

Establishment of an Interleukin 2-Dependent Human T Cell Line From a Patient With T Cell Chronic Lymphocytic Leukemia Who Is Not Infected With Human T Cell Leukemia/Lymphoma Virus

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We established an interleukin 2 (IL-2)-dependent human T cell line, Kit 225, from a patient with T cell chronic lymphocytic leukemia (T-CLL) with OKT3+, -T4+, -T8- phenotype. Southern blot analysis showed that Kit 225 is not infected with human T cell leukemia/lymphoma virus (HTLV) type I or II, and is probably derived from the major clone in the fresh leukemic cells. Kit 225 cells express a large amount of IL 2 receptors constitutively and their growth is absolutely dependent on IL 2. No other

stimuli, such as lectins or antigens, are required for maintaining the responsiveness to IL 2. As abnormal IL 2 receptor expression was also seen originally in the fresh leukemic cells, the establishment of this cell line with IL 2 suggests that IL 2-mediated T cell proliferation is involved in the leukemogenesis of some cases of HTLV-negative T-CLL.

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TCELL CHRONIC lymphocytic leukemia (T-CLL) is a heterogeneous disease clinically, morphologically, and immunologically.¹⁻⁵ Although it is believed to be a monoclonal expansion of mature peripheral T cells, the pathophysiology of this disease remains unclear. Our previous study showed that leukemic cells from T-CLL patients with an OKT3+, -T4+, -T8- phenotype express the interleukin 2 (IL 2) receptor without any stimuli and proliferate in response to IL 2.⁶ Because the IL 2/IL 2 receptor system plays an essential role in regulation of T cell growth, it is important to know whether failure of this regulatory mechanism is involved in some T cell malignancies. Recent studies⁷⁻⁹ revealed a close association between the abnormal expression of IL 2 receptor and the infection of human T cell leukemia/lymphoma virus type I (HTLV-I),^{10,11} which is believed to be a causative agent of adult T cell leukemia (ATL).¹² The mechanism and role of abnormal expression of IL 2 receptor in HTLV-negative T-CLL has not been well analyzed, however. It appears to be due partly to the lack of appropriate cell lines derived from T-CLL patients. In the present study we established an HTLV-negative and IL 2-dependent T cell line with T4+ phenotype from a T-CLL patient, which was demonstrated to be the same clone as the primary leukemic cells. Using this cell line as a model system, we discuss the implication of IL 2-mediated T cell proliferation in the leukemogenesis of some HTLV-negative T4+ T-CLL cases.

MATERIALS AND METHODS

Patient. A 62-year-old man was admitted on September 26, 1983, because of persistent lymphocytosis, systemic lymph node swelling, and erythroderma. Peripheral blood analysis revealed WBC count of $291 \times 10^9/L$ (leukemic cells 90%), RBC count of $4.81 \times 10^{12}/L$, and platelet count of $3.82 \times 10^{11}/L$. Bone marrow infiltration of 40% pathologic cells was seen. The serologic test was negative for the anti-ATLA antibody.¹² The diagnosis of T-CLL was made on the basis of clinical features, hematologic characteristics, and cell surface phenotypes.⁶

Cell surface marker analysis. Cell surface antigens were detected by indirect immunofluorescence staining and cytofluorometry as described previously.⁶ Monoclonal antibodies OKT3, -T4, -T6, -T8, -T9, -T11 and -Ia were obtained from Ortho Diagnostic Systems (Westwood, MA). Anti-Tac monoclonal antibody (appropriately diluted ascitic fluid) was used as an anti-IL 2 receptor antibody.^{13,14}

Cell separation and culture. The patient's peripheral blood mononuclear cells were separated from heparinized blood by Ficoll-Conray density gradient centrifugation. Separated leukemic cells were then cultivated in RPMI 1640 medium (Nissui Pharmaceutical, Tokyo) supplemented with 10% fetal calf serum (FCS) (GIBCO, Grand Island, NY), 20 $\mu g/mL$ tobramycin, and 20% crude IL 2 (culture supernatant of lectin-stimulated human spleen cells) at 37°C under a humid atmosphere with 5% CO₂. After 3 months, crude IL 2 was replaced by 1 U/mL recombinant human IL 2 generously provided by Takeda Chemical Industries Inc. (Osaka, Japan).

Radiolabeled IL 2 binding assay. Radiolabeled IL 2 binding assay was done as described elsewhere¹⁵ to determine the number of both high-affinity and low-affinity binding sites.

