Transformation of CD8+ T-Cells Producing a Strong Cytopathic Effect on CD4+ T-Cells through Syncytium Formation by HTLV-II

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Human T-cell leukemia virus type II (HTLV-II) is thought to play an important role in the development of CD8+ T-cell malignancies resembling hairy cell leukemia. In this study, dramatic cytopathic effects characterized by syncytium formation in various CD4+ T-cell lines were observed upon their cocultivation with HTLV-II infected T-cells. The HTLV-II infected T-cells, however, did not die as a result of syncytium formation. HTLV-II also transformed CD4+ T-cells and CD8+ T-cells at various coculture ratios. Furthermore, sera from antiHTLV-II antibody-positive specific carriers inhibited syncytium formation in the CD4+ T-cells. These results suggest that HTLV-II infection may contribute to the pathogenesis of associated CD8+ T-cell malignancies.

Key words: Cytopathic effect — CD8+ T-cell transformation — HTLV-II

Human T-cell leukemia virus type II (HTLV-II) has been isolated from a patient with an atypical variant of hairy cell leukemia.[1] The role of HTLV-II in human malignancy has not been defined, but two patients have been found to have HTLV-II infection in usual CD8+ T-cell malignancies resembling hairy cell leukemia.[1, 2] Recently, a high rate of HTLV-II infection has been found in seropositive intravenous (IV) drug users (DUs) and AIDS patients among human immunodeficiency virus type I (HIV-I) seropositive IV DUs.[3–5] Here we investigate the role of HTLV-II in human malignancy and report the transformation of CD8+ T-cells and CD4+ T-cell loss as cytopathic effects in syncytium formation caused by cocultivation with HTLV-II-infected T-cells.

We previously isolated the HTLV-II virus by cocultivation of leukocytes from a 35-year-old white male IV drug abuser in New York with those of a healthy Japanese female. The surface markers of the transformed cell line, HTLV-IIA, were CD3+, CD4– and CD8+.[6] The speed of growth of the transformed T-cell line decreased with the passage of culture time. To investigate the role of HTLV-II in human malignancy, we again carried out cocultivation to obtain a more stably growing HTLV-II-infected T-cell line. Human umbilical cord leukocytes from another healthy Japanese, a male infant, were separated by Ficoll-Conray gradient centrifugation. Separated leukocytes (10^6 cells) were cultured in a 35 mm Petri dish with RPMI 1640 medium supplemented with 10% fetal calf serum, 10% human cord serum and antibiotics. Simultaneously, 10^5 lethally irradiated (9,000 R) HTLV-II-infected T-cells (HTLV-IIA cell line) were added to the dish. Two weeks after the start of coculturing, scattered foci of cell aggregates appeared and increased thereafter in size and number, and a subculture was made one month later. The transformed cells were morphologically lymphoid, grew in suspension, and had a normal male karyotype. Expression of HTLV-II antigens was present in over 90% of the cells as determined by indirect immunofluorescence (IF) using anti-HTLV-II antibody-positive sera and negative Epstein-Barr virus nuclear antigen. The surface markers of the transformed cell line, designated as HTLV-IIC, were CD3 (91%), CD4 (70%) and CD8 (31%). To detect the HTLV-II provirus genome in the transformed cell line, DNA from the cells was analyzed by a previously described polymerase chain reaction (PCR) method combined with the digoxigenin (Dig)-enzyme linked immunosorbent assay (ELISA).[6] The position of the 103-nucleotide base pair (bp) HTLV-II-specific amplified DNA product is indicated in the transformed cell line (Fig. 1). To date (May 1991), this cell line has been maintained in continuous culture for over six months (Fig. 2a).

