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Transient induction of IL-2 receptor in cultured T cell lines by HTLV-1 LTR-linked *tax*-1 gene

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Human lymphotropic virus, HTLV-1, encodes in its proviral genome a transcriptional activator protein, *tax*-1, that may be responsible for the development of virus-induced adult T cell leukemia (ATL), possibly through the aberrant activation of the genes for interleukin-2 (IL-2) and one of its receptor (IL-2R) components, the IL-2 receptor α-chain (IL-2Rα). In the present study, an expression plasmid containing *tax*-1 cDNA under the control of HTLV-1 LTR was introduced into mouse and human CD4-positive T cell lines. Analysis of the established cell clones revealed a number of interesting features: (i) a limited fraction of the total cell population (<25% in each clone) was positive for IL-2Rα; (ii) the IL-2Rα expression was not permanent, as the IL-2Rα positive and negative cells could convert either way. The experimental data suggest that the observed heterogeneity in IL-2Rα expression in the transformants is due to a cell-cycle-regulated expression and function of *tax*-1. Furthermore, a proportion of the induced IL-2R in EL-4 was in high-affinity form, suggesting the association of the IL-2Rα and the IL-2Rβ chain (p70-75) components.

Key words: ATL/cell-cycle/IL-2R/high-affinity receptor/*tax*-1

Introduction

Adult T cell leukemia (ATL) is a malignancy involving mature, CD4-positive (i.e. helper) T cells (Uchiyama *et al.*, 1977). It is suggested that human T cell leukemia virus type-I (HTLV-1) is involved in the pathogenesis of ATL (Poiesz *et al.*, 1980; Hinuma *et al.*, 1981). One of the unique features of ATL cells and T cells transformed *in vitro* by HTLV-1 is the aberrant surface expression of interleukin-2 receptor α chain (IL-2Rα, Tac antigen or p55) (Gootenberg *et al.*, 1981; Hattori *et al.*, 1981; Depper *et al.*, 1984). In fact, some of the freshly isolated ATL cells require IL-2 for their *in vitro* growth (Maeda *et al.*, 1985; Arima *et al.*, 1987). Moreover, among these ATL cells some appear to produce IL-2 spontaneously; the cells grow by an autocrine mechanism (Arima *et al.*, 1987).

Recent studies have demonstrated that a viral protein, *tax*-1, which is encoded by the pX region of HTLV-1 proviral genome, functions as a *trans*-activator for the human IL-2 and IL-2Rα gene expression (Inoue *et al.*, 1986; Cross *et al.*, 1987; Maruyama *et al.*, 1987; Siekevitz *et al.*, 1987). It has been shown that the regulatory DNA sequences for IL-2 and IL-2Rα genes, both of which are otherwise silent in resting T cells, can be activated by the *tax*-1 protein in a cultured human T cell line, Jurkat (Cross *et al.*, 1987; Maruyama *et al.*, 1987; Siekevitz *et al.*, 1987). Furthermore, the IL-2 gene sequences are synergistically activated by the expression of *tax*-1 and the extracellular signal transduction through the antigen receptor complex (Maruyama *et al.*, 1987). However, these experimental data have been obtained by employing transient expression systems of the introduced genes.

In this study, we addressed the questions as to the circumstances in which the expression of the *tax*-1 gene by its cognate promoter (i.e. LTR) induces IL-2Rα, by stably introducing a 'mini proviral DNA' consisting of *tax*-1 cDNA that is linked to HTLV-1 LTR into cultured T cell clones. We provide evidence that *tax*-1 induces the expression of IL-2Rα in a cell-cycle-dependent manner in mouse EL-4 and human Jurkat cells. Furthermore, we demonstrate that a proportion of the induced IL-2R expressed in EL-4 manifests high affinity to IL-2, suggesting the expression of IL-2Rα and β chain components. Our results are discussed in light of the possible function of HTLV-1 in T cell leukemogenesis.

Results

Introduction of tax-1 expression vector into T cells and induction of IL-2Rα

We constructed an expression vector pHTLX that contains a 'mini proviral DNA' in which the *tax*-1 cDNA is ligated to the long terminal repeat (LTR) of HTLV-1 as depicted in Figure 1. The mouse CD4-positive T cell line, EL-4, and the human CD4-positive T cell line, Jurkat, which are known

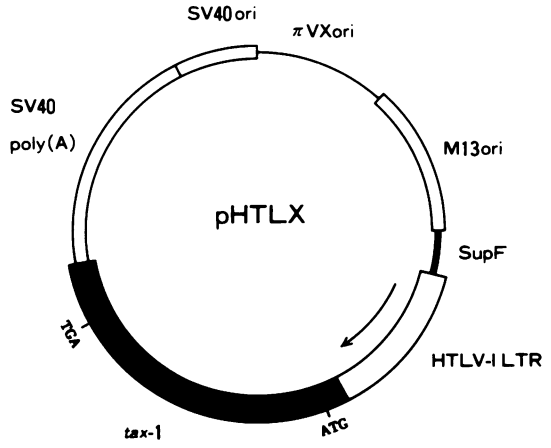

Fig. 1. Structure of *tax*-1 expression vector, pHTLX. The cDNA of pHTLX covers the entire coding region for *tax*-1 and is preceded by HTLV-1 LTR consisting of the U3, R and 5′ half of the U5.