Growth pattern of the cell line cells. To estimate the growth pattern of the cells, 2×10^5 cells in 4 mL culture medium with or without 10 U/mL recombinant human IL 2 were cultured in a set of seven Petri dishes, and the number of viable cells was counted by trypan blue dye exclusion every 24 hours for 7 days.

Southern blot hybridization. For detection of HTLV-I or HTLV-II provirus and abnormalities of IL 2 receptor gene and for analysis of clonal identity of the fresh leukemic cells and the established cell line, Southern blot hybridization was performed as described previously.¹⁶ In brief, high-mol-wt DNA was extracted, digested with restriction endonuclease (*EcoRI*), separated by agarose gel electrophoresis, and transferred onto a nitrocellulose membrane. After being baked, the membrane was hybridized with the nick-translated probes, washed, and autoradiographed. As the probes, *AccI-SmaI* fragment of HTLV-I (kindly provided by Dr T.

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Table 1. Cell Surface Markers

Source	Positive Cells (%)							
	OKT3	T4	T6	T8	T9	T11	Ia	Tac
Fresh leukemic cells	91	76	0	5	4	99*	84	4
Kit 225	69	96	0	0	63	97	94	96

*Determined on a freshly thawed sample of frozen cells.

Honjo, Kyoto University, Kyoto),¹⁷ LTR of HTLV-II (kindly provided by Dr K. Shimotohno, National Cancer Center, Tokyo), Sau 3A fragment of IL 2 receptor cDNA (Dr T. Honjo),¹⁸ and *Hind*III-*Eco*RI fragment of T cell receptor β chain cDNA (Dr T. Honjo)¹⁷ were used.

Chromosomal analysis. Cells in log phase of IL 2-dependent growth were exposed to 0.05 μ g/mL Colcemid for 1 hour. These cells were suspended in 0.075 mol/L KCl for 20 minutes at 37°C and then fixed with acetic acid/methanol (1:3). They were spread on slides to air-dry, and the chromosomes were stained with trypsin-Giemsa.

RESULTS

Hematologic and immunologic features of the fresh leukemic cells. May-Giemsa staining revealed large leukemic cells with azurophilic granules in abundant cytoplasm. The cell surface phenotype was OKT3+, -T4+, -T8-, as shown in Table 1. Fresh leukemic cells expressed a small amount of IL 2 receptors recognized by anti-Tac monoclonal antibody, and proliferated in response to IL 2 as was seen in normal phytohemagglutinin (PHA)-stimulated T cells (data shown as patient 2 in Fig 3 in the previous report.⁶).

Long-term culture of the leukemic cells with IL 2. Separated leukemic cells soon began to proliferate in the presence of 20% crude IL 2. After 3 months, crude IL 2 was replaced by 1 U/mL recombinant human IL 2, and the cells continued to grow in an IL 2-dependent manner. The established cell line, named Kit 225, has been cultured for >21 months. As shown in Fig 1, Kit 225 cells proliferated exponentially in the presence of IL 2 but died within several days without IL 2.

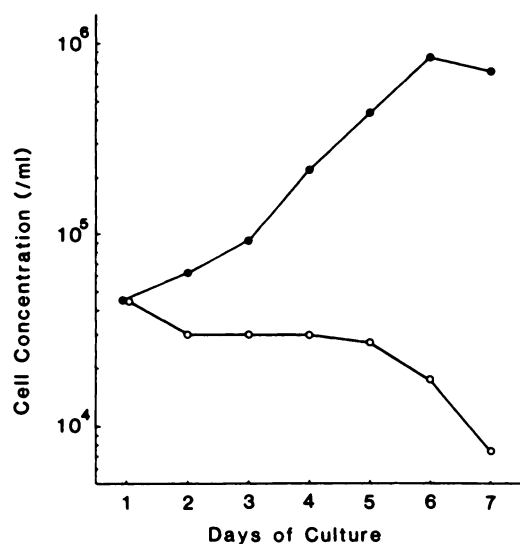


Fig 1. Growth curves of Kit 225 cells. Cells were cultured in medium alone (○) or in the presence of 10 U/mL recombinant human IL 2 (●).

No other stimuli such as antigens or lectins were required for maintaining the responsiveness to IL 2.

