

RAT LYMPHOID CELL LINES PRODUCING HUMAN T CELL LEUKEMIA VIRUS

II. Constitutive Expression of Rat Interleukin 2 Receptor

By JUNJI YODOI,* MASAFUMI OKADA,* YUTAKA TAGAYA,*
KEISUKE TESHIGAWARA,* KIYOSHI FUKUI,‡ NORIO ISHIDA,‡
KOH-ICHI IKUTA,‡ MICHİYUKI MAEDA,§ TASUKU HONJO,‡
HISAO OSAWA,|| TIBOR DIAMANTSTEIN,|| MASATOSHI TATENO,¶ AND
TAKASHI YOSHIKI¶

*From the *Institute for Immunology, ‡Department of Medical Chemistry, §Chest Disease Research Institute, Kyoto University, Faculty of Medicine, Sakyo-ku, Kyoto 606, Japan; ||The Immunology Research Unit, Klinikum Steiglitz, Freie Universitaet Berlin, Federal Republic of Germany; and ¶Sapporo City Hospital, Sapporo, Japan*

Adult T cell leukemia (ATL)¹ (1, 2), associated with human T cell leukemia/lymphoma virus (type I) (HTLV-I) (3), or ATL virus (ATLV) (4), is endemic to Japan, the Caribbean, and other areas (5). Beside integrating the proviral DNA of HTLV, ATL cell lines continuously express an antigen (Tac antigen) (6) associated with the human interleukin 2 (IL-2) receptor (7-9), the specific receptor for IL-2 (T cell growth factor) (10). An abnormality in the regulation of this receptor was discovered (9) when it was found that the Tac antigen on HTLV⁺ cells was not downregulated by anti-Tac monoclonal antibodies (mAb), whereas the same antigen on activated T cells was downregulated by the antibodies (11).

The striking association between the expression of Tac antigen and HTLV infection led us to the question of whether the abnormal induction of IL-2 receptor by HTLV infection is unique to human lymphoid tissues. Here, we report that the IL-2 receptor is constitutively expressed not only in HTLV⁺ human lymphoid cell lines, but also in rat cell lines transformed by HTLV-I (12). As was the case with human cell lines transformed by HTLV-I, these rat cell lines were shown to be in the T cell lineage, since they showed a rearrangement of the beta chain gene of the T cell antigen receptor (13). In contrast to human HTLV⁺ T cell lines reactive with anti-Tac antibodies (6), however, these HTLV⁺ rat cell lines failed to react with anti-Tac mAb. Instead, they reacted with antibodies against the rat IL-2 receptor (ART-18) (14, 15). These findings show that human as well as rat lymphoid cells transformed by HTLV constitutively express their own IL-2 receptor gene. The intimate relationship between HTLV

This work was supported by grants-in-aid from the Ministry of Education, Science, and Culture of Japan.

¹ *Abbreviations used in this paper:* ADF, ATL-derived factor; ATL, adult T cell leukemia; cDNA, complementary DNA; FITC-G/M, fluoresceinated goat anti-mouse IgG; HTLV, human T cell leukemia/lymphoma (lymphotrophic) virus; IL-2, interleukin 2; LTR, long terminal repeat; PAS, protein A-Sepharose; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

infection, IL-2 receptor gene expression, and transformation is discussed (16, 17).

Materials and Methods

Cells. Three HTLV⁺ rat lymphoid cell lines were obtained by cocultivating lymphoid cells of WKA/Hok rats with ATL cells from a typical ATL patient after first treating the ATL cells with 5-bromo-2'-deoxyuridine. TARS-1 and TART-1 cells were positive for the rat antigen Thy-1, whereas TARS-2 cells were negative (12). MT-1 (18) and ATL-2 cell lines (19) established from typical ATL patients were HTLV⁺, and expressed the Tac antigen (19).

Detection of T Cell Antigen Receptor Gene and HTLV Provirus Gene. The presence of mouse T cell antigen receptor gene was analysed using β chain complementary DNA (cDNA) (730 basepair Eco RI fragment of 86T5 [provided by Dr. M. M. Davis, Stanford University, Palo Alto, CA]) (13). High molecular weight DNA (2 μ g each) were digested with Eco RI and Bgl II, and fractionated by agarose gel electrophoresis. Southern blots of the gels were hybridized under the condition described previously (20) with nick-translated, ³²P-labeled β chain cDNA. The presence of the HTLV provirus genome was determined by a Southern blotting technique using DNA probes of the HTLV genome. Southern blots of the gels were hybridized with the nick-translated, ³²P-labeled long terminal repeat sequences (LTR) (Sma I/Sac I, 456 basepairs), and *gag* (Sma I/Sma I, 1,083 basepairs) probes of the HTLV/ATLV (21, 22). After hybridization, filters were washed in 0.1 \times SSC (SSC is 0.15 M NaCl in 0.015M sodium citrate) and 0.1% sodium dodecyl sulfate (SDS) at 65°C, and exposed for autoradiograph.

