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Retrovirus-induced expression of interleukin 2 receptors on cells of human B-cell lineage

[adult T-cell leukemia/human T-cell leukemia/lymphoma virus (HTLV)/Tac antigen]

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ABSTRACT **We provide direct evidence for interleukin 2 receptor (IL2R) induction by human T-cell leukemia/lymphoma virus (HTLV) in human B-cell lines. A lymphoblastoid B-cell line (LCL-Ter) was established by Epstein–Barr virus-induced transformation of peripheral blood lymphocytes derived from a healthy HTLV carrier and cloned *in vitro*. HTLV gp21 and/or p19 antigens were detected in eight LCL-Ter clones, all of which also expressed the IL2R antigens Tac and Hiei (defined by monoclonal antibodies). However, five other LCL-Ter clones, which were negative for the HTLV antigens, did not express the IL2R antigens. Furthermore, when the IL2R-negative B-cell line LCL-Kan, derived from a normal donor, was cocultured with HTLV-producer cells, three HTLV-carrying clones were obtained and found to constitutively express IL2R. IL2R induced by HTLV on these B-cell lines bound recombinant interleukin 2 and were similar in apparent molecular mass (≈60 kDa) to the IL2R of peripheral blood lymphocytes stimulated with phytohemagglutinin.**

It has recently been suggested that two viral oncogene products play an important role in proliferation mechanisms of transformed cells. One of these, the product of the v-*sis* gene of simian sarcoma virus, has a structural similarity to platelet-derived growth factor and exhibits growth-factor activity for 3T3 fibroblasts (1, 2). This similarity of v-sis to platelet-derived growth factor supports the hypothesis that autocrine secretion is involved in the abnormal growth control of transformed cells (3). The other oncogene product, the v-*erb*B gene product of avian erythroblastosis virus, has a close similarity to the epidermal growth factor receptor, and the enhanced expression of this receptor in the human epidermoid carcinoma cell line A431 was suggested to be due to amplification of the c-*erb*B gene (4, 5). Aside from the autocrine hypothesis, the overproduction of the receptor is considered to be closely related to malignant transformation of A431 cells.

The human retrovirus, human T-cell leukemia/lymphoma virus (HTLV), which is identical to adult T-cell leukemia virus, is known to induce human adult T-cell leukemia (ATL) (6). Various types of HTLV-carrying human T cells, including ATL cells and HTLV-transformed cells, have been shown to express interleukin 2 (IL2) receptors (IL2R) on the basis of IL2-dependent growth or of reactivity with monoclonal antibody against the IL2R antigen, Tac, (7–11). Therefore, we investigated whether IL2R expression on ATL cells or on HTLV-transformed cells might play a similar role to the expression of epidermal growth factor receptor on A431 cells. Recently, we reported the expression of Tac antigen on Epstein–Barr virus-transformed B-cell clones that were simultaneously infected with HTLV (12). This suggested to us that HTLV infection could induce expression of IL2R not only on human T cells but also on B cells or, alternatively, that HTLV infection could occur selectively in cells bearing IL2R. In this report, we show that infection with HTLV can induce IL2R expression in human B-cell lines and discuss possible mechanisms of this induction.

MATERIALS AND METHODS

Cell Lines. Cell lines used were LCL-Kan, LCL-Ter, ILT-OmI, TL-Su, MT-2, and NK7. LCL-Kan and LCL-Ter were established by Epstein–Barr virus-induced transformation of peripheral blood lymphocytes from a normal donor (Kan) and from a healthy HTLV-carrier (Ter), respectively (12). ILT-OmI is an HTLV-carrying IL2-dependent cell line derived from peripheral blood lymphocytes of an ATL patient. TL-Su is an HTLV-carrying IL2-independent T-cell line (12). MT-2 is an HTLV-carrying T-cell line established by Miyoshi *et al.* (13). NK7 is an IL2-dependent murine cell line established by Suzuki *et al.* (14). IL2-dependent cell lines were maintained in RPMI 1640 medium supplemented with 20% fetal calf serum, 2 mM L-glutamine, antibiotics, and either IL2 (50 units/ml) derived from Jurkat cells (Ajinomoto, Yokohama, Japan) or recombinant IL2 (rIL2, substituted at the same concentration; Shionogi, Osaka, Japan).