Cell surface phenotype and IL 2 receptor expression. Kit 225 had the cell surface phenotype of OKT3+, -T4+, -T8-, which was essentially the same as the fresh leukemic cells (Table 1). However, intense expression of Tac antigen/IL 2 receptor was noted in Kit 225 as compared with the fresh leukemic cells. To determine the number of IL 2 receptors, radiolabeled IL 2 binding assay was performed and Scatchard analysis revealed that Kit 225 cells expressed 3,000 receptors/cell with high affinity (kd 66 pmol/L) and 190,000 receptors/cell with low affinity (kd 7.0 nmol/L) (Fig 2).

Southern blot analysis. High-mol-wt DNA prepared from the fresh leukemic cells and Kit 225 cells was subjected to Southern blot analysis. As shown in Fig 3, Kit 225 cells have no HTLV-I or HTLV-II proviral DNA integration and have a rearrangement pattern of T cell receptor β chain gene identical to that of the fresh leukemic cells, suggesting that Kit 225 was derived from the major clone in the primary leukemic cells. The latter was supported by the results in another restriction enzyme (*Hind*III) digestion (data not shown). Neither gross rearrangement nor amplification of the IL 2 receptor gene was detected in the fresh leukemic cells and Kit 225 cells.

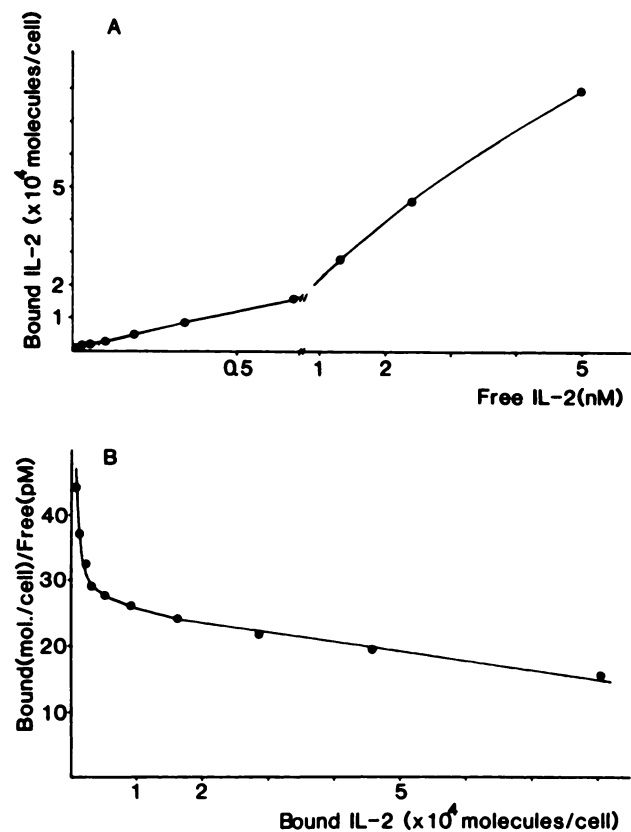


Fig 2. (A) Radiolabeled IL 2 binding to Kit 225 cells. (B) Scatchard plot of the radiolabeled IL 2 binding data shown in A. Estimated numbers of high-affinity and low-affinity IL 2 receptors were 3,000/cell and 190,000/cell, respectively.

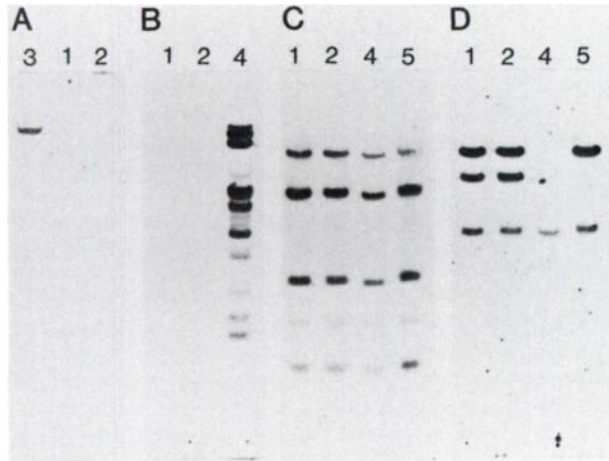


Fig 3. Southern blot analysis of the fresh leukemic cells and Kit 225. High-mol-wt DNA was prepared from the fresh leukemic cells (lane 1), Kit 225 (lane 2), adult T cell leukemia cells (lane 3), Mo, an HTLV-II-infected T cell line (lane 4), and normal human placenta (lane 5). Five micrograms of each DNA sample was digested with restriction enzyme *EcoRI*, separated by agarose gel electrophoresis, and transferred onto a nitrocellulose membrane. After being baked, the membrane was hybridized with the probes of HTLV-I (panel A), HTLV-II (panel B), IL 2 receptor (panel C), and T cell receptor β chain (panel D).

