ADULT T CELL leukemia (ATL) is a leukemia with characteristic clinical features, and was first shown to be caused by a human retrovirus, termed human T-cell leukemia virus type I (HTLV-I). The precise mechanism of the development of ATL and the neoplastic cell growth remains unclear although the constitutive expression of interleukin-2 receptor (IL-2R) is induced by the activity of p400kD of HTLV-I as a transacting transcriptional activator and has been postulated as one of the sequential key events leading to the overt ATL. We have been studying the characteristics of the cell growth of fresh leukemic cells isolated from peripheral blood (PB) of ATL patients focusing on the role of abnormally expressed IL-2R in the neoplastic cell growth. Others also reported different aspects of cell growth of in vitro immortalized HTLV-I-infected cell lines, most of which are, in fact, cell clones different from the leukemic cells. However, so far these studies do not appear to clarify the essential mechanism of neoplastic cell proliferation or leukemogenesis. In addition, we do not have an effective treatment of ATL and its prognosis, therefore, is still very poor. The appropriate model of in vivo proliferation of leukemic cells from ATL patients would provide us with more powerful experimental approaches to address these issues and important information not only to better understand the mechanism of leukemogenesis but also to develop an effective treatment of ATL. Useful animal models of the development of ATL and of the in vivo cell proliferation of ATL cells, to our knowledge, have not been developed, although the animal models of HTLV-I-infection or transmission have been reported. Severe combined immunodeficiency (SCID) mice, because of the defect of mature normal T and B cells, could be successfully engrafted with human hematopoietic cells or neoplastic cells. For example, in vivo tumor formation of Epstein-Barr virus (EBV)-infected human B cells in SCID mice has been reported and used as a useful model to study the mechanism of B-cell lymphomagenesis. We have been trying to make a model of in vivo cell proliferation of leukemic cells from ATL patients using SCID mice and recently succeeded in making such a model after failures of early studies in which we almost always observed the preferential cell growth of EBV-infected B cells from the patients in SCID mice. Here we present the methods to make a model of in vivo cell proliferation of ATL cells and the characterization of the cells proliferating in vivo and infiltrating into various organs.

MATERIALS AND METHODS

Mice. Immune-deficient SCID (CB17scid/scid) mice were obtained from Nihon Clea Inc (Tokyo, Japan). The mice were bred and maintained under specific pathogen-free conditions in the animal facility of the Institute for Virus Research, Kyoto University.

Preparation of the cells injected into SCID mice. PB and lymph node (LN) from ATL patients were obtained with informed consent. PB mononuclear cells (PBMC) were separated by Ficoll/Hyphaque (Pharmacia LKB, Uppsala, Sweden) density gradient centrifugation. For the preparation of LN cells (LNC), LN was cut into several pieces and resuspended in RPMI 1640 medium containing 10% fetal calf serum (FCS) (Bioproducts, Walkersville, MD) and LNC were separated from the remaining tissues. The depletion of B cells and monocytes from PBMC or LNC was performed using Dyna-beads (Dynal A.S., Oslo, Norway). PBMC or LNC were washed twice and resuspended in phosphate-buffered saline (PBS) containing 10% FCS and incubated with anti-B cell (Lym-1 from American Type Culture Collection, Rockville, MD), anti-CD11b cell clone. Histologic examination showed that the pattern of the infiltration of ATL cells into various organs in SCID mice was similar to that of an ATL patient. Such a model of in vivo cell proliferation of ATL cells will be useful for the study of the mechanism of neoplastic cell proliferation and for the development of a new and effective treatment of ATL.
Table 1. ATL Cells Injected Into SCID Mice