Cloning of LCL-Ter Cells. LCL-Ter cells were plated in microwells at an average of 0.3 cell per well; 2×10^4 irradiated LCL-Kan cells were added as feeder cells. Thirteen clonal cell lines thus obtained were maintained for >3 months in RPMI 1640 medium containing 10% fetal calf serum.

Cocultivation of Cells. LCL-Kan cells, which have now been maintained for >2 yr *in vitro*, were cocultured with 12,000-roentgen γ-irradiated TL-Su (TL-Su$_\gamma$) or LCL-Kan (LCL-Kan$_\gamma$) cells. LCL-Kan cells were seeded at 10 cells per well in microwells, and 2×10^4 TL-Su$_\gamma$ or LCL-Kan$_\gamma$ cells were added to each well. These cultures were maintained in RPMI 1640 medium containing 20% fetal calf serum at 37°C in a humidified 7% CO_2 atmosphere. Half of the medium was replaced every 3–4 days. Cells derived from three wells of a microculture plate containing TL-Su$_\gamma$ cells yielded three clonal cell lines, designated LCL-KanC1, LCL-KanC4, and LCL-KanC8. Similarly, eight clonal cell lines, LCL-Kan1–8, were obtained from wells containing LCL-Kan$_\gamma$ cells. All the cocultured cell lines were maintained in RPMI 1640 medium containing 10% fetal calf serum for >5 months.

Radioimmunoassay (RIA). An indirect RIA was used to quantitate cell-surface antigens as described (15). Mouse monoclonal antibodies used were F10, which is specific for HTLV gp21 (15); GIN14, for HTLV p19 (16); Tac, for IL2R (obtained from T. Uchiyama) (17); Hiei, for IL2R (18); OKT-9, for transferrin receptor (Ortho Pharmaceutical) (19); and anti-HLA-DR (Cappel Laboratories, Cochranville, PA).

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Abbreviations: ATL, adult T-cell leukemia; HTLV, human T-cell leukemia/lymphoma virus; IL2, interleukin 2; rIL2, recombinant IL2; IL2R, IL2 receptor.

Cells were incubated first with monoclonal antibodies diluted serially in duplicate and then with ^{125}I-labeled F(ab')$_2$ fragment sheep anti-mouse Ig (New England Nuclear). Radioactivity bound to cells was measured. The binding index of ^{125}I-labeled anti-mouse Ig was calculated; a binding index >10 was considered to be positive on the basis of statistical testing.

IL2 Absorption Test. The IL2-absorbing capability of cell lines was examined using rIL2. Cells (10^7) were incubated with 50 units of rIL2 for 1 hr at 37°C. The cells were removed by centrifugation, and supernatants were passed through a membrane filter of 0.22-μm pore size. Filtrates were assayed for IL2 activity, using NK7 cells according to the method of Suzuki *et al.* (14).

Binding of Iodinated rIL2. rIL2 was iodinated with Na^{125}I (Amersham) by the chloramine-T method (20) and then separated from the reagents by Sephadex G-25 column chromatography. Serial dilutions of ^{125}I-labeled rIL2 were each incubated with 10^6 test cells for 30 min at room temperature in the presence or absence of unlabeled rIL2. Specific binding of ^{125}I-labeled rIL2 to cells was calculated by subtracting radioactivity bound in the presence of 100-fold excess unlabeled rIL2 from that bound in the absence of unlabeled rIL2.

Radioimmunoprecipitation (RIP). Cells were surface-labeled with Na^{125}I (Amersham) using Iodo-gen reagent (21). ^{125}I-labeled cell lysates were immunoprecipitated by sequential incubation with mouse monoclonal antibody Hiei, anti-mouse IgG rabbit serum, and protein A-Sepharose. Immunoprecipitates were analyzed by NaDodSO$_4$/PAGE as reported previously (15).

Southern Blotting. Southern blot hybridization was performed as described (22). In brief, cellular DNA (5 μg), completely digested by *Eco*RI, was fractionated by electrophoresis on 0.8% agarose at 20 V for 16 hr. DNA on the gel was transferred to a nylon membrane filter (Biodyne; Pall, Irvine, CA) and hybridized to a nick-translated ^{32}P-labeled probe. The probe used was a 2.3-kilobase *Sst* I fragment of HTLV clone 42 (*gag-pXs*-U$_3$R) (22).