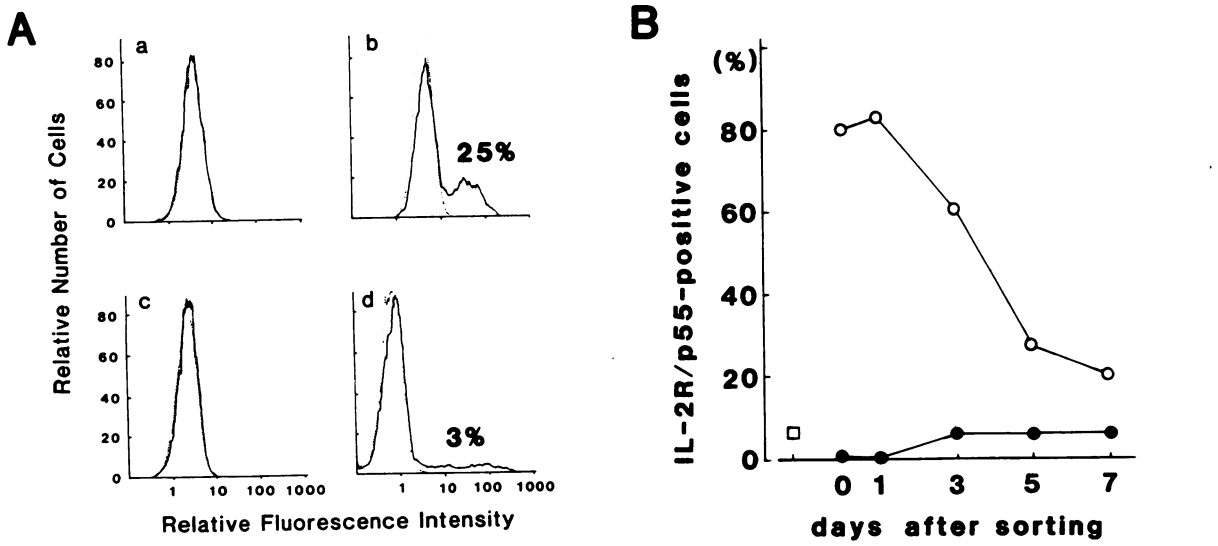

Fig. 2. (**A**) Flow-cytometric analysis of EL-4 transformants (**b,c**) and Jurkat transformants (**d**) expressing the IL-2Rα by using anti-IL-2Rα antibodies. **a**, EL-4; **b**, EH-9C; **c**, EH-1 (a G418-resistant, *tax*-1-negative clone); **d**, JH-12-SI. Dotted lines represent fluorescence pattern of cells treated with second-step antibody alone. The population of IL-2Rα-positive cells is represented in percentages. (**B**) The time-course kinetics of expression of IL-2Rα on the cell surface of the sorted IL-2Rα-positive or -negative cells. Using the clone EH-9C, IL-2Rα-positive (○——○) and -negative (●——●) cells were separated by a fluorescence-activated cell sorter. They were propagated for the indicated periods and the population of the cells expressing the IL-2Rα was analyzed by FACS. In clone EH-9C, ~5% of cells were IL-2Rα positive before the sorting (□).