<table>
<thead>
<tr>
<th>Case</th>
<th>Type</th>
<th>Source</th>
<th>No. (× 10^7)</th>
<th>Tumor Growth/ No. of Mice</th>
<th>sol-Tac* (pg/mL)</th>
<th>Survival (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. F.S.</td>
<td>Lymphoma</td>
<td>LNC</td>
<td>4.4</td>
<td>1/1</td>
<td>&gt;300,000</td>
<td>47</td>
</tr>
<tr>
<td>2. Y.K.</td>
<td>Chronic</td>
<td>PBMC</td>
<td>2.5</td>
<td>1/1</td>
<td>58,950</td>
<td>38</td>
</tr>
<tr>
<td>3. F.Y.</td>
<td>Lymphoma</td>
<td>LNC</td>
<td>5.8</td>
<td>1/2</td>
<td>22,9961</td>
<td>951</td>
</tr>
<tr>
<td>4. Y.N.</td>
<td>Chronic</td>
<td>PBMC</td>
<td>2.5</td>
<td>0/1</td>
<td>2,537</td>
<td>Alive</td>
</tr>
<tr>
<td>5. K.Ya.</td>
<td>Chronic</td>
<td>PBMC</td>
<td>2.1</td>
<td>1/1</td>
<td>35,025</td>
<td>32</td>
</tr>
<tr>
<td>6. N.S.</td>
<td>Acute</td>
<td>PBMC</td>
<td>11.2</td>
<td>0/4</td>
<td>1,539t</td>
<td>Alive</td>
</tr>
<tr>
<td>7. K.K.</td>
<td>Lymphoma</td>
<td>LNC</td>
<td>2.0</td>
<td>1/1</td>
<td>17,073</td>
<td>44</td>
</tr>
<tr>
<td>8. K.Yu.</td>
<td>Lymphoma</td>
<td>LNC</td>
<td>2.2</td>
<td>1/1</td>
<td>477,290</td>
<td>21</td>
</tr>
</tbody>
</table>

* Serum soluble human IL-2Ra chain (Tac) levels.
† Values of the mouse engrafted with ATL cells.
‡ Mean value of 4 mice.

Injection of the cells into SCID mice. Cells (2.0 ~ 11.2 × 10^7) isolated from ATL patients and prepared as described above were intraperitoneally injected into SCID mice pretreated with 1 mg/mouse of TM-β1 MoAb. In addition, the mice were intraperitoneally injected with 20 ng/mouse/d of human recombinant IL-2 (a kind gift from Shionogi & Co Ltd, Osaka, Japan) every day from day 1 to day 60 after the inoculation of the cells. Mice were killed and autopsied when they showed the elevated serum soluble human IL-2Ra chain (Tac) levels and the signs such as weight loss, lethargy, ruffled fur, and a hunched posture. The tumors and other tissues of interest were dissected into two or more pieces that were fixed in 10% formalin for immunohistologic analysis and used for preparing viable cell suspensions for FACS analysis and DNA analysis.

Measurement of serum-soluble human IL-2Ra chain (Tac) levels. Blood periodically obtained from the tail and also blood from the heart of a killed mouse were used for the measurement of soluble human IL-2Ra chain (Tac). Serum-soluble human IL-2Ra chain (Tac) levels were measured by enzyme-linked immunosorbent assay (ELISA) as previously described, with a slight modification. In brief, peroxidase-conjugated streptavidin (Dakopatts) and α-phenylene diamine were used as enzyme and substrate, respectively.

FACS analysis. Fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated murine MoAbs to human CD2 (Leu5b), CD3 (Leu4), CD4 (Leu2a), CD8 (Leu2a), CD20 (Leu16), and HLA-DR were purchased from Becton Dickinson (San Jose, CA). The MoAbs to the α chain and β chain of human IL-2R, anti-Tac, and 2R-B MoAbs, respectively, were used as FITC-conjugated or biotin-conjugated IgG. Cell suspensions from the murine tumors, spleen, PB, and bone marrow (BM) were washed in Hanks’ balanced salt solution (HBSS) containing 1% bovine serum albumin (BSA) and 0.1% NaN3, and stained with saturating amounts of MoAbs as previously described. The stained cells were analyzed on a FACS (Becton Dickinson & Co, Mountain View, CA).