We noted a few syncytia during cocultivation of human leukocytes in vitro (Fig. 2b). To investigate the mechanism of syncytium formation, cocultivation was attempted with HTLV-IIC cells (donor cells) and various hematopoietic cell lines, five CD4+ T-cell lines (Molt-4, Jurkat, MKB-1, HD-Mar-2 and KE-37), an HTLV type I (HTLV-I)-infected CD4+ T-cell line

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(MT-2), a CD4− T-cell line (Molt-16), a B-cell line (B-ATL3 derived from an adult T-cell leukemia (ATL) patient),[7] non-T non-B cell lines (HL-60 and K562) and an HIV-1-infected T-cell line (HIV-1/Molt-4)(recipient cell). Recipient cells (4.0×10^5 cells from each of the various hematopoietic cell lines) were seeded into a 24-well plate. Simultaneously, 2.0×10^5 donor cells (HTLV-
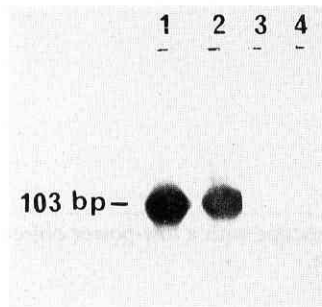

Fig. 1.   Detection of the HTLV-II provirus genome in the HTLV-IIC cell line. Lane 1: positive control (HTLV-IIA cell line).[6] Lane 2: HTLV-IIC cell line. Lane 3: HTLV-I-infected T-cell line, MT-2. Lane 4: negative control (anti-HTLV-I antibody-negative healthy male). The primers, SK58 and SK59 and a probe, SK60, were used in the PCR reaction.[6, 14]

IIC cell line and HIV-1/Molt-4 cell line) were seeded into each well. Cocultivation was done for 24 h. Syncytium formation was observed in all five CD4+ T-cell lines and the HIV-1/Molt-4 cell line (Fig. 2c, d, e, f, g and h) (Table I). After a week of cocultivation of the five CD4+ T-cell lines at 37°C in 5% CO_2, living cells appeared *in vitro*. Chromosome analysis was performed to examine their origin. Most of the cell karyotypes, with the exception of leukocytes cocultivated with cells of the KE-37 cell line, were human normal male 46,XY derived from the donor cells (HTLV-IIC cell line). We then examined the growth curves of the cells and did a chromosome analysis of HTLV-IIC cocultivated with the five CD4+ T-cell lines. The populations of the CD4+ T-cell lines, with the exception of the KE-37 cell line, decreased dramatically with the passage of coculture time. The population of the KE-37 cell line decreased slowly, while HTLV-IIC cells slowly increased (Fig. 3). When the HTLV-IIC cells and cells of the five CD4+ T-cell lines were cocultived, a great deal of small cell debris from the CD4+ T-cells, due to the cytopathic effects of syncytium formation, was observed. This observation indicates that the HTLV-IIC cells remained, because the cells of each of the five CD4+ T-cell lines were rapidly destroyed. To determine the effect on the CD8+ T-cell and HTLV-II-
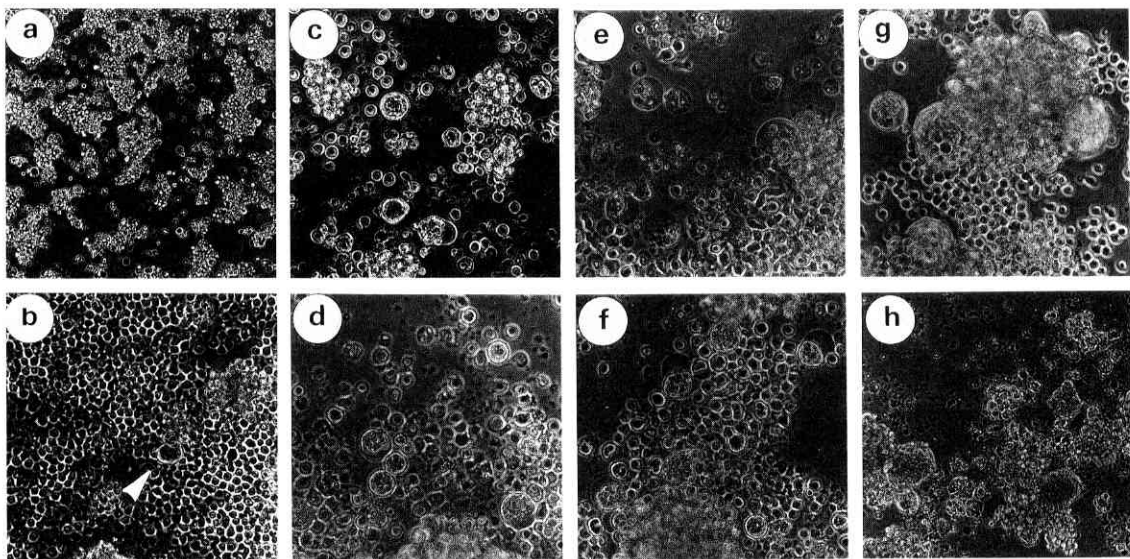