¹²⁵I-labeled IL-2. *E. coli*-derived recombinant IL-2 (provided by Aji-nomoto Co., Ltd.) was labeled with ¹²⁵I using B.H. reagent (23). Effective radioactivity bindable to the ATL-2 cells that expressed IL-2 receptors was 25% of total activity.

Scatchard Plot Analysis of IL-2 Receptor. 3 \times 10⁶ cells were incubated with ¹²⁵IL-2 (1,000 cpm/ng) at room temperature for 40 min. After washing three times in 1 ml of medium, by centrifugation at 1,200 rpm for 10 min, the cells were centrifuged at 10,000 rpm for 3 min on the layer of fetal calf serum. The radioactivity bound to the cells was counted by a gamma counter. Each value was calculated by subtracting nonspecific binding counts in the presence of a 500-fold excess of cold IL-2.

Detection of IL-2 Receptor by Anti-IL-2 Receptor mAb. 5 \times 10⁵ cells were incubated with 20 μ l of appropriately diluted IgG fraction of anti-rat IL-2 receptor (ART-18) or anti-human IL-2 receptor (anti-Tac) at 4°C for 30 min. After staining with fluoresceinated goat anti-mouse IgG (FITC-G/M) (Cappel Laboratories, Cochranville, PA) as the second antibody, the fluorescence intensity of the cells was analyzed by flow cytometry using a fluorescence-activated cell sorter (Becton Dickinson, Mountain View, CA). The data are given on a linear- or log-fluorescence scale. Positive fluorescence intensity was set so that three percent of the cells were positive when X5563 murine myeloma IgG2a was used as a negative control.

SDS-polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis with Anti-IL-2 Receptor. The cells were radiolabeled externally with ¹²⁵I, lysed with the nonionic detergent Nonidet P-40 (NP-40), and immunoprecipitated with anti-Tac or ART18. The radiolabeled materials bound to protein A-Sepharose (PAS) beads were analyzed by SDS-PAGE in a 7.5% acrylamide gel, under reducing conditions. 5 \times 10⁷ cells were suspended in pH 8.0 phosphate buffered saline containing 20 mM glucose, and incubated with 10 μ l of lactoperoxidase (2 mg/ml), 20 μ l of glucose oxidase (10 U/ml), and 0.5 mCi ¹²⁵I-NaI (17.4 Ci/mg; New England Nuclear, Boston, MA) for 30 min at room temperature. The cells were lysed in an extraction buffer (0.5% Nonidet P-40, 10 mM Tris-HCl, 0.15 M NaCl, 1 mM phenylmethylsulfonyl fluoride, pH 7.5). After preabsorption with PAS and Sepharose beads conjugated with human Ig, the lysates were incubated overnight at 4°C with PAS and 5 μ g/ml of either anti-Tac or ART-18 as an Ig fraction. After washings, the materials bound to the beads were eluted with SDS sample buffer and analyzed with SDS-PAGE (7.5% gel).

Results

Presence of HTLV Provirus Genome. The presence of proviral DNA of HTLV-1 in an HTLV⁺ rat cell line (TARS-1) was determined by Southern blot hybridization using DNA fragments of *pol*, *env*, and *pX* sequences of HTLV. Both the TARS-1 cell line and leukemic cells in the peripheral blood of the ATL patient contained multiple copies of the viral genome. As shown in Fig. 1, they contained both complete and defective viral genomes. Although the data are not shown here, the genome of TARS-1 did not contain the human repetitive *Alu* sequence (24). This result strongly suggests that there has been no transfer of human genome associated with the introduction of HTLV genome during the cocultivation of rat spleen cells and ATL cells.