RESULTS

The LCL-Ter cell line, which was established by Epstein–Barr virus transformation of peripheral blood lymphocytes from an HTLV-carrier, was cloned <3 months after it was established. Thirteen LCL-Ter clones were examined for expression of HTLV antigens and IL2R antigens using the mouse monoclonal antibodies F10 or GIN14 and Tac or Hiei, respectively (Table 1). Eight clones were positive for HTLV antigens, and all of these also expressed Tac and Hiei antigens. The five HTLV-antigen-negative clones did not express the IL2R antigens. This indicates that HTLV-carrying LCL-Ter clones express a significant quantity of the IL2R antigens recognized by Tac and Hiei.

We then examined whether HTLV could induce expression of Tac and Hiei antigens on cells of an Epstein–Barr virus-transformed line, LCL-Kan, which was derived from normal peripheral blood lymphocytes. LCL-Kan cells were cocultured with γ-irradiated cells of an HTLV-producer line, TL-Su, in microwells. Growing cells were obtained from 16 wells, and of these, cells from 10 wells were significantly Hiei-antigen positive during at least the next three months in culture. Subsequently, cells derived from 3 wells out of the 10 wells were randomly selected and three clonal cell lines, LCL-KanC1, -C4, and -C8 were thus obtained. These three cell lines were shown to carry an HTLV provirus genome by Southern blot hybridization with an HTLV probe derived from MT-2 cells (Fig. 1). The integration site of the provirus genome in cellular DNA seems not to be identical among the cell lines. Then, LCL-KanC1, -C4, -C8 and the parental

Table 1. Expression of cell-surface antigens on LCL-Ter clones

	Binding index			
	HTLV antibody		IL2R antibody	
Clone	F10	GIN14	Hiei	Tac
21	50	20	32	38
23	33	19	28	20
24	80	25	45	21
29	28	22	18	26
32	20	20	17	14
35	22	11	44	12
38	32	10	46	17
39	21	24	38	16
28	7	7	8	4
31	7	8	5	2
40	0	2	2	6
42	0	0	0	3
43	6	6	4	0

Binding indexes >10 indicate significant binding.

LCL-Kan cells were examined for expression of the IL2R antigens recognized by Tac and Hiei, using monoclonal antibodies Ia and OKT9 as controls (Fig. 2). The three clones were positive for all these antigens, while the parental LCL-Kan cells were negative for Tac and Hiei antigens. The quantitative expression of Ia and OKT9 antigens did not significantly vary between the parental LCL-Kan and LCL-KanC1, -C4, and -C8. Additionally, as a control, we similarly obtained eight other clones of LCL-Kan cells from coculture of LCL-Kan and LCL-Kan$_\gamma$ cells. The cloning efficiency was not significantly different from that of the coculture with TL-Su$_\gamma$ cells. All of the eight clones (LCL-Kan1–8), like the parental LCL-Kan cells, were negative for Tac and Hiei antigens as well as HTLV antigens (Table 2). Furthermore, clones LCL-KanC1, -C4, and -C8, although carrying HTLV provirus, expressed insignificant amounts of HTLV antigens (Table 2). These results suggest that expression of Tac and Hiei antigens was induced by HTLV-infection of the LCL-Kan cells.

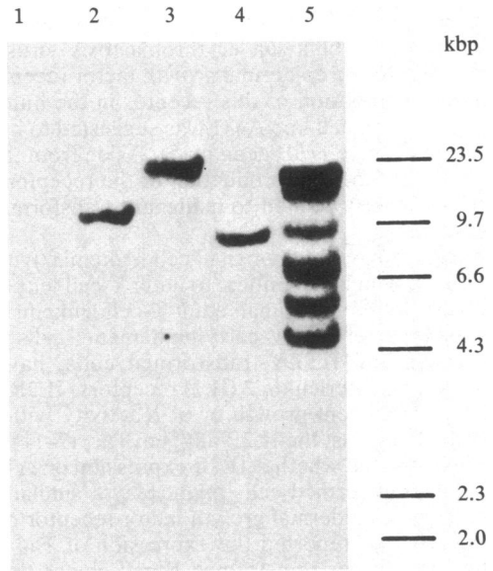

FIG. 1. Southern blotting of cellular DNAs with an HTLV-specific probe. DNAs of LCL-Kan (lane 1), LCL-KanC1 (lane 2), LCL-KanC4 (lane 3), LCL-KanC8 (lane 4), and MT-2 cells (lane 5) were completely digested with *Eco*RI and hybridized with the ^{32}P-labeled HTLV-specific fragment probe. *Hin*dIII-digested λ DNA was used as a molecular size standard. kbp, Kilobase pairs.