Chromosomal analysis. The karyotype is described in detail (International System for Human Cytogenetic Nomenclature-High Resolution Banding, 1981) as follows: 47, XY, -5, -6, -14, +19, ins inv(1)(pter \rightarrow p36.3::p34.1 \rightarrow p31.2::p36.3 \rightarrow p34.1::p31.2 \rightarrow p22.1::q12 \rightarrow p22.1::q12 \rightarrow qter), inv(3)(pter \rightarrow p26.2::q27.1 \rightarrow p26.2::q27.1 \rightarrow qter), +der(5)t(5;7)(7qter \rightarrow 7q22::5p15.3 \rightarrow cen \rightarrow 5qter), del(6)(pter \rightarrow q21::q23.3 \rightarrow qter), +der(6)t(6;?) (q21;?), +der(14)t(6;14)6q21 \rightarrow 6q23.3::14p12 \rightarrow cen \rightarrow 14qter). No abnormalities were detected in chromosome 10, where the gene that encodes IL 2 receptor is located.¹⁹

DISCUSSION

In the present study, we established an IL 2-dependent human T cell line, Kit 225, from a T-CLL patient with T4+ phenotype. Southern hybridization analysis demonstrated that Kit 225 cells are not infected with HTLV-I or HTLV-II and are probably derived from the major clone in the original leukemic cells.

Some murine IL 2-dependent T cell lines exist.^{20,21} Also, in humans, many IL 2-dependent HTLV-infected T cell lines have been established, some of which are reported to become independent of IL 2 during the passage of culture. Establishment of HTLV negative and IL 2-dependent human T cell lines that require no other stimulation has not been reported however, as far as we know.

The IL 2/IL 2 receptor system is considered to regulate normal T cell proliferation.²² T cells activated by antigens synthesize and release IL 2 as well as express IL 2 receptors on their cell surface membranes, and the binding of IL 2 to its receptors induces the clonal expansion of T cells. The two phenomena, IL 2 production and IL 2 receptor expression, always occur transiently in activated T cells in a normal state, which prevents unlimited growth of T cells. Conversely, it is quite possible that some failure of the IL 2/IL 2 receptor system may cause a neoplastic T cell proliferation.

We previously reported that leukemic cells from most HTLV-negative T-CLL patients with OKT3+, -T4+, -T8- phenotype express IL 2 receptor without any stimuli and proliferate in response to IL 2.⁶ Therefore, they seem to be ready to grow by the paracrine mechanism wherever IL 2 is available, even in in vivo environments. In this context, the establishment or immortalization of an IL 2-dependent T cell line, Kit 225, from a T-CLL patient suggests that IL 2-mediated T cell proliferation is involved in the leukemogenesis of some HTLV-negative T-CLL cases with T4+ phenotype. So far, we have succeeded in establishing an IL 2-dependent T cell line from one of eight T-CLL cases we tried. The duration of maintained cell cultures from the remaining seven cases varied from 2 to 16 weeks, which may reflect the heterogeneity of T-CLL in IL 2-dependent growth of leukemic cells.

Recent studies revealed a close association between abnormal expression of the IL 2 receptor and HTLV-I or HTLV-II infection. In such cases, pX region of the virus is speculated to play a key role in augmenting the IL 2 receptor gene expression.⁹ However, the mechanism underlying the abnormal expression of the IL 2 receptor on Kit 225 cells as well as HTLV-negative leukemic cells from T-CLL patients remains to be clarified. Kit 225 is expected to serve as a model system of the abnormal expression of IL 2 receptor that may trigger the development of some HTLV-negative T-CLL cases. Further analyses concerning the mechanism of the unregulated IL 2 receptor expression in Kit 225 will be required, including the search for an unknown virus.