Rearrangement of T cell Receptor Gene. Since ATL is a human leukemia of peripheral T cells, we analyzed the possible rearrangement of the T cell receptor β chain gene in these HTLV-1⁺ rat cell lines. DNA from these cell lines and from rat liver (germline control) were analyzed using a β chain DNA probe in Southern blot hybridization. As shown in Fig. 2, β chain genes were all rearranged in these rat cell lines compared with germline DNA. The loss of one of the two bands in these cell lines suggested that the 3' constant region gene was rearranged, while the 5' constant region gene was deleted. The rearrangement appeared to be biallelic. Although we could not conclude whether the rearrangement was a VDJ

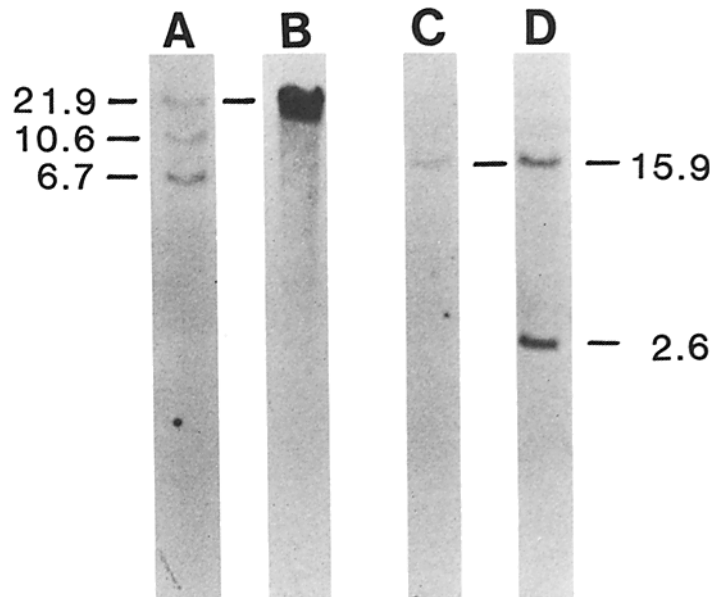


FIGURE 1. Proviral DNA of HTLV in TARS-1 cells and peripheral blood lymphocytes of the ATL patient that had been used for the establishment of the rat cell line. DNA were obtained from TARS-1 cells and the leukemic cells in peripheral blood of the ATL patient used for the coculture to establish HTLV⁺ rat cell lines. After digestion with restriction enzymes, and electrophoresis in 0.5% agarose gel, they were hybridized with LTR and *gag* probes. (A and B) High molecular weight DNA (2 μ g each) from TARS-1 cell line; (C and D) High molecular weight DNA from the leukemic cells of the ATL patient. Numbers on the left and right indicate sizes (in kilobases) of the hybridizing bands, based on the migration of λ Hind III markers.

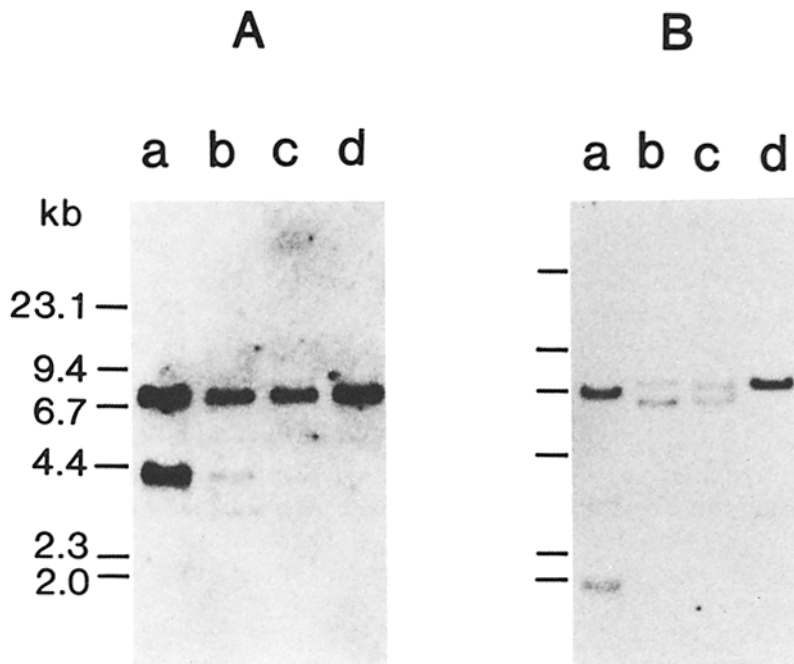


FIGURE 2. Rearrangement of T cell receptor gene in HTLV-1⁺ rat cell lines. DNA from rat liver, TARL-2, TART-1, and TARS-1 cells were digested with appropriate restriction enzymes and fractionated by 0.7% agarose gel electrophoresis. Southern blots of the gels were hybridized with mouse β chain cDNA probe (86T5). Size marker is Hind III digest of λ DNA. (A) Eco RI digestion; (B) Bgl II digestion; (lane a) rat liver; (lane b) TARL-2; (lane c) TART-1; (lane d) TARS-1.