to display both IL-2 and IL-2Rα gene induction upon mitogen stimulation (Fujita *et al.*, 1985; Miller *et al.*, 1985; Maruyama *et al.*, 1987), were each co-transfected by electroporation with the expression plasmid and the neomycin-resistant gene (pSTneoB; Katoh *et al.*, 1987). The transfected cells were selected in the presence of G418 and the resistant cells were cloned by the limiting dilution method. The transfection efficiency was 1×10^{-5} per cell and 5×10^{-5} per cell for Jurkat and EL-4 cells respectively. Those clones were screened for expression of *tax*-1 by immunoblotting using an anti-*tax*-1 monoclonal antibody, MI73 (Mori *et al.*, 1987) and four and three positive clones which are respectively derived from Jurkat and EL-4 cells were selected. Those transformants were then examined for the expression of IL-2Rα by staining the cells with anti-human IL-2Rα monoclonal antibody, anti-Tac (Uchiyama *et al.*, 1981), or anti-mouse IL-2Rα monoclonal antibody, AMT-13 (Osawa and Diamantstein, 1984). Figure 2A shows representative profiles of IL-2Rα expression in those transformants. Although each of these cells was derived from single clones, they could be divided into receptor-positive and receptor-negative subpopulations. The receptor-positive subpopulations were 25, 20 and 5% in the EL-4 transformants, EH-9C, EH-10S-11 and EH-11S-15 respectively. Such a difference may be reflecting the different magnitude of the *tax*-1 expression in these cell clones. Less than 3% of the cell population in each Jurkat transformant clone expressed IL-2Rα. Parental cells as well as the *tax*-1-negative, G418-resistant clones did not contain cells expressing detectable amount of IL-2Rα (Figure 2A). To assure the clonal origin of the cells analyzed, the cloning was repeated further. Again, the resultant subclones all exhibited identical FACS profiles as the parental clone for the IL-2Rα expression (data not shown). In order to study the nature of the IL-2Rα positive and negative cell populations, we first sorted out positive and negative cells from a population of an EL-4 transformant clone, EH-9C,

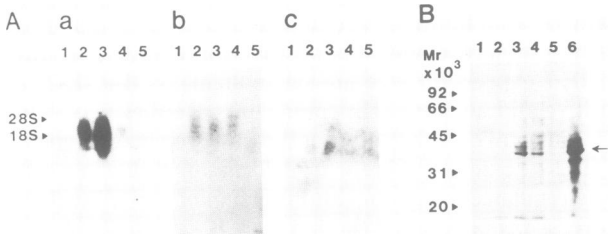

Fig. 3. (**A**) RNA blotting analysis of *tax*-1 (**a**) and *tax*-1-induced IL-2Rα (**b**) and IL-2 (**c**) mRNAs expression. The poly(A) $^{+}$RNA prepared from EL-4 transformants were electrophoresed, blotted and hybridized with the ^{32}P-labeled *tax*-1 cDNA or with the ^{32}P-labeled *Pst*I−*Eco*RI fragment of the mouse IL-2Rα cDNA (Miller *et al.*, 1985) or with the ^{32}P-labeled *Eco*T14I−*Pst*I fragment of mouse IL-2 cDNA. **Lane 1**, EH-1; **lane 2**, EH-11S-15; **lane 3**, EH-9C; **lane 4**, EH-10S-11; **lane 5**, EL-4. (**B**) Immunoblotting analysis of the *tax*-1 protein. Nuclear lysates prepared from each transformant were subjected to SDS−PAGE (7.5% gel), transferred to Durapore membrane, reacted with monoclonal anti-*tax*-1 antibody (MI73) and ^{125}I-labeled anti-mouse immunoglobulin. **Lane 1**, EL-4; **lane 2**, EH-1; **lane 3**, EH-9C; **lane 4**, EH-10S-11; **lane 5**, EH-11S-15; **lane 6**, Hut102 (HTLV-1-infected human T cell clone). Arrow indicates the position of *tax*-1 protein.

and then examined the induction of the receptor expression during cell propagation (Figure 2B). Interestingly, during the cultivation of the sorted cells, the ratio of the receptor-positive versus negative cells decreased from the positive cell population, while receptor-positive cells gradually appeared from the negative cell population. This observation indicated that cells expressing *tax*-1 cDNA under the control of HTLV-1 LTR express IL-2Rα only under certain circumstances. Induction of IL-2Rα was not detected in the parental EL-4 and Jurkat cells when they were cultivated in the presence of conditioned medium from transformant cells, indicating that the IL-2Rα induction is not mediated by a secreted factor(s) by the transformants (data not shown).

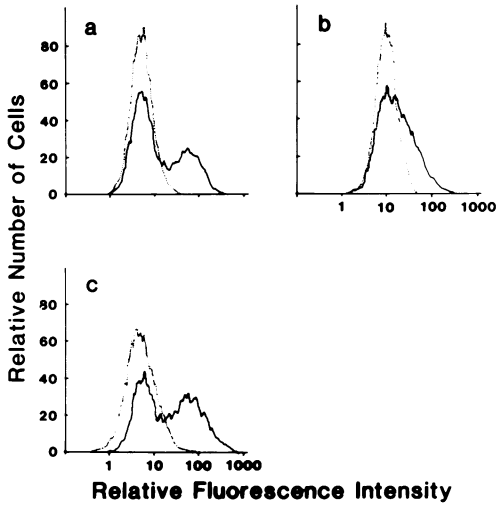

Fig. 4. Effects of serum starvation on IL-2Rα expression. EL-4 transformant, EH-9C (**a**), was cultured in serum-free medium for 48 h (**b**) and was transferred into the medium containing 10% FCS and was cultured for a further 24 h (**c**). Expression of IL-2Rα was analyzed by FACS.