Fig. 2.   Appearance of syncytium formation in peripheral blood leukocytes, five CD4+ T-cell lines and the HIV-1/Molt-4 cell line cocultivated with the HTLV-IIC cell line for 24 h. a: HTLV-IIC cell line only. b: About 10^6 peripheral blood leukocytes were seeded in a 35 mm Petri dish. Simultaneously, 10^5 lethally irradiated (9,000 rad) HTLV-II infected T-cells were added to the dish. About two weeks after coculturing, a few syncytia appeared. The arrow indicates a syncytium. c–g: 2.0×10^5 HTLV-IIC cells were seeded into each well of a 24-well plate (Falcon dish). Simultaneously, 4.0×10^5 cells of five CD4+ T-cell lines, Molt-4 (c), Jurkat (d), MKB-1 (e), HD-Mar-2 (f) or KE-37 (g), were added to the dish. Cocultivation was done for 24 h. (h) 4.0×10^5 HIV-1/Molt-4 cells were seeded into a well of a 24-well plate. Simultaneously, 2.0×10^5 HTLV-IIC cells were added to the well. Cocultivation was done for 24 h.

Table I. Syncytium Induction in Various Human Hematopoietic Cell Lines Cocultivated with HTLV-IIC Cell Line for 24 h

Donor cells	Recipient cells (CD4, CD8)	Coculture ratio donor cells:recipient cells	Syncytium induction
HTLV-IIC	Molt-4 (CD4+, CD8+)	1:2	++
"	Jurkat (CD4+, CD8−)	1:2	++
"	MKB-1 (CD4+, CD8−)	1:2	++
"	HD-Mar-2 (CD4+, CD8+)	1:2	++
"	KE-37 (CD4+, CD8−)	1:2	++
"	MT-2 (CD4+, CD8−)	1:2	−
"	Molt-16 (CD4−, CD8−)	1:2	−
"	B-ATL3 (CD4−, CD8−)	1:2	−
"	HL-60 (CD4−, CD8−)	1:2	−
"	K562 (CD4−, CD8−)	1:2	−
"	HIV-1/Molt-4 (CD4−, CD8+)	1:2	++
HIV-1/Molt-4	H9 (CD4+, CD8−)	1:2	+

Whole syncytia in one well of a 24-well plate were counted on an inverted microscope with a low-power objective (×40). ++, more than 500 syncytia; + less than 500 syncytia; − absence of syncytia.
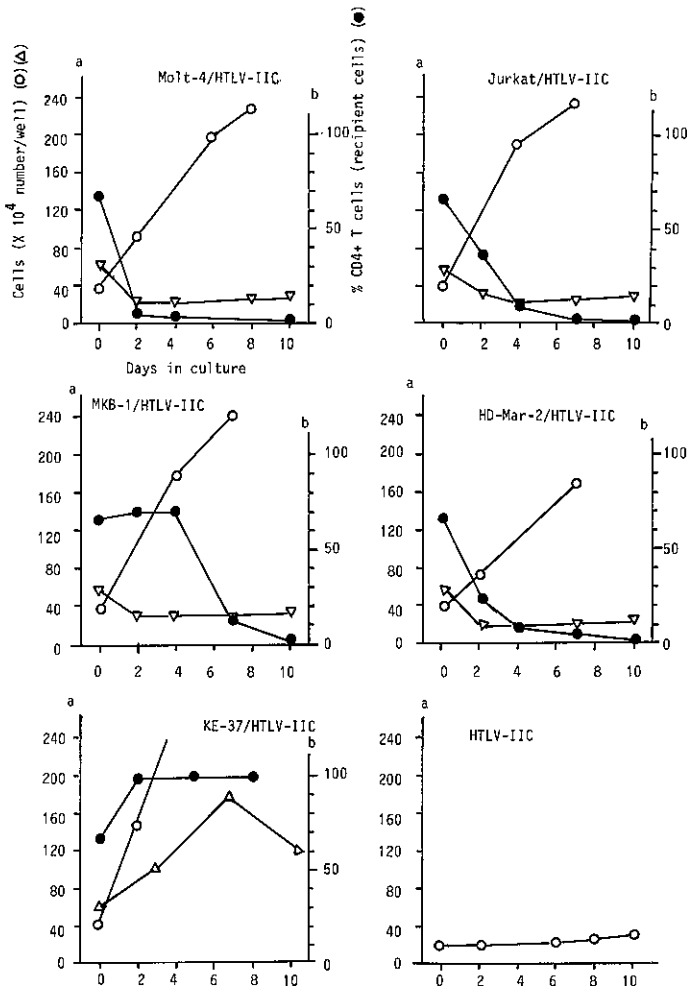