(variable-diversity-joining) recombination or a DJ recombination, the data suggested that these cell lines were in the T cell lineage.

Affinity of IL-2 Receptor for ^{125}I -IL-2. The presence of IL-2 receptor of the TART-1 cell line was demonstrated by its binding with ^{125}I -IL-2. The association constant (K_a), and the number of receptor sites were calculated from a Scatchard plot. The results are shown in Fig. 3. The results show that there are at least two different levels of affinity for IL-2 receptor. Receptors with a high affinity for IL-2 have a K_a of $1.3 \times 10^{11}/\text{M}$, and those with low affinity have a K_a value of $8.8 \times 10^9/\text{M}$. The number of receptor sites was also calculated using this analysis. There are 1.8×10^4 high affinity receptor sites, and 5.6×10^4 low affinity receptor sites per cell. TARS-1 and TARL-2 cell line cells also expressed $\sim 7.1 \times 10^3$ and 7.8×10^3 IL-2 binding sites per cell, respectively, while the HTLV⁺ human T cell line, ATL-2, had 3.9×10^5 IL-2 binding sites per cell (data not shown). As expected, there was no significant IL-2 binding in unstimulated rat spleen cells.

Detection of IL-2 Receptor by Anti-IL-2 Receptor mAb. To clarify whether a host IL-2 receptor was expressed in HTLV⁺ cell lines, the antigenic properties of IL-2 receptor on HTLV⁺ rat and human T cell lines were compared. The antigenic properties of IL-2 receptors were determined by mAb recognizing rat IL-2

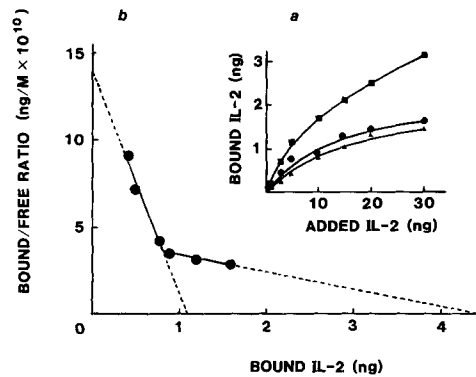


FIGURE 3. Scatchard plot of TART-1. (a) Binding curves of ¹²⁵I-labeled IL-2 to TART-1 (■). Total binding of IL-2 to TART-1; (●) specific binding; (▲) nonspecific binding. Scatchard plot is calculated by these curves. (b) Scatchard plot. The K_a value for receptors with a high affinity is $1.3 \times 10^{11}/M$; the K_a for receptors with a low affinity is $8.8 \times 10^9/M$. The number of high affinity receptor sites is 1.8×10^4 per cell, and that of low affinity receptor is 5.6×10^4 per cell.

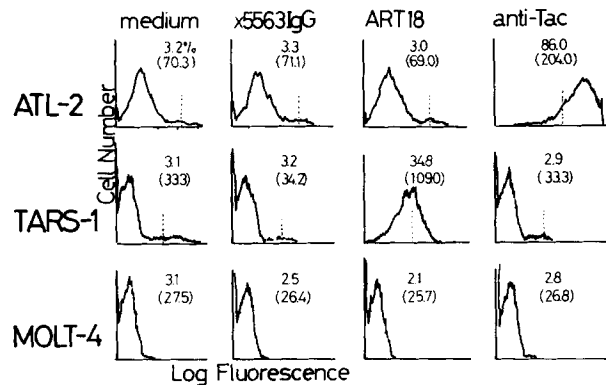


FIGURE 4. Expression of surface antigens associated with IL-2 receptor on rat and human T cell lines. HTLV⁺ human ATL-2, rat TARS-1 T cell lines, and HTLV⁻ human MOLT-4 T cell lines were assessed for the expression of antigens reactive with anti-rat IL-2 receptor (ART-18) and anti-human IL-2 receptor (anti-Tac). The fluorescence intensity higher than the vertical dashed line represents the positive labelling. The line is set so that a background nonspecific fluorescence with irrelevant x5563 IgG shows ~3% positivity. The proportion of positive cells is shown in as percent positive, while the mean fluorescence intensity is shown in parentheses. The data is expressed in the log fluorescence scale.