Thus the results demonstrated that *tax*-1 induces endogenous IL-2Rα gene expression not only in the human but also in the murine T cell lines. Since EL-4-derived transformant clones contained a larger amount of receptor-positive cells than those derived from Jurkat (Figure 2A), more detailed analyses were carried out with the EL-4 transformants in the subsequent experiments.

The Southern blot analysis of DNAs obtained from the EL-4 transformants revealed that the integration sites of the *tax*-1 cDNA are random (data not shown), indicating that induction of the IL-2Rα is not dependent on the integration site of the transfected gene. As shown in Figure 3, expression of the introduced *tax*-1 cDNA is observed in both mRNA and protein levels. The IL-2Rα and IL-2 mRNAs are induced in those transformants without extracellular stimulation. It was revealed that expression level of *tax*-1 protein correlated with those of IL-2Rα. Since we could not detect *tax*-1 protein on a single-cell basis because of the low sensitivity of our immunofluorescence staining, it was unclear whether cells expressing IL-2Rα express *tax*-1 protein (data not shown).

Correlation between the cell-cycle phase and induction of IL-2Rα

The transient nature of IL-2R induction in the transformed EL-4 cells raised the possibility that expression of the *tax*-1 and IL-2Rα is regulated by the cell cycle. As the first test for this possibility, quiescent EH-9C cells were obtained by culturing in the absence of serum, then expression of IL-2Rα was analyzed by FACS. As shown in Figure 4, IL-2Rα expression levels decreased in the serum-depleted cells and addition of serum to the culture medium resulted in the observed increase. These data indicate that induction of IL-2Rα correlates with the growth cycle of cells. In order to examine in which phase of cell cycle the induction of IL-2Rα occurs, we treated the EH-9C cells with various cell-cycle blocking agents and analyzed the expression levels of IL-2Rα and *tax*-1. The agents used were: (i) sodium butyrate, which is known to inhibit cell transition from G_1a (early G_1) to G_1b (late G_1) (Darzynkiewicz *et al.*, 1980; Nishimura *et al.*, 1987); (ii) hydroxyurea, which inhibits DNA synthesis (Darzynkiewicz *et al.*, 1980; Yagita *et al.*, 1986); and (iii) vinblastine, a spindle toxin which blocks mitosis (Noronha and Richman, 1984).

As shown in Figure 5A, treatment of the cells with sodium butyrate (enrichment of cells entering G_1a) caused a significant accumulation of *tax*-1 protein, whereas treatment with hydroxyurea (enrichment of cells entering G_1b) resulted in a decrease of the *tax*-1 level. In contrast to *tax*-1, the IL-2Rα expression was almost undetectable in cells treated with sodium butyrate. The number of the IL-2Rα-positive cells increased dramatically by the hydroxyurea treatment (Figure 5B). Vinblastine treatment only slightly increased the receptor-positive population. Similar results were obtained in other EL-4 transformant clones treated with the reagents, whereas treatment of the parental EL-4 cells with those drugs did not induce IL-2Rα expression (data not shown). These results indicate that *tax*-1 protein accumulates in G_1a phase and decreases in G_1b phase, and that expression of IL-2Rα occurs in G_1b phase. Although we could not examine whether the expression of IL-2Rα in Jurkat transformants depends on the cell cycle, because of low-level IL-2Rα induction (Figure 2A), it is likely that similar events also occur in the Jurkat transformants.

Expression of high-affinity IL-2R in tax-1-expressing EL-4 cells

It is well documented that signals by IL-2 are transduced by the high-affinity receptor which consists of at least two membrane components: IL-2Rα and IL-2Rβ (also referred to p70−75) chains (Sharon *et al.*, 1986; Tsudo *et al.*, 1986; Teshigawara *et al.*, 1987; Hatakeyama *et al.*, 1989). In this regard, we next characterized the nature of the induced IL-2R by the following experiments. First, [^{125}I]IL-2 binding assay was carried out (Hatakeyama *et al.*, 1985). As shown in Figure 6A, the EL-4 transformants expressed both high- and low-affinity forms of IL-2R. The dissociation constants (K_d) of high- and low-affinity IL-2R were 7−15 pM and 15−50 nM respectively. These K_d values are comparable to those expressed in mitogen-activated T cells (Robb *et al.*, 1984). Next, chemical crosslinking studies were carried out with [^{125}I]IL-2 as well as with homobifunctional non-cleavable crosslinker, disuccinimidyl subserate (DSS). Figure 6B demonstrates the existence of two major bands migrating at ~70 and 85 kd. Those bands correspond to IL-2 crosslinked to the IL-2Rα and to the IL-2Rβ respectively, indicating that the *tax*-1-induced high-affinity IL-2R primarily consists of a non-covalently associated heterodimer of IL-2Rα and IL-2Rβ. As shown in this figure, a broad band >150 kd was also detectable and may represent a trimolecular complex of IL-2, IL-2Rα and IL-2Rβ (Saragovi and Malek, 1987; Hatakeyama *et al.*, 1989).

