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

By Toshiyuki Hori, Takashi Uchiyama, Mitsuru Tsudo, Hiroshi Umadome, Hitoshi Ohno, Shirou Fukuhara, Kenkichi Kita, and Haruto Uchino

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.

Cell separation and culture. The patient’s peripheral blood mononuclear cells were separated from heparinized blood by Ficol-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 µg/ml streptomycin, 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 µU/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 elsewhere10 to determine the number of both high-affinity and low-affinity binding sites.

Growth pattern of the cell line cultures. To estimate the growth pattern of the cells, 2 x 10^6 cells in 4 mL culture medium with or without 10 U/mL recombinant human IL 2 were cultivated in a set of seven Petri dishes, and the number of viable cells was counted by trypsin 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.14 In brief, high-mol-wt DNA was extracted, digested with restriction enzymes, separated by agarose gel electrophoresis, and transferred onto a nitrocellulose membrane. After being baked, the membrane was hybridized with the nick-translation probes, washed, and autoradiographed. As the probes, Accl-SmaI fragment of HTLV-I (kindly provided by Dr T.

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 x 10^9/L (leukemic cells 90%), RBC count of 4.81 x 10^12/L, and platelet count of 3.82 x 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.4

Cell surface marker analysis. Cell surface antigens were detected by indirect immunofluorescence staining and cytofluorometry as described previously.4 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.15,14

Established cell line. Southern blot hybridization was performed as described previously.14 In brief, high-mol-wt DNA was extracted, digested with restriction enzymes, separated by agarose gel electrophoresis, and transferred onto a nitrocellulose membrane. After being baked, the membrane was hybridized with the nick-translation probes, washed, and autoradiographed. As the probes, Accl-SmaI fragment of HTLV-I (kindly provided by Dr T.

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1069
Table 1. Cell Surface Markers

<table>
<thead>
<tr>
<th>Source</th>
<th>OKT3</th>
<th>T4</th>
<th>T6</th>
<th>T8</th>
<th>T9</th>
<th>T11</th>
<th>la</th>
<th>Tac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh leukemic cells</td>
<td>91</td>
<td>76</td>
<td>0</td>
<td>5</td>
<td>4</td>
<td>99</td>
<td>84</td>
<td>4</td>
</tr>
<tr>
<td>Kit 225</td>
<td>98</td>
<td>96</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>97</td>
<td>94</td>
<td>96</td>
</tr>
</tbody>
</table>

* Determined on a freshly thawed sample of frozen cells.

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). 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 (HindIII) 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.
HTLV(-) AND IL-2 DEPENDENT HUMAN T CELL LINE

Some murine IL-2-dependent T cell lines exist. 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. 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. 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. 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


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