## *Brief Definitive Report*

# ORIGIN OF HUMAN T-LYMPHOTROPHIC VIRUS I–POSITIVE T CELL LINES IN ADULT T CELL LEUKEMIA

## Analysis of T Cell Receptor Gene Rearrangement

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Adult T cell leukemia (ATL), endemic in Japan and other countries, is thought to be caused by type I human T cell leukemia/lymphoma (lymphotrophic) virus (HTLV-I) (1–5). In the majority of instances, both the leukemic cells and the established T cell lines have a surface phenotype of helper T cells, and express interleukin 2 (IL-2) receptors (2, 6–9). The abnormal IL-2 receptor expression on these cell lines is due to the constitutive transcription of the IL-2 receptor gene (10, 11). It is important to examine the clonal origin of the established ATL cell lines, particularly because leukemic cells of acute ATL cases do not proliferate in response to IL-2, despite the expression of the IL-2 receptor (12). A mature T cell has unique rearrangements of cellular genes encoding the T cell antigen receptor (13, 14). Since ATL is a leukemia of mature peripheral T cells (1, 2), the rearrangement profile of the T cell receptor gene is a suitable genetic marker for tracing the clonal origin of ATL cells and the cell lines established from the patients. Using the genomic fragment encoding the $\beta$ chain of the human T cell receptor ($T_\beta$) as probe, we analyzed the rearrangement profiles of $T_\beta$ gene in leukemic cells and cell lines from various ATL cases. Based on the findings, we discuss the origin of HTLV-I$^+$ T cell lines derived from ATL patients and the role of IL-2/IL-2-receptor system (15, 16) on the leukemogenesis in ATL.

## Materials and Methods

*Cell Culture.* Cells were cultured in RPMI-1640 medium supplemented with 10–20% of fetal calf serum (FCS) (Rehatuin, Armour Pharmaceutical Co. Ltd., Kankakee, IL) IL-2-dependent T cells were cultured in petri dishes with crude or recombinant IL-2 (see below) in humidified atmosphere of 5% $CO_2$ in air at 37°C.

*Interleukin 2.* To obtain crude IL-2 $2 \times 10^6$ spleen cells/ml were cultured for 48–72 h in the presence of 0.1% phytohemagglutinin-P (PHA-P) (Difco Laboratories, Detroit, MI), $10^{-6}$ M indomethacin (Sigma Chemical Co., St. Louis, MO), and 2% FCS. Recombinant IL-2 produced by yeast or by *E. coli* was kindly provided by Suntry Co., Osaka,

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Japan, and Takeda Pharmaceutical Co., Osaka, respectively. IL-2 activity was titrated using the mouse IL-2-dependent cytotoxic T lymphocyte (CTLL-2) cell line (15).

*Southern Blot Hybridization.* Human T cell receptor $\beta$ chain gene was isolated from normal human placenta DNA (17) by crosshybridization with mouse $T_\beta$ chain cDNA provided by M. Davis (14). A 2.6 kilobase (kb) Hind III fragment, containing constant region of the human $\beta$ chain, was used as the $T_\beta$ probe. For the detection of the provirus of HTLV-I, the long terminal repeats (LTR) (Sma I–Sac I, 456 base pairs [bp]) (18) was used as the probe. High–molecular weight DNA extraction from the cells, digestion with appropriate restriction enzymes (Eco RI or Bam HI), electrophoresis in 0.6% agarose, blotting to nitrocellulose filter, and hybridization (15 h, 65°C) with $^{32}$P-labeled, nick-translated probes were carried out as described (19). Filters were washed with 0.3× standard sodium citrate (SSC) with 0.1% sodium dodecyl sulfate (SDS) ($T_\beta$ probe) or 0.1× SSC with 0.1% SDS (HTLV probe) at 65°C (19).

*T Cell Lines Derived from ATL Patients.* Leukemic cells separated from ATL patients were cultured in the presence of crude IL-2 or recombinant IL-2. IL-2-dependent T cell lines were relatively easily established from ATL patients but not from HTLV-I$^-$ healthy donors. All the IL-2-dependent T cell lines have been maintained for at least 8 mo. The surface phenotypes of the T cell lines were analyzed by using mouse monoclonal antibodies reactive with T cell antigens, as previously described (7). The intensity of the fluorescence was analyzed by flow cytometry using Ortho Spectrum III (Ortho Pharmaceutical Co., NJ).