receptor (ART-18) and human IL-2 receptor (anti-Tac). As shown in Fig. 4, cells from the HTLV⁺ ATL-2 cell line expressed a determinant reactive with anti-Tac antibody. There was no significant staining with either ART-18 or X5563 mouse Ig, used as a control, confirming a previous observation that the IL-2 receptor on human HTLV⁺ cell lines does not react with ART-18 (14). As expected, the HTLV⁻ MOLT-4 T cell line failed to react with any of the antibodies. In TARS-1 cells, there was no significant staining with anti-Tac, despite the presence of IL-2 receptor, as detected by ¹²⁵I-IL-2 binding. In contrast, >30% of the cells were stained with ART-18. As shown in Fig. 5, all three HTLV⁺ rat T cell lines were positive for ART-18, but not for anti-Tac,

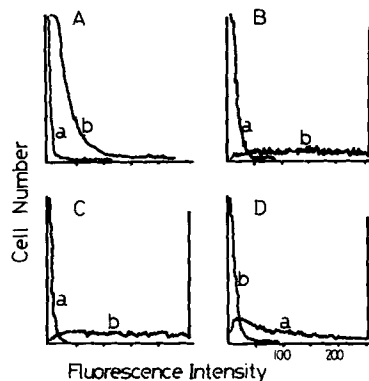


FIGURE 5. The staining of three HTLV⁺ rat cell lines with anti-human and anti-rat IL-2 receptor mAb. (A) TARS-1; (B) TARL-2; (C) TART-1; (D) ATL-2. These cell line cells were stained with anti-Tac (a) and ART-18 (b), followed by FITC-G/M antibodies. The fluorescence intensity is expressed in a linear fluorescence scale.

proving that the IL-2 receptor expressed on HTLV⁺ rat T cell lines has the antigenic determinant reactive with ART-18 but not with anti-Tac. This result was confirmed by the finding that the binding of ¹²⁵I-IL-2 to these cells is inhibited by ART-18 but not by anti-Tac, whereas the binding to ATL-2 cells is inhibited by anti-Tac but not by ART-18 (not shown).

Characterization of the IL-2 Receptor on TARS-1 Cells by SDS-PAGE. To compare the properties of the IL-2 receptor molecules expressed on human and rat HTLV⁺ cell lines, MT-1 cells and TARS-1 cells were internally labeled with [³⁵S]-methionine. Their IL-2 receptors were immunoprecipitated with anti-Tac and ART-18, and characterized by SDS-PAGE analysis. As shown in Fig. 6, anti-Tac precipitated 60–65 kilodalton (kD) molecules from MT-1 cell lysates, but not from lysates of TARS-1 cells. It is known (25) that the 60–65 kD band is obtained from normal T cells, HTLV⁻, as well as HTLV⁺ human cell lines bearing IL-2 receptor. On the other hand, ART-18 precipitated 50–55 kD molecules from TARS-1, but not from MT-1 cells. The size of ART-18-reactive molecules corresponds to the size of molecules from normal activated T cells in rat (15).

Discussion

The expression of IL-2 receptor on rat cell lines transformed by HTLV-I was clearly demonstrated in our study by both the binding assay with ¹²⁵I-IL-2, and by flow cytometry with anti-IL-2 receptor mAb. The results clearly show that HTLV infection induced IL-2 receptor expression on both human (7–9) and rat lymphoid cells. The analysis of the T cell receptor β chain gene (Fig. 2) showed that these cell lines had a rearrangement in the β chain gene. The data suggested that these HTLV-I-transformed rat cell lines were in the T cell lineage, despite the fact that one of the cell lines, TARL-2, had been lacking for any T cell marker on the cell surface (12).

It is generally known that HTLV⁺ human cell lines (6–9) express the human IL-2 receptor. IL-2 receptor is strongly expressed in ATL cells after a short-

