RAT LYMPHOID CELL LINES PRODUCING HUMAN T CELL LEUKEMIA VIRUS

II. Constitutive Expression of Rat Interleukin 2 Receptor

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Adult T cell leukemia (ATL) (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

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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.
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 TARL-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 µg 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× 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 × 10⁸ cells were incubated with 125IIL-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 × 10⁸ cells were incubated with 20 µl 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 × 10⁷ cells were suspended in pH 8.0 phosphate buffered saline containing 20 mM glucose, and incubated with 10 µl of lactoperoxidase (2 mg/ml), 20 µl 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 µg/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).
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-I+ 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 2. Rearrangement of T cell receptor gene in HTLV-I+ 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 125I-IL-2. The presence of IL-2 receptor of the TART-1 cell line was demonstrated by its binding with 125I-IL-2. The association constant (Kₐ), 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 Kₐ of 1.3 × 10¹¹/M, and those with low affinity have a Kₐ value of 8.8 × 10⁹/M. The number of receptor sites was also calculated using this analysis. There are 1.8 × 10⁴ high affinity receptor sites, and 5.6 × 10⁸ low affinity receptor sites per cell. TARS-1 and TARL-2 cell line cells also expressed ~7.1 × 10⁸ and 7.8 × 10⁹ IL-2 binding sites per cell, respectively, while the HTLV+ human T cell line, ATL-2, had 3.9 × 10⁶ 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
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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 $1.3 \times 10^9/M$; the $K_a$ for receptors with a low affinity is $8.8 \times 10^9/M$. The number of high affinity receptor sites is $1.8 \times 10^4$ per cell, and that of low affinity receptor is $5.6 \times 10^4$ 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 $\sim 3\%$ positivity. The proportion of positive cells is shown as percent positive, while the mean fluorescence intensity is shown in parentheses. The data is expressed in the log fluorescence scale.

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-
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 leuke-mogenesis 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, TARL-2, and TART-1) (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, TART-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.

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