## Results and Discussion

*Clonal Continuity between In Vitro Cell Lines and Leukemic Cells in a Chronic ATL Case.* HTLV-I$^+$ T cell lines were established from a 34-y-old man with chronic ATL. In contrast to many ATL cases with acute clinical courses, he manifested no deterioration of his general clinical state for 10 y, except for massive erythrodermia and sustained leukocytosis. Southern blot hybridization with HTLV-I U3R probe showed a common band in two DNA of the leukemic T cells obtained at an interval of 2 mo. The result indicated that the leukemic cells were comprised of a monoclonally expanded HTLV-I-infected T cell clone. When peripheral blood lymphocytes (PBL) of the patient were cultured in the presence of an IL-2-containing conditioned medium, the cells proliferated promptly within a day of culture. IL-2-dependent growth continued and a cell line was established. The cell line, ED-40515, bearing the T4 marker, has been maintained for >1 y. Using recombinant IL-2, independent cultures were initiated 3 mo after the first trial, and two more T cell lines with the T4$^+$ phenotype were established and maintained for 10 mo. Surface phenotype of these three T cell lines was T3$^+$, T4$^+$, T8$^-$, T11$^+$, and positive for Tac antigen, which were identical to that of original leukemic cells. All the cell lines remained dependent on IL-2. Because of the peripheral T cell property of the leukemic cells and the established T cell lines of ATL, we analyzed the rearrangement profiles of the $T_\beta$ gene. Rearrangements of $T_\beta$ gene were clearly shown by a decrease in intensities of the germline bands (Eco RI, 10.5 kb; Bam HI, 23 kb; as shown in Fig. 1*C*), and by the appearance of one or two new bands. There was an identical rearrangement pattern of the $T_\beta$ gene among all of the IL-2-dependent T cell lines and the original leukemic cells (Fig. 1*A*). In the DNA of original leukemic cells and the three established T cell lines, the 10.5 kb Eco RI fragment of germline disappeared, while a rearranged 7 kb fragment appeared. In Bam HI digests, the 23 kb germline fragment was lost, and two rearranged fragments of smaller size