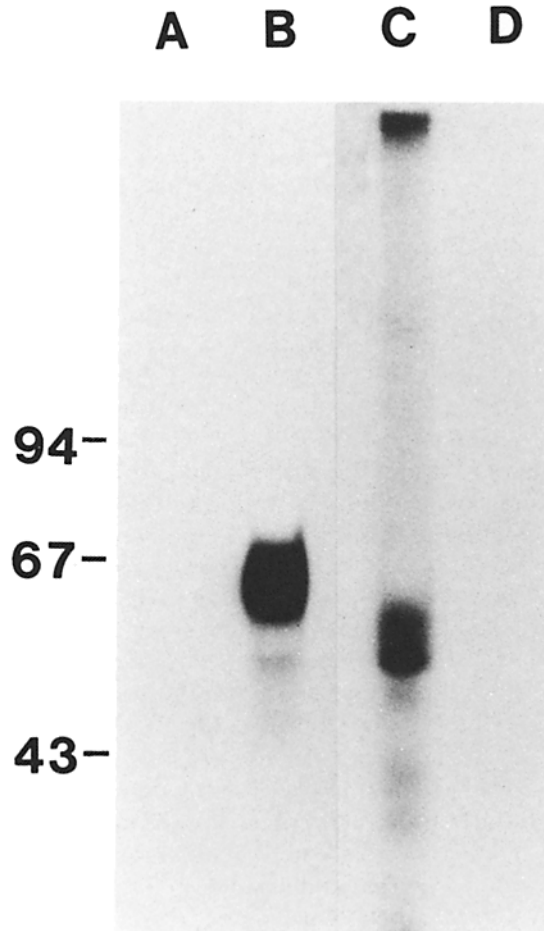


FIGURE 6. SDS-PAGE analysis of antigens on rat TARS-1 and human MT-1 cells transformed with HTLV/ATLV. Lysates of TARS-1 (A) and MT-1 cells (B) were immunoprecipitated with anti-Tac; those of TARS-1 (C) and MT-1 cells (D) were immunoprecipitated with ART-18 antibodies.

term incubation *in vitro* (8). In such cases, there are no gross abnormalities in the IL-2 receptor molecules, as determined by SDS-PAGE (25). Functionally, however, the IL-2 receptor on the ATL-derived cell lines (MT-1 and Hut-102) have a significantly lower affinity for IL-2 than that of the receptors on HTLV⁻ cell lines (our unpublished observations). However, TART-1 cells proved to have receptors with a high affinity for IL-2.

Rat IL-2 receptor, not human IL-2 receptor, was expressed on HTLV⁺ rat cell lines, as demonstrated by the fact that the IL-2 receptor on the rat cells does not react with anti-Tac, but does react with ART-18 (14). The collective evidence indicates that the IL-2 receptors on these cells are not the product of the extrinsic gene sequence transferred from human chromosomes during cocultivation with human ATL cells. It appears that HTLV infection is associated with the constitutive expression of a host gene coding for IL-2 receptor, regardless of the species. Our results are quite consistent with the data using cDNA for recently

cloned human IL-2 receptor genes (26, 27). In ATL-derived cell lines expressing IL-2 receptor/Tac antigen, there was continuous transcription of the cellular IL-2 receptor gene. We have not detected any gross abnormality of the IL-2 receptor gene in ATL cell lines.

The mechanism that leads to the expression of the host IL-2 receptor gene in HTLV⁺ lymphoid cell lines has not yet been elucidated. There are no common integration sites for HTLV, and the virus does not have typical oncogenes (20). One possible explanation is the trans-acting transcriptional activity of the HTLV-I genome, which may activate the transcription of host genes, including the IL-2 receptor gene (28). On the other hand, it was recently found that many HTLV⁺ human T cell lines with helper phenotype produce a unique lymphokine, which enhances the synthesis and expression of human IL-2 receptor. The lymphokine, named ATL-derived factor (ADF) (19, 29), augmented the synthesis and expression of the ART-18 antigen, associated with rat IL-2 receptor, on TARS-1 cells, across the species barrier (our unpublished data). It was also found that ADF and the viral proteins having trans-activating effects (28) seemed to have approximately the same molecular size, 40 kD. One may speculate that ADF may be related to the latter viral protein. Alternatively, the trans-activating protein may activate the cellular ADF gene to produce ADF, which, in turn, stimulates expression of the IL-2 receptor in susceptible cell lines. Indeed, our preliminary study indicated that ADF enhanced IL-2 receptor gene expression (unpublished).

There is no direct evidence yet that IL-2 receptor plays a role in the leukemogenesis in ATL. Since the majority of HTLV⁺ cell lines neither produce a significant amount of IL-2 (17) nor contain messenger RNA for IL-2 (18), an autocrine mechanism regulating IL-2 does not seem to be involved in the transformation in ATL. However, recent findings (30) of homology between the *erb* B oncogene and the receptor gene for epidermal growth factor strongly suggest that an abnormally increased density of growth factor receptors, such as IL-2 receptor, may play a crucial role in the abnormal proliferation of cells. Using a cDNA probe for IL-2 receptor gene, the analysis of the constitutive expression of IL-2 receptor gene in ATL cell lines transformed by HTLV will be facilitated.