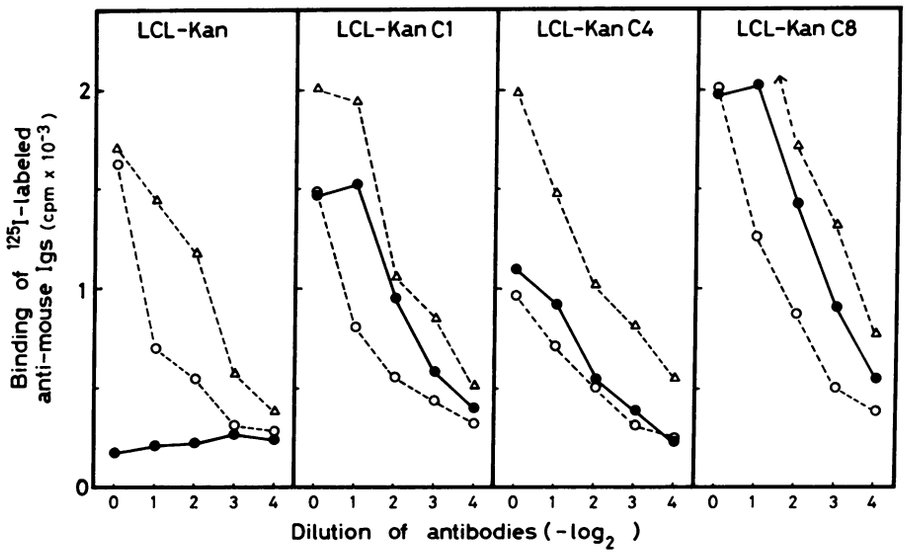

FIG. 2. Radioimmunoassay of cell-surface antigens. Cell-surface antigens, Tac (●), Ia (○), and OKT9 (△), were quantitated by an indirect RIA in LCL-KanC1, LCL-KanC4 and LCL-KanC8 cells, and in parental LCL-Kan cells.

We also examined the IL2-absorbing capability of LCL-KanC1, -C4, and -C8 cells. As shown in Table 3, these Tac and Hiei antigen-positive cells were able to absorb significantly more rIL2 than the antigen-negative parental LCL-Kan cells. However, their IL2-absorbing capability was much less than that of IL2-dependent human (ILT-OmI) or murine (NK7) cell lines. Similar results were obtained using ^{125}I-labeled rIL2 in direct IL2-binding assays (Fig. 3). These results suggest that the Tac and Hiei antigens on these lymphoblastoid cells are IL2R.

IL2R induced on lymphoblastoid cells by HTLV infection was compared with that on phytohemagglutinin-stimulated peripheral blood lymphocytes by NaDodSO$_4$/PAGE of immunoprecipitates obtained using the anti-IL2R antibody Hiei (Fig. 4). Hiei antibody detected an ^{125}I-labeled cell-surface antigen of ≈60 kDa on IL2R-bearing lymphoblastoid clones and on the stimulated lymphocytes.

DISCUSSION

There is increasing evidence for a close relationship between HTLV and IL2R recognized by Tac antibodies. Cultured HTLV-carrying ATL cells and HTLV-transformed T-cell lines always expressed Tac antigen or IL2R (10–12). It has also been reported that purified HTLV virions contain Tac antigen (23), and the anti-IL2R monoclonal antibody Hiei used in the present study was derived from spleen cells of mice immunized with purified HTLV (18). Furthermore, we previously detected expression of Tac antigen not only on HTLV-carrying T-cell lines but also on HTLV-carrying B-cell clones derived from peripheral blood lymphocytes of healthy HTLV carriers (12). The present study confirms these observations of IL2R expression on B-cell lines by showing that it also occurs in a number of LCL-Ter clones carrying HTLV. These results suggest two possibilities: (*i*) HTLV could preferentially infect cells expressing IL2R or (*ii*) infection with HTLV could induce IL2R expression on the cell surface. It has been reported that HTLV is able to infect nonlymphoid cells as well as lymphoid cells of human and nonhuman origin (24, 25). A human osteosarcoma cell line has been shown to be infected with HTLV *in vitro* but is Tac-antigen negative (26). This implies that the target cells for HTLV might not be restricted to IL2R-bearing cells. The present study supports the second possibility: IL2R-negative LCL-Kan cells were converted into IL2R-positive cells when infected with HTLV by cocultivation with HTLV-producer cells. It is possible that a minor population of LCL-Kan cells expressing IL2R could be preferentially infected with HTLV and subsequently proliferate, as it has been shown that a human B-cell line derived from hairy-cell leukemia expresses Tac antigen (27). However, this is not likely because all eight randomly selected LCL-Kan clones derived from cocultivation with LCL-Kan$_\gamma$ were negative for