Discussion

In this report, we present evidence that the expression of *tax*-1 protein in T-lymphoid cells, mouse EL-4 and human Jurkat cells, leads to the activation of IL-2 and IL-2Rα genes by employing a stable transformation system. Induction of the receptor molecules correlates with the level of expression of *tax*-1 protein in these transformants. Previously we presented evidence that *tax*-1 activated IL-2Rα gene in Jurkat

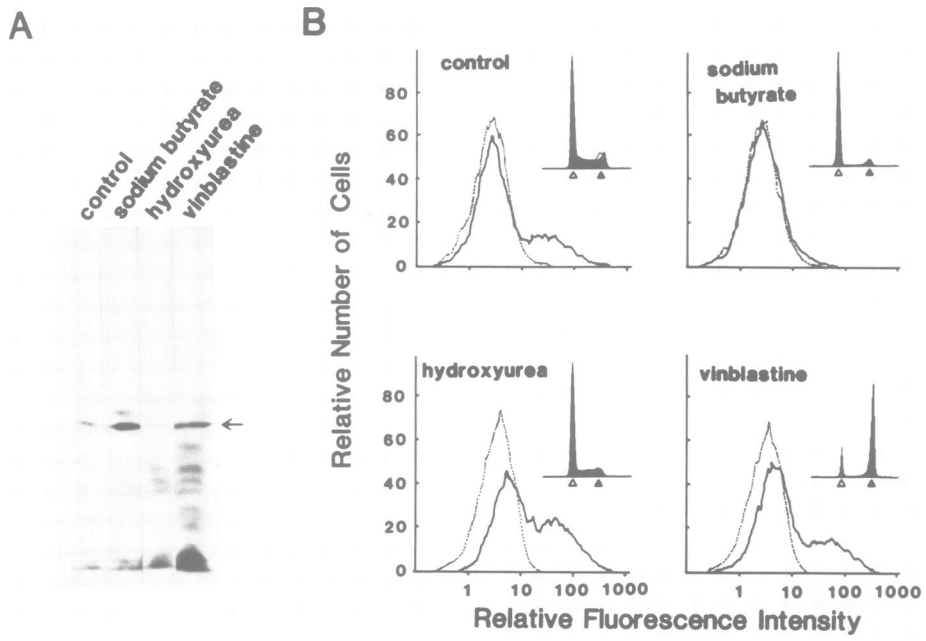

Fig. 5. Effects of cell-cycle blocking agents on *tax*-1 expression (**A**) and IL-2Rα expression (**B**). EL-4 transformant, EH-9C, was treated with 5 mM hydroxyurea, 5 mM sodium butyrate or 0.2 μg/ml of vinblastine sulfate. (**A**) Nuclear lysates prepared from cells treated with those agents were subjected to SDS−PAGE and immunoblotting analysis, as described in Materials and methods. The arrow indicates the position of *tax*-1 protein. (**B**) Expression of IL-2Rα was analyzed by FACS. Insets: DNA content of drug-treated EH-9C cells was measured by staining with propidium iodide. The open and closed triangles mark 2 N and 4 N DNA content respectively.

but not in EL-4 cells by using a transient gene expression system (Maruyama *et al.*, 1987). However, studies presented here show that *tax*-1 also induces IL-2Rα in EL-4 cells. The observed difference may be explained by the difference of DNA transfection efficiency in those two different cell lines; in the transient expression system, Jurkat cells manifest much higher DNA transfection efficiency than EL-4 cells. On the other hand, it appears that the EL-4 transformants reported in this study produce higher levels of *tax*-1 protein as compared with the Jurkat transformants in the expression system employed (data not shown).

In addition, analysis of the stable transformants has given new insight on the induction mechanism of IL-2Rα by *tax*-1. In those cell clones, only a certain subpopulation of cells was found to express endogenous IL-2Rα. The receptor expression is reversible and seems to be related to the cell cycle. This notion is supported by the experiment showing that serum starvation of cells led to a dramatic decrease in the receptor-positive subpopulation, and the receptor expression is strongly induced following the addition of serum to the culture medium (Figure 4). Although the precise mechanism of the cell-cycle-related expression of the IL-2Rα remains uncertain, the results obtained by cell-cycle-blocking reagents suggest that cells entering early G_1 phase may express a higher level of the *tax*-1 protein, which is responsible for the subsequent induction of endogenous IL-2Rα expression in cells entering late G_1 phase. Such a regulated expression of the *tax*-1 protein may reflect the cell-cycle-dependent activation of the promoter function of HTLV-1 LTR, as has been shown in the case of the HIV LTR (Nabel and Baltimore, 1987; Tong-Starkson *et al.*, 1987). On the other hand, since the cell cycle blockers also have other effects unrelated to cell cycle, further experiments will be required to consolidate the observations.

