV-II provirus genome in the primary cells and the transformed cell lines, DNA from these cells was analyzed by a previously described polymerase chain reaction (PCR) method combined with the digoxigenin (Dig)-enzyme-linked immunosorbent assay (ELISA) method.[4] Briefly, DNA was prepared from samples of approximately 10^6 cells of each type by SDS-proteinase K treatment, followed by phenol and chloroform extraction. Then one microgram of DNA was subjected to 30 cycles of PCR amplification. A primer pair complementary to a conserved region of *pol* (SK58/SK59) was used in the amplification of HTLV-II DNA sequences.[5] The designated primer SK58 is complementary to the viral minus strand and SK59 to the plus strand. After amplification, 10 μl of the reaction

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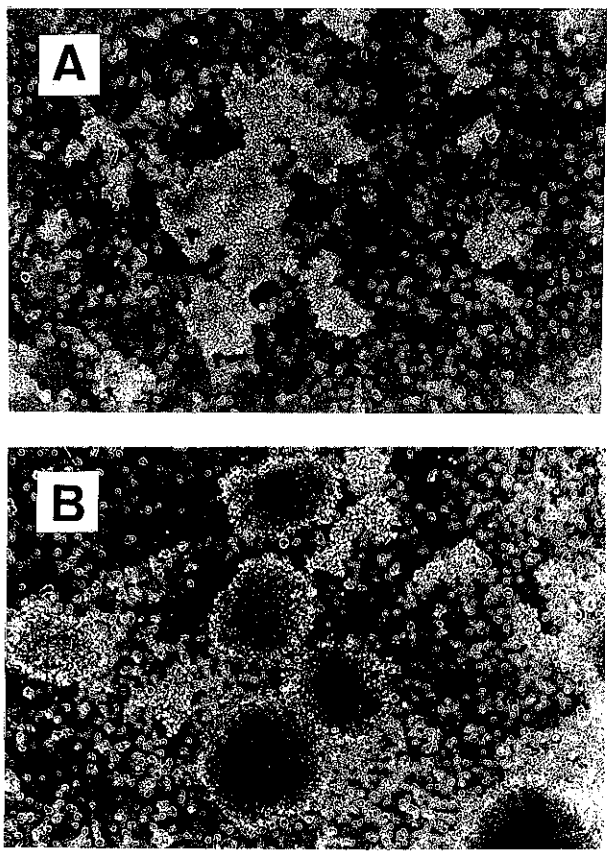
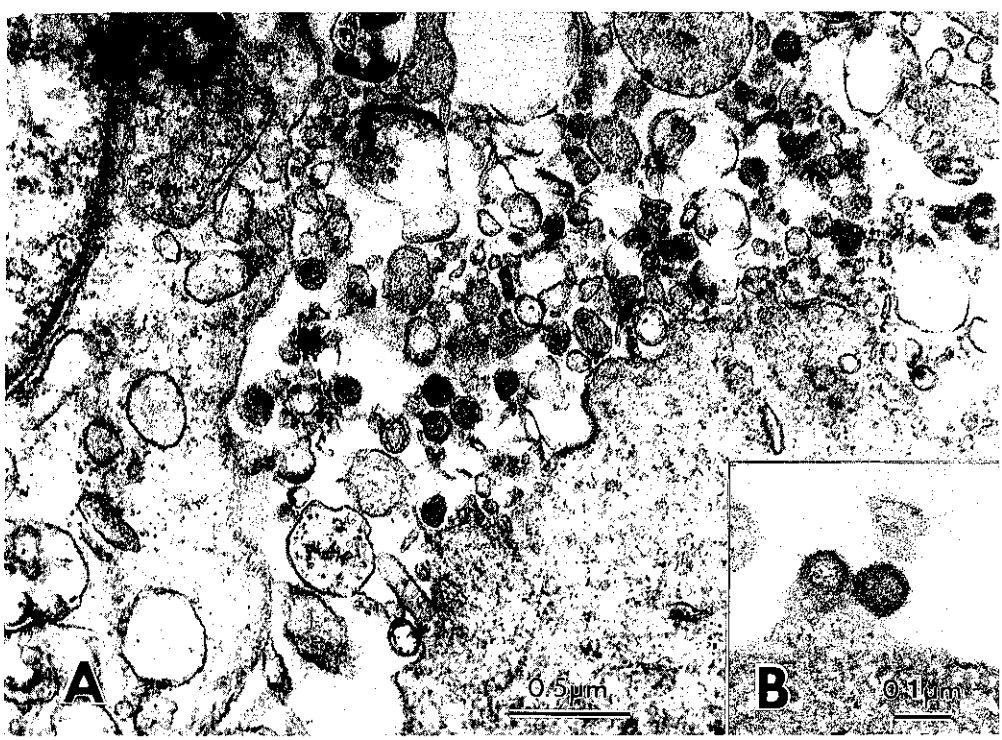