Fig. 3. Growth curves of each of the five CD4+ T-cell lines, Molt-4, Jurkat, MKB-1, HD-Mar-2 and KE-37 (○), and HTLV-IIC cell line cells (○) and the total numbers of cells remaining following cocultivation of the cells of each of the five CD4+ T-cell lines with HTLV-IIC cells (△). Percentage of cells of each of the five CD4+ T-cell lines remaining on various days of cocultivation (●). a: 4.0× 10^5 cells of each of the five CD4+ T-cell lines and 2.0×10^5 cells of HTLV-IIC cell line per well were seeded into a 24-well plate. The cells from each of the CD4+ T-cell lines and the HTLV-IIC cells were cultivated for 2–10 days. The total cell numbers were counted on various days of the culture (○). Each of the five CD4+ T-cell lines (4.0×10^5 cells) was seeded into a 24-well plate. Simultaneously, 2.0 ×10^5 HTLV-IIC cells were added to each well. The initial total cell numbers were 6.0×10^5 (4.0×10^5 cells of each of the five CD4+ T-cell lines +2.0×10^5 HTLV-IIC cells) on day 0 of the culture. Cocultivation was performed for 10–11 days (△). The open circles represent total numbers of cells of each of the five CD4+ T-cell lines and HTLV-IIC cells. The inverted open triangles represent the total number of remaining cells after cocultivation of cells of each of the five CD4+ T-cell lines and HTLV-IIC cells. b: After the HTLV-IIC cells had been cocultivated with cells of each of the five CD4+ T-cell lines, chromosome analysis was done to distinguish the donor cells (HTLV-IIC cells) from the recipient cells (the five CD4+ T-cell lines cells) on various coculture days. The karyotype of the donor cell was normal human male, 46, XY and the recipient cells showed structural abnormalities and numerical abnormalities in hyperdiploid or near-diploid ranges. About 500 metaphase cells were examined by the Q-banding method. The results are expressed as the percentage of recipient metaphase cells/recipient metaphase cells + donor metaphase cells (●).

infected T-cell interrelation, a monoclonal antibody against CD4+ T-cells was added to the cocultures of the CD4+ CD8+ Molt-4 cell line and the HTLV-IIC cell line. Syncytium formation was blocked by the monoclonal antibody. When HTLV-IIC cells were cocultivated with the HIV-1/Molt-4 cell line, the number of syncytia observed was more than double that noted when HIV-1-infected T-cells were cocultivated with the CD4+ T-cell line, H9 cells. In this case, both types of cells died due to cytopathic effects during the first week of cocultivation.