It has been reported that the IL-2Rα induction occurs during G_1 phase in mitogen-activated T cells (Cotner *et al.*, 1983). Thus the IL-2Rα induction by *tax*-1 in G_1b phase is indicative that *tax*-1 bypasses the T cell activation pathway, by inducing and/or activating the factors involved in the IL-2 and IL-2Rα gene expression. Maruyama *et al.* (1987) presented evidence that expression of *tax*-1 in Jurkat cells followed by mitogens or anti-CD3 results in much higher induction of the IL-2 and IL-2Rα gene transcription than either stimuli alone. The simultaneous *tax*-1 expression and mitogenic stimulation may synergize in the induction of those genes in the G_1b phase of the cells, causing over-expression of those genes.

Establishment of cells stably expressing *tax*-1 cDNA also made it possible to examine the nature of IL-2R induced by the viral protein in the absence of extracellular mitogenic stimulation. IL-2 binding assay and chemical crosslinking studies revealed unequivocally the induction of high-affinity

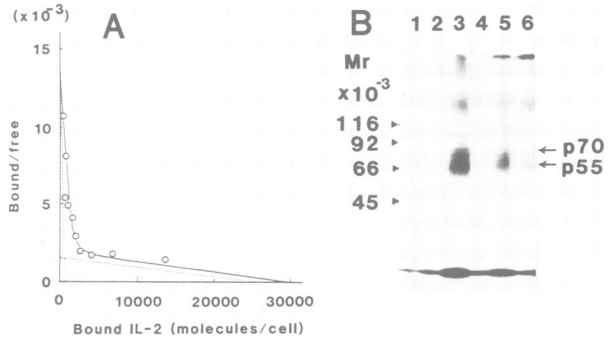

Fig. 6. (**A**) Scatchard plot of the binding of ^{125}I-labeled IL-2 to EL-4 transformant, EH-9C. (**B**) SDS−PAGE (7.5% gel) of ^{125}I-labeled IL-2 cross-linked to EL-4 tranformants. After incubation with 500 pM [^{125}I]IL-2 in the absence (**lanes 1−3** and **5**) or presence (**lanes 4** and **6**) of unlabeled IL-2, cells were reacted with DSS, solubilized and subjected to SDS−PAGE. **Lane 1**, EL-4; **lane 2**, EH-1; **lanes 3** and **4**, EH-9C; **lanes 5** and **6**, EH-10S-11.

IL-2R in those cells. Assuming that the T lymphoid cells tested in this study are representing their normal cellular counterparts (i.e. normal T cells), the finding suggests that infection of HTLV-1 and expression of *tax*-1 in T cells leads to the expression of functional, high-affinity IL-2R. It remains to be demonstrated whether or not the gene encoding IL-2Rβ is also induced by *tax*-1. Our previous experiments showed that the EL-4 cells transfected with human IL-2Rα cDNA express both high- and low-affinity IL-2R, while the parental EL-4 cells do not express the endogenous IL-2Rα (Hatakeyama *et al.*, 1985). The observation may be explained as such that either EL-4 cells already express mouse IL-2Rβ molecules that is not detected by human IL-2 (i.e. human IL-2 may not be able to interact with mouse IL-2Rβ), or expression of the IL-2Rα protein induces expression of IL-2Rβ. In this regard, cDNAs encoding the human IL-2Rβ have been recently isolated (Hatakeyama *et al.*, 1989). Availability of the mouse IL-2Rβ gene may provide further insights on IL-2R induction by *tax*-1 in the EL-4 transformants.

The *tax*-1-producing mouse T cells also expressed mRNA for IL-2. Since the level of mRNA was quite low, we could not directly identify the biological activity of IL-2 in the culture supernatant. The results, however, suggest the spontaneous generation of IL-2 autocrine loop in cells infected with HTLV-1 under certain circumstances.