Table 2. Cell-surface antigens on LCL-Kan clones

Clone* or cell line	Binding index			
	HTLV antibody		IL2R antibody	
	F10	GIN14	Hiei	Tac
C1	10	8	45	65
C4	9	3	30	72
C8	5	11	48	62
1	0	0	2	4
2	1	1	7	4
3	0	0	0	2
4	3	0	1	2
5	1	2	3	1
6	3	5	3	0
7	2	0	5	0
8	0	0	6	1
LCL-Kan	0	2	0	4

Binding indexes >10 indicate significant binding.
*LCL-KanC1, -C4, and -C8 were derived from parental LCL-Kan cells cocultured with TL-Su$_\gamma$ cells, and LCL-Kan1–8, from coculture with LCL-Kan$_\gamma$ cells.

Table 3. IL2-absorbing activities of cell lines

Cell line*	Hiei/Tac antigens on cell surface	IL2 activity, units/ml
None		50
LCL-Kan	−	38
LCL-KanC1	+	0.64
LCL-KanC4	+	0.87
LCL-KanC8	+	0.13
ILT-OmI	+	<0.01
NK7	−	<0.01

rIL2 was incubated with 10^7 cells and unabsorbed rIL2 activity was titrated.
*ILT-OmI and NK7 are IL2-dependent human and murine cell lines, respectively.

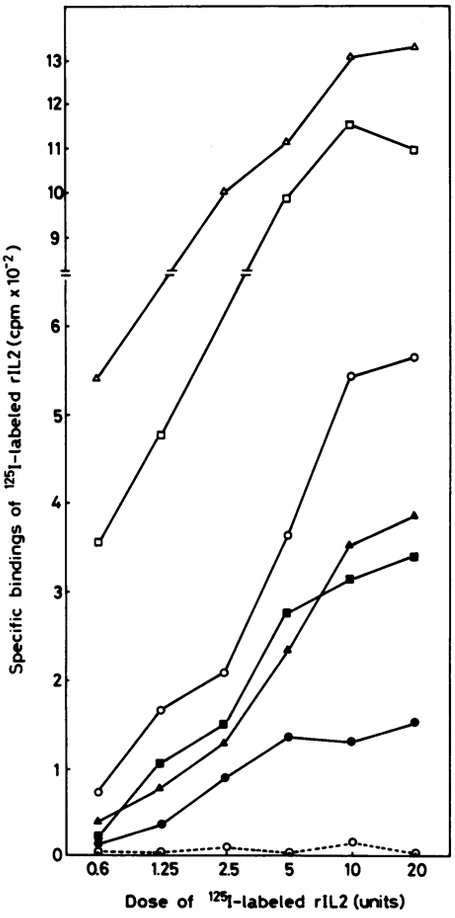

FIG. 3. IL2 binding assays to various cell lines. Binding of ^{125}I-rIL2 to cells was examined for clones LCL-KanC1 (●), -C4 (■), and -C8 (▲), for clones LCL-Ter21 (○), and LCL-Ter23 (□), and for parental LCL-Kan (---○---) and ILT-OmI (△) cell lines.

Tac and Hiei antigens. Furthermore, one of the IL2R-positive clones, LCL-KanC8, did not express these antigens initially and only became positive for them ≈3 months later. The conversion of LCL-Kan cells to IL2R-positive cells was probably not due to factors derived from the TL-Su, cells, other than infection by HTLV, as these irradiated cells were diluted out during the maintenance and reculture of the converted cell lines. The IL2R expressed on LCL cells has IL2-binding capability and a molecular weight was similar to that of IL2R on phytohemagglutinin-stimulated peripheral blood lymphocytes. Thus, it appears that HTLV can induce IL2R expression on Epstein–Barr virus-transformed B-cell lines.