Summary

Three rat lymphoid cell lines (TARS-1, TARS-2, and TARS-3) (12) transformed by human T cell leukemia/lymphoma virus I (HTLV-I) had rearrangement of the β chain gene of the T cell antigen receptor, and had integrated proviral DNA from HTLV-I in their genomes. As is the case with adult T cell leukemia (ATL)-derived human T cell lines transformed by HTLV-I, these rat cell lines unequivocally expressed interleukin 2 (IL-2) receptor, as determined by radiolabeled IL-2 binding. By Scatchard plot analysis, one of the cell lines, TARS-1, proved to have high affinity receptors ($K_a = 1.3 \times 10^{11}/M$ and $8.8 \times 10^9/M$). Rat IL-2 receptor, not human IL-2 receptor, was expressed on HTLV⁺ rat cell lines, as demonstrated by the fact that they expressed antigens reactive with monoclonal antibodies (ART-18) against rat IL-2 receptor, but not with anti-Tac antibodies. The collective evidence indicates that the endogenous IL-2 receptor gene is activated in human and rat lymphoid cell lines with HTLV-I

production. The mechanism of abnormal IL-2 receptor expression in HTLV infection is discussed.

We thank Dr. T. Uchiyama (Department of Internal Medicine, Faculty of Medicine, Kyoto University) and Dr. J. Hamuro (Aji-no-moto Co. Ltd.) for kindly supplying anti-Tac mAb and recombinant IL-2, respectively. We also appreciate the supply of 86T5 T cell receptor β chain cDNA given by Dr. M. M. Davis, Stanford University. We appreciate the excellent technical help of Dr. T. Nikaido, T. Noma, and Miss A. Kasahara.

Received for publication 5 September 1984 and in revised form 17 January 1985.

References

1. Yodoi, J., K. Takatsuki, and T. Masuda. 1974. Two cases of T-cell chronic leukemia in Japan. *New Engl. J. Med.* 290:572.
2. Uchiyama, T., J. Yodoi, K. Sagawa, K. Takatsuki, and H. Uchino. 1977. Adult T-cell leukemia: Clinical and hematologic features of 16 cases. *Blood.* 50:481.
3. Poiesz, B. Z., F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, and R. C. Gallo. 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T cell lymphoma. *Proc. Natl. Acad. Sci. USA.* 77:7415.
4. Hinuma, Y., K. Nagata, M. Hanaoka, M. Nakai, T. Matsumoto, K. Kinoshita, S. Shirakawa, and I. Miyoshi. 1981. Adult T-cell leukemia; Antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proc. Natl. Acad. Sci. USA.* 78:6476.
5. Gallo, R. C., and F. Wong-Staal. 1982. Retroviruses as etiologic agents of some animal and human leukemias and lymphomas and as tools for elucidating the molecular mechanism of leukemogenesis. *Blood.* 60:545.
6. Uchiyama, T., S. Broder, and T. A. Waldmann. 1981. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. I. Production of anti-Tac monoclonal antibody and distribution of Tac(+) cells. *J. Immunol.* 126:1393.
7. Robb, R. J., A. Munck, and K. A. Smith. 1981. T cell growth factor receptors. Quantitation, specificity and biological relevance. *J. Exp. Med.* 154:1455.
8. Hattori, T., T. Uchiyama, T. Toibana, K. Takatsuki and H. Uchino. 1981. Surface phenotype of Japanese adult T-cell leukemia cells characterized by monoclonal antibodies. *Blood.* 58:645.
9. Tsudo, M., T. Uchiyama, H. Uchino, and J. Yodoi. 1983. Failure of regulation of Tac antigen/TCGF receptor on adult T cell leukemia cells by anti-Tac monoclonal antibody. *Blood.* 61:1014.
10. Morgan, D. A., F. W. Ruscetti, and R. C. Gallo. 1976. Selective in vitro growth of T-lymphocytes from normal human bone marrows. *Science (Wash. DC).* 193:1007.
11. Tsudo, M., T. Uchiyama, K. Takatsuki, H. Uchino, and J. Yodoi. 1982. Modulation of Tac antigen on activated human T cells by anti-Tac monoclonal antibody. *J. Immunol.* 129:592.
12. Tateno, M., N. Kondo, T. Itoh, T. Chubachi, T. Togashi, and T. Yoshiki. 1984. Rat lymphoid cell lines with human T cell leukemia virus production. I. Biological and serological characterization. *J. Exp. Med.* 159:1105.
13. Hedrick, S. M., E. A. Nielsen, J. Kavalier, D. I. Cohen, and M. M. Davis. 1984. Sequence relationships between putative T-cell receptor polypeptides and immunoglobulins. *Nature (Lond.).* 308:153.
14. Osawa, H., and T. Diamantstein. 1983. The characteristics of a monoclonal antibody