Based on the observation that CD4+ T-cell loss is a cytopathic effect of syncytium formation caused by HTLV-II-infected T-cells, we investigated whether HTLV-II can transform CD4+ T-cells or CD8+ T-cells at various coculture ratios of donor cells (lethally irradiated HTLV-IIC cell line) and recipient cells (leukocytes from three healthy adult females) in vitro. Recipient cells (1.5×10^6) (case 1) stimulated by phytohemagglutinin for three days were seeded in 35 mm Petri dishes. Simultaneously, various numbers of donor cells $(5 \times 10^4 - 6 \times 10^5)$ were added to the dishes. Cocultivation was done for five weeks. Similarly, 1×10^6 recipient cells (case 2) were seeded in 35 mm Petri dishes and various numbers of donor cells $(5 \times 10^4 - 6 \times 10^5)$ were added to the dishes. Cocultivation was done for five weeks. In case 3, 2×10^6 recipient cells were seeded in 35 mm Petri dishes and various numbers of donor cells $(1 \times 10^5 - 2 \times 10^6)$ were added. Cocultivation was done for eight weeks. The surface markers of the transformed cells were altered from CD8+ T-cells to CD4+ T-cells by increasing the coculture ratio of donor cells and recipient cells (Table II). The results indicate that

HTLV-II can transform CD4+ T-cells and/or CD8+ T-cells by cell-to-cell fusion. When the coculture ratio is small, CD8+ T-cells can be transformed because of the cytopathic effects of syncytium formation on uninfected CD4+ T-cells. On the other hand, when the coculture ratio is large, the cytopathic effects may be weak because of the decrease of uninfected CD4+ T-cells caused by HTLV-II infection of CD4+ T-cells and CD8+ T-cells. Therefore, HTLV-II-infected CD4+ T-cells grow dominantly in vitro.

We also measured the neutralizing activity of sera from anti-HTLV-II antibody-positive IV DUs, anti-HTLV-I antibody-positive healthy carriers, an ATL patient and anti-HIV-1 antibody-positive hemophiliac patients by observing the inhibitory effects on syncytium formation of HTLV-IIC cells cocultivated with the Molt-4 cell line. Molt-4 cells (8×10^4) were cocultivated with 4 $\times 10^4$ HTLV-IIC cells per well in a 96-well plate and sera were added to each sample at the final concentrations indicated (1:10-1:>2560) at the time of seeding. Cocultivation was done for 24 h. The sera titers of anti-HTLV-II antibody-positive carriers are the reciprocal of the highest dilution causing >80% reduction of syncytium formation.

Neutralizing antibodies specific to HTLV-II were found in the sera of anti-HTLV-II antibody-positive IV DUs in titers varying from 1:20 to 1:>2560 but none was found in the sera of HTLV-I antibody-positive carriers, an ATL patient or anti-HIV-1 antibody-positive hemophiliac patients. Neutralizing antibodies specific to HTLV-II in the sera of IV DUs did not correlate closely with the antibody titers for internal viral antigens (Table III).

Table II. Characterization of Transformed Cells by Cocultivation with Various Donor Cells (X-Ray-irradiated HTLV-IIC Cell Line) and Recipient Cells (Leukocytes of Three Healthy Females) at Various Ratios

Case no.	Donor cell line	Recipient cell source age/sex	Coculture ratio donor cells: recipient cells	Time in coculture (weeks)	Surface markers				HTLV-II antigens (%)	Karyotype
					CD3 (%)	CD4 (%)	CD8 (%)	CD4/CD8 ratio		
1	HTLV-IIC	44/F	1:30	5	88	31	67	0.46	>80	46,XX
			1:8	//	87	45	55	0.82	>80	46,XX
			1:2.5	//	96	66	34	1.94	>80	46,XX
2	HTLV-IIC	40/F	1:20	//	97	51	49	1.04	>80	46,XX
			1:5	//	97	78	21	3.71	>80	46,XX
			1:1.7	//	96	79	18	4.38	>80	46,XX
3	HTLV-IIC	58/F	1:10	8	90	35	76	0.46	>80	46,XX
			1:7	//	84	50	66	0.76	>80	46,XX
			1:1	//	88	68	32	2.12	>80	46,XX

Culture medium was RPMI 1640 + 5% fetal calf serum + 5% human cord serum. Transformation was defined as a state of the cells allowing subcultivation. Expression of the HTLV-II antigens was examined by immunofluorescence test. Anti-HTLV-II antibody-positive carriers' sera were used.