DNA analysis. DNA was prepared from the cells or tissues by proteinase K digestion followed by phenol chloroform extraction. DNA was then digested with the restriction enzyme EcoRI or BamHI (Toyobo, Osaka, Japan), electrophoresed in a 0.7% agarose gel, and blotted to a nylon filter. Filters were hybridized to randomly primed 32P-labeled DNA probes, washed under appropriate conditions, and subjected to autoradiography. Two probes were used in the present study. One was a 0.96-kb AccI/Smal fragment of HTLV-I pX region, and the other was a 3.5-kb HindIII/EcoRI fragment that contained human TCR Cδ gene.
other tissues of interest fixed in 10% formalin were processed to 2. Y.K. 45 0 2

3-mouse with the white blood cell (WBC) count of 8. K.Yu. 5 80 72 85

IN VIVO CELL GROWTH MODEL

4-µm paraffin wax-embedded sections for staining with hematoxylin and eosin (H&E). Immunohistochemical studies were performed on paraffin sections by the avidin-biotin-peroxidase complex technique of Hsu et al.44 with minor modifications. A panel of monoclonal and polyclonal antibodies used in the present study and dilutions used were: CD3 (polyclonal, ×100 with protease pre-treatment; Dakopatts),45 UCHL1 (CD45RO) (monoclonal, ×25; Bio-Science Products, Emmenbrücke, Switzerland),46 and L26 (CD20) (monoclonal, ×100, Dakopatts).47

RESULTS

Characteristics of patients with ATL. Neoplastic cells from 8 ATL patients, including four male and four female patients, were used in the present study. Their ages ranged from 46 to 83 years (mean = 61.6). They consisted of 1 acute type, 3 chronic type, and 4 lymphoma type ATL patients. Patient 4 showed the signs of crisis developing after a chronic clinical course when examined. They received no therapy before cell sampling. PBMC were obtained from acute and chronic type ATL patients, and LNC from lymphoma type ATL patients, respectively (Table 1).

Serum-soluble human IL-2Rα chain (Tac) levels as indicators of cell proliferation in vivo. Serum-soluble human IL-2Rα chain (Tac) levels periodically measured were moderately to markedly elevated in mice that were found to be engrafted with ATL cells (mean = 151,889 pg/mL) in contrast to the low levels in the non-engrafted mice (mean = 2,523 pg/mL) (Table 1 and Fig 1).

Histopathologic findings of the mice engrafted with ATL cells. We found the tumor growth in SCID mice injected with ATL cells from 6 (cases 1, 2, 3, 5, 7, and 8) of 8 ATL patients 5 to 7 weeks after the inoculation of ATL cells. Two mice injected with ATL cells from patient 3 or 7 (tentatively called case 3-mouse and case 7-mouse, respectively) were found dead of tumor and, therefore, could not be examined in detail except for histologic examination of case 7-mouse. Other mice engrafted with ATL cells from patient 1, 2, 5, or 8 were killed, autopsied, and further examined. We found the small tumors at the root of mesentery and the enlargement of lymphoid organs (spleen, thymus, and lymph nodes) in cases 1-, 2-, 5- and 8-mouse at autopsy. Cases 1- and 5-mouse with the white blood cell (WBC) count of 30,000/mm³ became leukemic. Histologic examination showed the infiltration of atypical lymphoid cells that were reactive with human CD3 and UCHL1 (CD45RO) antibodies but not with L26 (CD20) MoAb into portal regions of the liver, interstitial regions of the lung, and the kidney in cases 1- and 5-mouse. In contrast, the study of case 2-mouse with a systemic lymph node enlargement showed the histopathologic characteristics of a lymphoma type ATL. The enlarged lymph nodes showed the histologic characteristics of a pleomorphic type lymphoma with scattered human CD3+ giant cells, which is often observed in the LN of an ATL patient. Consistent with these findings, the spleen of the mouse showed the infiltration of human CD3+ cells mainly into the white pulp region. However, the infiltration of the lymphoid cells into other organs such as the liver, kidneys, and lungs was minimal (Table 2 and Fig 2). Case 8-mouse also showed the pattern of cell infiltration that was similar to that of a lymphoma-type ATL.