The observations that production of *tax*-1 in T-lymphoid cells results in the simultaneous activation of endogeneous IL-2 and IL-2Rα genes argues for the possibility that the IL-2 system is involved in ATL leukemogenesis at a certain stage (Inoue *et al.*, 1986; Cross *et al.*, 1987; Maruyama *et al.*, 1987; Siekevitz *et al.*, 1987; this work). Furthermore, the fact that the induced receptor contains high-affinity IL-2R indicates the presence of IL-2 autocrine growth stimulation in such processes. Since established ATL is of clonal origin, one may envisage that such an aberrant activation of the IL-2 system is insufficient for the ultimate development of ATL. From our present and previous data, it is possible that T cell activation signals in the HTLV-1 infected T cells potentiate the dysregulated operation of IL-2 system by synergizing with *tax*-1. Such an event may allow a certain infected T cell clone to grow by an aberrant autocrine mechanism. Such an autocrine growth may predispose cells to acquire additional genetic changes that may be required for further malignant characteristics of the cells.

The fact that *tax*-1 induces activation of both IL-2 and IL-2Rα genes indicates the existence of common regulatory mechanism in those genes. In fact recent studies have suggested the existence of a NF-κB-like transcriptional factor involved in the activation of both IL-2 and IL-2Rα genes (Shibuya *et al.*, 1989). Interestingly, the *tax*-1 seems to affect the expression and/or function of this factor to activate those genes. Elucidation of the nature of such cellular factors will further uncover the molecular mechanism involved in the *tax*-1 mediated activation of IL-2 system and, possibly, its relationship to ATL development.

Materials and methods

Construction and transfection of tax-1 cDNA expression vector

The cDNA for *tax*-1 was cloned from a λgt10 library prepared by using the MT-2 cell-derived poly(A)$^+$ RNA. Positive clones were identified by using a synthetic oligonucleotide probe, 5′-CCAACACCATGGCCCAC-TTCCCAG-3′. The isolated cDNAs were all incomplete so that two cDNAs containing either the 5′ portion or the 3′ portion of the *tax*-1 cDNA were linked by using the single *Tth*111I site that was present in the overlapping region to generate cDNA containing the entire coding region of *tax*-1 (pMTX). The 5′ end of the pMTX cDNA was extended up to 80 bp upstream of the initiator ATG sequence. The *tax*-1 cDNA was treated with T4 DNA polymerase to generate blunt ends, inserted into *Hin*cII site of pUC19 plasmid, and then excised from the plasmid by *Hin*dIII and *Hin*cII digestion. The *Hin*dIII−*Hin*cII fragment (1.32 kb) of *tax*-1 cDNA was ligated into the backbone fragment of the *Hin*dIII−*Xba*I-digested πH3M vector in which the *Xba*I site had been rendered flush by the T4 DNA polymerase (Aruffo and Seed, 1987). The resultant plasmid was termed pCDS. The *Sma*I−*Bgl*I fragment, consisted of the U3, R and 5′ half of the U5, of HTLV-1 LTR was excised from pHLR3 plasmid kindly provided by Drs Y.Hinuma and M.Nakamura (Ohtani *et al.*, 1987). In isolating the fragment, a *Hin*dIII linker was attached to the *Bgl*I site. The resultant *Sma*I−*Hin*dIII fragment was then inserted into the backbone fragment of the *Nru*I−*Hin*dIII-digested pCDS to generate pHTLX.

Transfection of the pHTLX was carried out by electroporation method (Potter *et al.*, 1984). Briefly, Jurkat or EL-4 cells (1×10^7) were suspended in 1 ml of Hanks' balanced salt solution containing 0.1% bovine serum albumin, 100 μg of pHTLX linearized by *Bam*HI digestion and 1 μg of pSTneoB plasmid (Katoh *et al.*, 1987) digested with *Xho*I. After incubation for 10 min on ice, the cells were exposed to a 1500 V pulse for 20−50 ms (X-Cell 2000, Promega Biotec), allowed to remain in the buffer for 10 min on ice, and cultivated in a 24-well dish. Transfected cells were selected in the RPMI-1640 medium containing 10% fetal calf serum (FCS) and 1 mg/ml of G418.

Flow-cytometric analysis

Expression of IL-2Rα was detected by using indirect immunofluorescence either with anti-Tac antibody (Uchiyama *et al.*, 1981) or with a rat monoclonal anti-mouse IL-2Rα antibody, AMT-13 (Osawa and Diamantstein, 1984) as described previously (Hatakeyama *et al.*, 1985). The IL-2Rα-postive and -negative cells were sorted with a fluorescence-activated cell sorter (FACS440, Becton Dickinson).

RNA blotting analysis

Total RNA was isolated from each EL-4 transformant by the guanidium/cesium chloride method (Chirgwin *et al.*, 1979), and poly(A)$^+$ RNA was enriched by oligo(dT)−cellulose.