Table III. HTLV-II-neutralizing Antibody in IV DUs, HTLV-I Carriers, an ATL Patient and Anti-HIV-1 Antibody-positive Hemophiliac Patients

Serum sample	Serum source	PA method Titer values	IF method Titer values	Titer values Syncytium inhibition
1	IV DU	$> \times 512$	$> \times 10$	$\times 20$
2	IV DU	$> \times 512$	$> \times 10$	$> \times 2560$
3	IV DU	$\leqq 512$	$> \times 10$	$\times 320$
4	IV DU	$> \times 512$	$> \times 10$	$\times 160$
5	IV DU	$> \times 512$	$> \times 10$	$\times 80$
6	IV DU	$\leqq 512$	$> \times 10$	$\times 160$
7	IV DU	$\times 256$	$> \times 10$	$\times 160$
8	HTLV-I carrier	$> \times 128$	$> \times 10$	$< \times 10$
9	//	$> \times 128$	$> \times 10$	$< \times 10$
10	//	$> \times 128$	$> \times 10$	$< \times 10$
11	ATL patient	$> \times 128$	$> \times 10$	$< \times 10$
12	Hemophiliac patient	$> \times 128$	$> \times 10$	$< \times 10$
13	//	$> \times 128$	$> \times 10$	$< \times 10$
14	//	$> \times 128$	$> \times 10$	$< \times 10$

Human sera determined to be positive by the particle agglutination test (PA) kit (SERODIA.HTLV-I and SERIDIA. HIV) (Fujirebio Inc.) and an indirect immunofluorescence (IF) method using MT-2/Molt-4 mixed cells and HIV-1/Molt-4 cell line were titrated after heat inactivation (56°C for 30 min). Serum samples Nos. 1–7 are from IV DUs in New York. These samples were confirmed to be HTLV-II genome-positive IV DUs.

HTLV-I and HTLV-II have been associated with specific forms of malignancy in humans.[1, 8-10] HTLV-I can transform human CD4+ T-cell or CD8+ T-cells in culture.[6, 11] HTLV-I is a retrovirus associated with disorders of the CD4+ subset of T lymphocytes. Here we have shown that the cytopathic effects of HTLV-II-infected T-cells characterized by syncytium formation occur in various CD4+ T-cell lines. Also, it is indicated that HTLV-II can transform CD8+ T-cells and CD4+ T-cells at various coculture ratios. The results suggest that HTLV-II infection may contribute to the pathogenesis of the associated CD8+ and CD4+ subsets of T lymphocyte malignancies. Recently, a high rate of HTLV-II infection has been found in AIDS patients among HIV-1-seropositive IV DUs. IV DUs infected with both viruses are three times more likely to die from AIDS during follow-up than are those infected with HIV-1 only.[5] Also, Lefrere et al.[12] reported rapid progression to AIDS in dual HIV-1/HTLV-II infection. The progression towards disease of the patient was evidenced by a decrease in CD4+ T-cell count. However, serum HIV-1 p24 antigen remained negative during follow-up, as is sometimes observed in the pre-disease phase.[12] The mechanism involved is unknown. The effects of coinfection with HTLV-II on the survival of HIV-1 antibody-positive AIDS patients may be accounted for by the acceleration of CD4+ T-cell loss from cytopathic effects characterized by syncytium formation caused by HTLV-II-infected T-cells. We also found neutralizing antibodies specific to HTLV-II, varying in titer from 1:20 to 1: >2560, in the sera of anti-HTLV-II antibody-positive IV DUs. Previously, Clapham et al. reported that plaque assays of vesicular stomatitis viruses, HTLV-I and HTLV-II pseudo types, were employed to determine the neutralizing antibodies in the serum of ATL patients and a hairy cell leukemia patient.[13] The methodology used here and previously described methods may be useful in screening for neutralizing antibodies in patients and carriers, in distinguishing anti-HTLV-I anti-HTLV-II and anti-HIV-1 antibodies from each other, and in monitoring future vaccine approaches.

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