REFERENCES

- Brouet JC, Flandrin G, Sasportes M, Preud'Homme JL, Seligmann M: Chronic lymphocytic leukaemia of T-cell origin. Immunological and clinical evaluation in eleven patients. *Lancet* 2:890, 1975
- Nair KG, Han T, Minowada J: T-cell chronic lymphocytic leukemia. Report of a case and review of the literature. *Cancer* 44:1652, 1979
- Reinherz EL, Nadler LM, Rosenthal DS, Moloney WC, Schlossman SF: T-cell subset characterization of human T-CLL. *Blood* 53:1066, 1979
- Boumsell L, Bernard A, Reinherz EL, Nadler LM, Ritz J, Coppin H, Richard Y, Dubertret L, Valensi F, Degos L, Lemerle J, Flandrin G, Dausset J, Schlossman SF: Surface antigens on malignant Sezary and T-CLL cells correspond to those of mature T cells. *Blood* 57:526, 1981
- Pandolfi F, De Rossi G, Semenzato G, Quinti I, Ranucci A, De Sanctis G, Lopez M, Gasparotto G, Aiuti F: Immunologic evaluation of T chronic lymphocytic leukemia cells: Correlations among phenotype, functional activities, and morphology. *Blood* 59:688, 1982
- Tsuda M, Uchiyama T, Umadome H, Wano Y, Hori T, Tamori S, Uchino H, Kita K, Chiba S, Mitsutani S, Nesumi N:

Expression of interleukin-2 receptor on T cell chronic lymphocytic leukemia cells and their response to interleukin-2. *Blood* 67:316, 1986

7. Uchiyama T, Hori T, Tsudo M, Wano Y, Umadome H, Tamori S, Yodoi J, Maeda M, Sawami H, Uchino H: Interleukin-2 receptor (Tac antigen) expressed on adult T cell leukemia cells. *J Clin Invest* 46:446, 1985

8. Yodoi J, Okada M, Tagaya Y, Teshigawara K, Fukui K, Ishida N, Ikuta K, Maeda M, Honjo T, Osawa H, Diamantstein T, Tateno M, Yoshiki T: Rat lymphoid cell lines producing human T cell leukemia virus II; constitutive expression of rat interleukin-2 receptor. *J Exp Med* 161:924, 1985

9. Inoue J, Seiki M, Taniguchi T, Tsuru S, Yoshida M: Induction of interleukin 2 receptor gene expression by p40^r encoded by human T-cell leukemia virus type I. *EMBO J* 5:2883, 1986

10. Poesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC: 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, 1980

11. Hinuma Y, Nagata K, Hanaoka M, Nakai T, Matsumoto T, Kinoshita K, Shirakawa S, Miyoshi I: 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, 1981

12. Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H: Adult T cell leukemia: Clinical and hematological features of 16 cases. *Blood* 50:481, 1977

13. Uchiyama T, Broder S, Waldmann TA: 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, 1981

14. Leonard WJ, Depper M, Uchiyama T, Smith KA, Waldmann TA, Greene WC: A monoclonal antibody that appears to recognize the receptor for human T-cell growth factor: Partial characterization of the receptor. *Nature (Lond)* 300:267, 1982

15. Hori T, Uchiyama T, Umadome H, Tamori S, Tsudo M, Araki K, Uchino H: Dissociation of interleukin-2-mediated cell proliferation and interleukin-2 receptor upregulation in adult T cell leukemia cells. *Leuk Res* 10:1447, 1986

16. Honjo T, Obata M, Yamawaki-Kataoka Y, Kataoka T, Kawakami T, Takahashi N, Mano Y: Cloning and complete nucleotide sequence of mouse immunoglobulin γ 1 chain gene. *Cell* 18:559, 1979

17. Maeda M, Shimizu A, Ikuta K, Okamoto H, Kashiwara M, Uchiyama T, Honjo T, Yodoi J: Origin of human T-lymphotropic virus I-positive T cell lines in adult T cell leukemia: Analysis of T cell receptor gene rearrangement. *J Exp Med* 162:2169, 1985

18. Nikaido T, Shimizu A, Ishida N, Sabe H, Teshigawara K, Maeda M, Uchiyama T, Yodoi J, Honjo T: Molecular cloning of cDNA encoding human interleukin-2 receptor. *Nature* 311:631, 1984

19. Leonard WJ, Donlon TA, Lebo RV, Greene WC: Localization of the gene encoding the human interleukin-2 receptor on chromosome 10. *Science* 228:1547, 1985

20. Gillis S, Smith KA: Long term culture of tumour-specific cytotoxic T cells. *Nature (Lond)* 268:154, 1977

21. Jones-Villeneuve E, Phillips RA: Potentials for lymphoid differentiation by cells from long-term culture of bone marrow. *Exp Hematol* 8:65, 1980

22. Cantrell DA, Smith KA: The interleukin-2 T-cell system: A new cell growth model. *Science* 224:1312, 1984



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