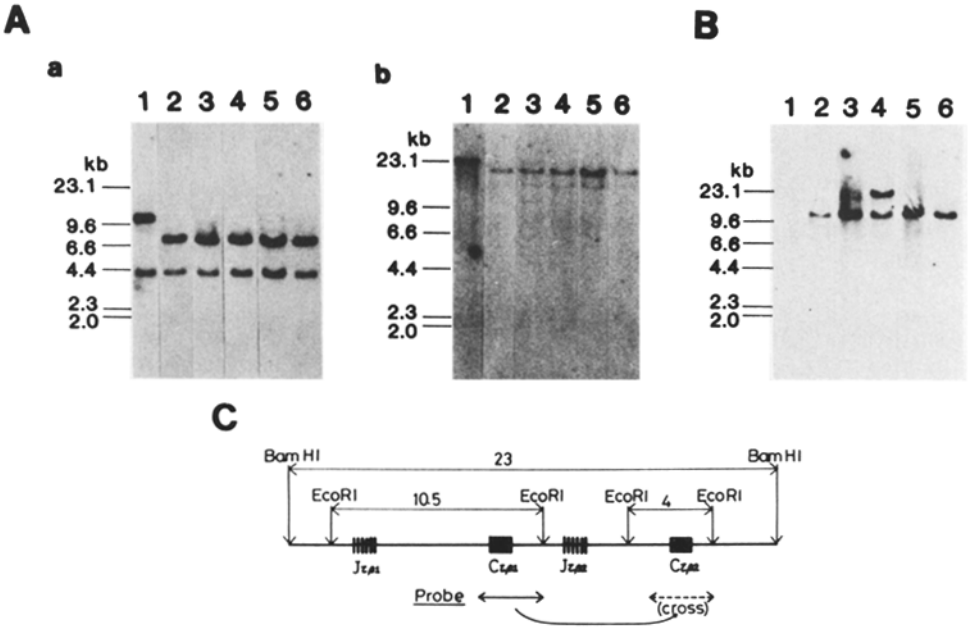


FIGURE 1. Clonality analysis of chronic ATL leukemic cells and T cell lines derived from them by Southern blot hybridization using $T_\beta$ probe and HTLV U3R probe. (*A*) Analysis of T cell receptor gene rearrangement by $T_\beta$ probe. Restriction enzymes used were: *a*, Eco RI; *b*, Bam HI. (*B*) Analysis of HTLV integration site by HTLV U3R probe. DNA were digested with restriction enzyme Eco RI. Origin of DNA were: lane 1, human placenta; 2, PBL of a chronic ATL patient T. Ya.; 3–6, cell lines derived from patient T. Ya. [3, ED-40515, 2 mo in culture; 4, ED-40515, 4 mo in culture; 5, ED-40810(S); 6, ED-40810 (T)]. (*C*) Schematic demonstration of T cell receptor gene rearrangement determined by $T_\beta$ probe.

appeared, indicating the rearrangement on both chromosomes. Coincidence of the genotype between the T cell lines and the original leukemic cells was confirmed by Southern blot hybridization with HTLV U3R probe (Fig. 1*B*). There was a band of the same size as that of original leukemic cells in all of three lines, and an additional band in ED-40515 cell DNA. The results strongly indicate that the three independently established T cell lines were derived from the major clone in the original leukemic cells, which had monoclonally expanded in vivo.

*Established T Cell Lines Represent Clones that Differ from Major Leukemic Clones in Acute ATL.* We also established IL-2-dependent T cell lines from PBL of five acute ATL patients. Four T cell lines had a helper surface phenotype ($T4^+T8^-$) consistent with that of the original leukemic cells of ATL. However, in one patient, the established cell line had a phenotype of killer/suppressor, although original leukemic cells carried the $T4^+T8^-$ phenotype. As was the case with the chronic ATL case, one or two rearranged fragments of the $T_\beta$ gene was detected in each T cell line, as well as the leukemic cells of these acute ATL cases (Fig. 2*A*). When the profiles of the $T_\beta$ gene rearrangement are compared between the cell line and the original leukemic cells, it is evident that the patterns of the $T_\beta$ gene rearrangement were different between pairs of the cell line and the original leukemic cells (ATL-2, ATL-7, and ATL-12) (Fig. 2*A*). Moreover, the HTLV genome was integrated in multiple copies in all these cell lines, as shown

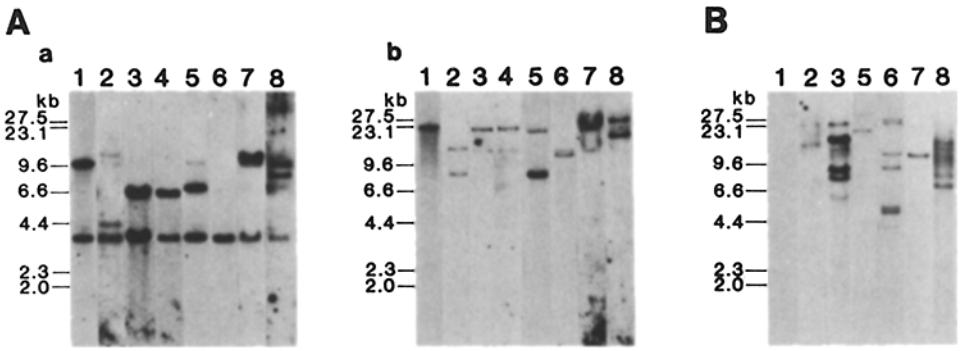


FIGURE 2. Clonality analysis of ATL-derived T cell line cells and its original ATL leukemic cells. (*A*) T cell receptor gene rearrangement by $T_\beta$ probe. DNA were digested with restriction enzymes: *a*, Eco RI; *b*, Bam HI. (*B*) HTLV integration site by HTLV U3R probe. Origin of DNA were: lane 1, human placenta; 2, PBL of patient T. Y.; 3, ATL-2 cell line derived from patient T. Y. (4 mo in culture); 4, ATL-2 cell line (6 mo in culture); 5, PBL of patient R. T.; 6, ATL-7 cell line derived from patient R. T.; 7, PBL of patient K. N.; 8, ATL-12 cell line derived from patient K. N.