One microgram of poly(A)$^+$ RNA was electrophoresed on formaldehyde gel, transferred to a nylon membrane (Nytran NY13, Schleicher and Schuell), and hybridized with the ^{32}P-labeled *tax*-1 cDNA (a *Mlu*I−*Sma*I fragment from pHTLX) or a ^{32}P-labeled *Pst*I−*Eco*RI fragment of mouse IL-2Rα cDNA derived from pmIL2Pr1 (Miller *et al.*, 1985), or a ^{32}P-labeled *Eco*T141−*Pst*I fragment of mouse IL-2 cDNA, by using the Multiprime DNA labeling system (Amersham).

Immunoblotting analysis of the tax-1 protein

Nuclear lysate was prepared from each transformant by a method described previously (Slamon *et al.*, 1985). Briefly, cells (6×10^6) were homogenized in 20 mM Hepes, pH 7.4, containing 1 mM $MgCl_2$ and 5 mM KCl, and crude nuclei were pelleted by centrifugation for 5 min at 700 *g*. The nuclear pellets were suspended in the above buffer and centrifuged through a sucrose cushion (25 mM Tris−HCl, pH 7.5, 30% sucrose, 1 mM EDTA). Pellets were solubilized with lysis buffer [10 mM Tris−HCl, pH 7.5, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, 150 mM NaCl and 1 mM phenylmethylsulfonyl fluoride (PMSF)] on ice for 30 min, applied to SDS−PAGE (10% gel), transferred onto Durapore membrane (Millipore) and reacted with mouse monoclonal anti-*tax*-1 antibody, MI73 (kindly provided by Dr S.Hatanaka, Kyoto University) and ^{125}I-labeled anti-mouse immunoglobulin (Amersham).

Cell-cycle analysis

Transformant cells were maintained in RPMI-1640 medium containing 10% FCS. To obtain the quiescent cells, cells were washed with serum-free RPMI-1640 and were cultured in serum-free medium. After 48 h, some of the cells were transferred into the medium containing 10% FCS and were cultured for a further 24 h. Expression of IL-2Rα on these treated cells was analyzed by FACS.

For the cell-cycle analysis, cells were cultivated in the presence of various cell-cycle blocking agents, i.e. sodium butyrate (5 mM), hydroxyurea (5 mM) and vinblastine sulfate (0.2 μg/ml). In each case, cells were exposed to one of these agents for 15 h. The viability of the cells was >90%. The distribution of DNA content of each treated cell was measured by staining with propidium iodide (Crissman and Steinkamp, 1973). The drug-treated

cells (5×10^6) were fixed in 50% methanol overnight at −20°C. After rinsing with 30% methanol (stocked at −20°C) and with phosphate-buffered saline (PBS), cells were treated with 1 mg/ml of RNase A for 30 min at 37°C. The cells were washed with PBS, stained 50 μg/ml of propidium iodide in PBS on ice for 2 h in the dark, and subsequently analyzed by flow cytometry (FCS-1, Japan Spectroscopic).

IL-2 binding assays

Human recombinant IL-2 kindly provided by Dr H.Matsui (Ajinomoto Co. Ltd), was iodinated with $Na^{125}I$ by the chloramine-T method as described previously (Fujii *et al.*, 1986). The specific activity of ^{125}I-labeled IL-2 was 3×10^4 c.p.m./ng. The IL-2 binding assay of the EL-4 transformant was performed as previously described (Hatakeyama *et al.*, 1985). Briefly, cells were incubated with serial dilutions of ^{125}I-labeled IL-2 at 37°C for 40 min. After incubation, cell-bound and free IL-2 were separated by centrifugation on a layer of 80% di-*n*-butyl phthalate/20% olive oil. The tip of the tubes was cut off and the radioactivities measured. Non-specific IL-2 binding was determined in the presence of a 300-fold molar excess of unlabeled IL-2. Specific binding was obtained by subtracting non-specific binding (Hatakeyama *et al.*, 1985).

[^{125}I]IL-2 cross-linking studies

The [^{125}I]IL-2 cross-linking study was performed as described, using ^{125}I-labeled human recombinant IL-2 obtained from New England Nuclear (sp. act. 30−50 μCi/μg) (Sharon *et al.*, 1986). Briefly, cells (5×10^6) were incubated with 500 pM [^{125}I]IL-2 for 1 h and then treated with 0.5 mM disuccinimidyl suberate in PBS (pH 8.3) containing 1 mM $MgCl_2$. After incubation at 4°C for 20 min, the reaction was quenched with 10 mM Tris−HCl, pH 7.4, containing 150 mM NaCl and 1 mM EDTA. The cells were subsequently solubilized with extraction buffer (50 mM Tris−HCl, pH 7.4, containing 150 mM NaCl, 0.5% Nonidet P-40, 1 mM PMSF, 1 μg/ml pepstatin, 0.05% NaN_3, 8 mM iodoacetamide and 0.5 mm EDTA) for 1 h on ice. After centrifugation, supernatants were subjected to SDS−PAGE (7.5% gel).

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