- that binds specifically to rat T lymphocytes and inhibits IL-2 receptor functions. *J. Immunol.* 30:51.
15. Osawa, H., and T. Diamantstein. 1984. Partial characterization of the putative rat-interleukin-2-receptor. *Eur. J. Immunol.* In press.
 16. Yodoi, J., T. Uchiyama, and M. Maeda. 1983. T-cell growth factor receptor in adult T-cell leukemia. *Blood.* 62:509.
 17. Arya, S., F. Wong-Staal, and R. C. Gallo. 1984. T-cell growth factor gene. Lack of expression in human T-cell leukemia-lymphoma virus infected cells. *Science (Wash. DC).* 223:1086.
 18. Miyoshi, I., I. Kubonishi, M. Sumida, S. Hiraki, T. Tsubota, I. Kimura, K. Miyamoto, and J. Sato. 1980. A novel T-cell line derived from adult T-cell leukemia. *Gann.* 71:155.
 19. Yodoi, J., K. Teshigawara, T. Uchiyama, Y. Wano, M. Tsudo, T. Nikaido, K. A. Smith, and M. Maeda. 1984. Adult T-cell leukemia-derived factor augmenting TCGF (IL-2) receptor expression. In *Cancer Cells. Volume 3, Human T-Cell Leukemia Viruses.* R. C. Gallo, M. E. Essex, and L. Gross, editors. Cold Spring Harbor Laboratory, New York. 255-259.
 20. Yaoita, Y., and T. Honjo. 1980. Detection of immunoglobulin heavy chain genes accompanies the class switch rearrangement. *Biomed. Res.* 1:164.
 21. Seiki, M., S. Hattori, Y. Hirayama, and M. Yoshida. 1983. Human adult T-cell leukemia virus: Complete nucleotide sequence of the provirus genome integrated in leukemia cell DNA. *Proc. Natl. Acad. Sci. USA.* 80:3618.
 22. Fukui, K., T. Noma, K. Takeuchi, N. Kobayashi, M. Hatanaka, and T. Honjo. 1983. Origin of adult T-cell leukemia virus. Implication for its zoonosis. *Mol. Biol. Med.* 1:447.
 23. Bolton, A. W., and Hunter, W. M. 1973. The labelling of proteins to high specific radioactivities by conjugation to a ¹²⁵I-containing acylating agent—application to the radioimmunoassay. *Biochem. J.* 133:529.
 24. Jelinek, W. R., T. P. Toomey, L. Leinwand, C. H. Duncan, P. A. Biro, P. V. Choudary, S. M. Weissman, C. M. Rubin, C. M. Houck, P. L. Deininger, and C. W. Schmid. 1980. Ubiquitous, interspersed repeated sequences in mammalian genomes. *Proc. Natl. Acad. Sci. USA.* 77:919.
 25. Wano, Y., T. Uchiyama, H. Uchino, M. Maeda, K. Fukui, and J. Yodoi. 1984. Characterization of human interleukin 2 receptor (Tac antigen) in normal and leukemic T cells: Co-expression of normal and aberrant receptors on Hut-102 cells. *J. Immunol.* 132:3005.
 26. Nikaido, T., A. Shimizu, N. Ishida, H. Sabe, K. Teshigawara, M. Maeda, T. Uchiyama, J. Yodoi, and T. Honjo. 1984. Molecular cloning of cDNA encoding human interleukin 2 receptor. *Nature (Lond.).* 311:631.
 27. Leonard, W. J., J. M. Depper, G. R. Crabtree, S. Rudikoff, J. Pumphrey, R. J. Robb, M. Kronke, P. B. Svetlik, N. J. Peffer, T. A. Waldmann, and W. C. Greene. 1984. Molecular cloning and expression of cDNAs for the human interleukin-2 receptor: Evidence for alternate mRNA splicing and the use of two polyadenylation sites. *Nature (Lond.).* 311:626.
 28. Haseltine, W. A., J. Sodroski, R. Patarca, D. Briggs, D. Perkins, and F. Wong-Staal. 1984. Structure of 3' terminal region of type II human T lymphotropic virus:evidence for new coding region. *Science (Wash. DC).* 225:419.
 29. Teshigawara, K., M. Maeda, K. Nishino, T. Nikaido, T. Uchiyama, T. Tsudo, M.

- Wano, and J. Yodoi. 1985. Adult T leukemia cells produce a lymphokine that augments interleukin-2 receptor expression. *J. Mol. Cell. Immunol.* In press.
30. Downward, J., Y. Yarden, E. Mayes, G. Scrace, N. Totty, P. Stockwell, A. Ullrich, J. Schlessinger, and M. D. Waterfield. 1984. Close similarity of epidermal growth factor receptor and *v-erb-B* oncogene protein sequences. *Nature (Lond.)*. 307:521.