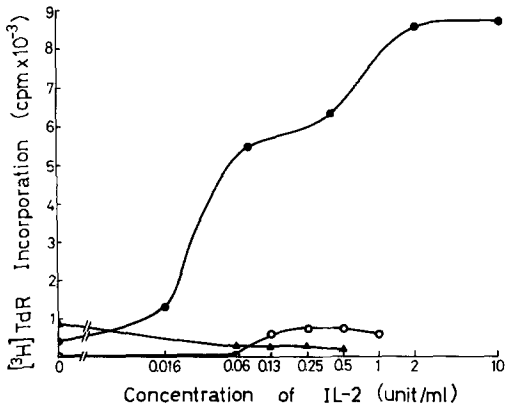


FIGURE 3. The effect of IL-2 on the DNA synthesis by ATL leukemic cells. $5 \times 10^4$ Ficoll-Hypaque-separated peripheral blood mononuclear cells were cultured in a culture medium for 72 h in the presence of different concentration of purified IL-2. Cells were pulsed with methyl-[$^3$H]thymidine ([$^3$H]TdR) (2 Ci/mM) for the last 4 h, and the cells were harvested on glass filter paper using cell harvester (Bellco Glass Co., Vineland, NJ) and [$^3$H]thymidine incorporation was counted by Packard Mark II scintillation counter. (●) PBL from a chronic ATL patient T. Ya. (○) and (△) PBL from two typical ATL patients. Peripheral blood cell count of the three patients were 23,000, 70,000 and 127,500 cells/ml, respectively.

in Fig. 2*B*. The patterns of HTLV-I provirus integration also differ between each pair of the leukemia cells and T cell line, in agreement with the difference in the T cell receptor gene rearrangement. These results demonstrate unequivocally that there is no clonal continuity between the leukemic cells of three acute ATL patients and the corresponding established HTLV-I$^+$ IL-2-dependent T cell lines.

*IL-2-Reactive and -Nonreactive Leukemic Cells in ATL Cases.* As expected, a prompt proliferative response to IL-2 was observed in the leukemic cells of this chronic ATL patient (Fig. 3). In contrast, IL-2 failed to induce a detectable proliferative response in leukemic cells freshly isolated from the typical ATL

patients (Fig. 3). Probably because of this unresponsiveness to IL-2, leukemic cells of the majority of acute ATL cases did not grow in vitro (12). Moreover, the lack of clonal continuity between the cell lines established from the patients with acute ATL, as evidenced by the data accumulated in this report, indicates that most established acute ATL cell lines do not represent the vast majority of leukemic cell clones expanded in vivo. Consequently, the acute ATL cell lines examined thus far may have been selected for survival and growth in vitro from the IL-2-reactive T cells, which might have been infected with HTLV-I either in vivo or during in vitro culture (20). Although the role of the IL-2 receptor system in HTLV-I-induced leukemogenesis remains ill-defined, apparently ATL cells differ markedly from normal T cells, which only express IL-2 receptors transiently upon T cell antigen receptor activation. Our results on the IL-2-responsive chronic ATL case may indicate that there is an IL-2-reactive stage in HTLV-1 leukemogenic process. Further studies on the IL-2 receptor on ATL cells and the accumulation of similar IL-2-reactive ATL cases is required to clarify this possibility.

## Summary

Using the clone-specific rearrangement of the T cell receptor gene as the genetic marker of the clonotype, we analyzed the clonal origin of the interleukin 2 (IL-2)-dependent human T-lymphotrophic virus I (HTLV-I)–positive T cell lines established from various adult T cell leukemia (ATL) patients. From a patient with chronic ATL, whose leukemic cells proliferated in vitro in response to IL-2, we repeatedly established leukemic T cell clones having the same rearrangement profile of the $T_\beta$ chain gene as the leukemic cells. By contrast, established cell lines from acute ATL patients had different $\beta$ chain gene rearrangements from those of the leukemic cells. These HTLV-$I^+$ T cell lines might not be the direct progeny of the leukemic cells, but that of T cells infected either in vivo or in vitro. These IL-2-reactive nonleukemic T cells might have been selected in vitro, because their leukemic cells failed to respond to IL-2, despite the expression of IL-2 receptor. The analysis of the T cell receptor gene rearrangement may give a new approach for the elucidation of the mechanism of leukemogenesis and the origin of the HTLV-$I^+$ T cell lines in ATL.

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