← Fig. 1. Phase-contrast micrograph of the Ra-IIA, Si-IIA and Ham-IIA cell lines. A, Ra-IIA; B, Si-IIA; C, Ham-IIA.

↓ Fig. 2. Electron micrograph of transformed Si-IIA cells showing type C virus particles. A, ×32,000; B, ×73,000.



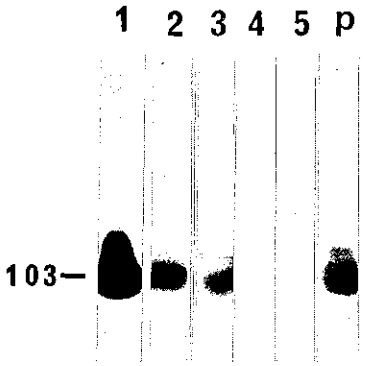


Fig. 3. Detection of HTLV-II provirus genome in transformed animal cell lines. Lane 1: Ra-IIA cell line. Lane 2: Si-IIA cell line. Lane 3: Ham-IIA cell line. Lane 4: MT-2 cell line. Lane 5: healthy anti-HTLV-I antibody-negative cells. Lane P: positive control (HTLV-IIA cell line).[4] The primer SK58 is situated at the position from 4198 to 4217 in the *pol* region of the HTLV-II virus sequence and the primer SK59 from 4281 to 4300. The nucleotide sequences of SK58 and SK59 are 5′-ATCTACCTCCACCATGTCCG-3′ and 5′-TCAGGGGAACAAGGGGAGCT-3′. The designated SK60 probe is complementary to the viral plus strand. SK60 is situated at the position from 4237 to 4276 in the *pol* region of the HTLV-II virus genome sequence, and its nucleotide sequence is 5′-GACCCAATTTCCACCTTCAATGAATACACAGACTCCCTTA-3′.

mixture was used for further analysis by 1.8% agarose gel electrophoresis, followed by denaturing in alkaline solution and transfer onto a 'Hybond N' (Amersham) nylon membrane. After pre-hybridization and hybridization with the 3′-end Dig-11-dUTP-labeled SK60 probe, the filter was washed, blocked with blocking reagent, reacted with polyclonal sheep anti-Dig Fab fragments conjugated to alkaline phosphatase and colored with nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate solutions. The SK60 probe was an oligonucleotide of a *pol* region in the HTLV-II provirus DNA sequence.[5] The position of the 103-nucleotide HTLV-II-specific amplified DNA product is indicated in the transformed cell lines (Fig. 3). These results indicate that HTLV-II immortalizes not only human T-cells but also the leukocytes and spleen cells of such animals as the rabbit, monkey and hamster.

Similarly, HTLV-I immortalized not only human T cells [6] but also the lymphocytes of such animals as the monkey,[7] rabbit,[8] cat,[9] hamster[10] and rat.[11] The established HTLV-II-infected animal cell lines we have described here may be useful for the induction of a specific disease with associated HTLV-II infection or may contribute to elucidation of the mechanisms involved in the pathogenesis of multiple maladies involving HTLV-II.

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