Previously, an IL2 autocrine hypothesis for ATL leukemogenesis was proposed on the basis of observations that ATL cells expressed IL2R, that an HTLV-carrying leukemia cell line, HUT102, was a producer of IL2, and that *in vitro* transformation of hematopoietic cells was induced at high frequency by HTLV not carrying a typical v-*onc* gene (28). However, this hypothesis was recently withdrawn because the transcription of *IL2* genes could not be demonstrated in any other cell lines derived from ATL (29). It was reported, however, that the Tac antigen on ATL leukemia cells was not down-regulated by anti-Tac antibody, whereas that on concanavalin A-activated peripheral blood lymphocytes was. This suggested that the constitutive expression of Tac antigen on ATL cells could play an important role in ATL leukemogenesis (30). This is an interesting proposal, as it has been suggested that the epidermal growth factor receptor gene, which may correspond to the c-*erb*B oncogene, is amplified and rearranged in A431 human carcinoma cells, resulting in overproduction of the receptor protein (5). However, we previously observed that IL2R expression was often not detectable on fresh ATL cells (12), and it has not been detected in an HTLV-associated B-cell malignancy other than in the present study. Therefore, it is still possible that the IL2R expression on ATL cells could be merely a coexpression phenomenon after HTLV infection and not related to the mechanism of leukemogenesis.

Whether or not IL2R induction by HTLV is directly related to ATL leukemogenesis, it is of interest from the aspect of regulation of IL2R expression. There are several possible mechanisms of IL2R induction by HTLV. First, IL2R could be coded for by the HTLV genome. This is unlikely because the HTLV genome consists of virus-specific *gag*, *pol*, and *env* genes, and the gene called *pX*, which was demonstrated to have no homology with the human genome (31, 32). Second, the HTLV proviral genome could promote expression of the cellular *IL2R* gene by integrating at a site adjacent to the gene in cellular DNA. The integration site of the HTLV proviral genome seems not to be identical among at least three LCL-KanC clones (Fig. 1), but we cannot rule out the presence within the cellular genome of multiple loci that may affect the expression of the *IL2R* gene. Third, an HTLV gene product(s) could induce the expression of IL2R by affecting cells intracellularly or extracellularly. Just as antigenic stimulation or treatment with lectins or antibodies to T-cell-surface antigens is known to induce IL2R on T cells (33–36), the viral product could be an IL2R-inducing factor. However, we could not detect HTLV antigens in the IL2R-positive LCL-KanC clones (Table 2), although it is possible that amounts of HTLV gene product(s) that are undetectable by our methods may induce IL2R expression. Fourth, as

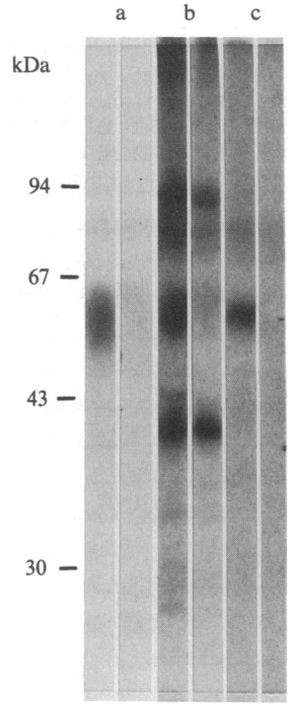

FIG. 4. NaDodSO$_4$/PAGE analysis of immunoprecipitates of various cell lysates with monoclonal antibody Hiei. ^{125}I-labeled cell lysates of phytohemagglutinin-stimulated peripheral blood lymphocytes (lanes a) and of LCL-KanC4 (lanes b) and LCL-Ter23 (lanes c) cell lines were immunoprecipitated with anti-Hiei antibody (left lanes) or negative controls (right lanes) and analyzed by NaDodSO$_4$/PAGE on 10% gels followed by autoradiography. Positions of molecular mass marker proteins (not shown) are indicated on the left.

HTLV-carrying T-cell lines are known to produce various lymphokines (37–39), it is possible that HTLV-carrying cells produce factor(s) not encoded by the HTLV genome that induce IL2R expression.

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