FACS analysis of the cells proliferating in SCID mice. We next examined the cell-surface phenotype of the cells recovered from the tumors and other tissues of the mice to determine whether the cells proliferating in the mice were the leukemic cells derived from ATL patients. The cells obtained from the tumors consisted predominantly of human CD4+ cells (32%, 45%, 85%, and 94% of the cells from cases 1-, 2-, 5-, and 8-mouse, respectively), a considerable proportion of human CD8+ cells, and a small proportion of human CD20+ cells (Table 3). In case 5-mouse, the proportion of CD4+ cells plus that of CD8+ cells were greater than 100%, which indicated the presence of CD4+CD8+ (double positive) cells in the cells examined. Double-staining study showed that the proportion of cells reactive with both antihuman CD4 MoAb and anti-Tac MoAb was 20%, 47%, 67%, and 86% of the tumor cells examined (Fig 3). In other words, the study showed the recovery from the murine tumor of cells with the same cell-surface phenotype as that of original ATL cells because the leukemic cells from all ATL patients examined in the present study were CD4+ Tac+, except the cells from patient 4, which were CD4+CD8+ Tac+. FACS analysis of the cells from the PB, BM, and spleen also showed human CD4+ cells in cases 1- and 5-mouse. However, in accord with the histopathologic findings, no human CD4+ cells were demonstrable in PB of cases 2- and 8-mouse (Table 2).

DNA analysis. Southern blot hybridization analysis using pX of HTLV-1 and Cβ1 of human T-cell receptor (TCR) gene as probes was performed to confirm that the clone of the cells proliferating in SCID mice was the same as that of

Table 2. Infiltration of ATL Cells Into Organs of SCID Mice

<table>
<thead>
<tr>
<th>Case</th>
<th>Tumor</th>
<th>PB BM</th>
<th>Spleen</th>
<th>LN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. F.S.</td>
<td>32</td>
<td>16</td>
<td>17</td>
<td>28</td>
</tr>
<tr>
<td>2. Y.K.</td>
<td>45</td>
<td>0</td>
<td>2</td>
<td>NE</td>
</tr>
<tr>
<td>3. F.Y.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. K.Ya.</td>
<td>85</td>
<td>80</td>
<td>72</td>
<td>85</td>
</tr>
<tr>
<td>7. K.K.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. K.Yu.</td>
<td>94</td>
<td>0</td>
<td>0</td>
<td>NE</td>
</tr>
</tbody>
</table>

Histologic Study

<table>
<thead>
<tr>
<th>Spleen</th>
<th>Liver</th>
<th>Kidney</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>++</td>
<td>--</td>
<td>--</td>
<td>++</td>
</tr>
<tr>
<td>NE</td>
<td>++</td>
<td>++</td>
<td>NE</td>
</tr>
<tr>
<td>--</td>
<td>++</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Abbreviations: ++++, markedly infiltrated; ++, moderately infiltrated; +, slightly infiltrated; --, not infiltrated; NE, not examined.
Fig 2. Histologic findings of the tissues from mice engrafted with ATL cells. (A) Lymphoid cells heavily infiltrated into the interstitial regions of the lung (case 1-mouse) (original magnification X 10, H&E staining). (B) The LN showed the characteristics of a diffuse pleomorphic type lymphoma with scattered giant cells (case 2-mouse) (original magnification X 40, H&E staining). (C) The spleen showed the infiltration of lymphoid cells mainly in the white pulp region (case 2-mouse) (original magnification X 10, H&E staining). (D) Immunohistochemical analysis of the same region as that of photo C showed that the infiltrating cells were reactive with antihuman CD3 antibody (case 2-mouse) (original magnification X 10).

As shown in Fig 4, the clear band(s) was detected at the same position of the filter both in leukemic cells of PB and LN of the patient and murine tumor and tissues, confirming their clonal identity. Of note, clear bands other than that of ATL cells were found in the lanes of tumor and PBMC DNA of case 5-mouse, indicating the clonal proliferation of the cells other than the original ATL cell clone.

As summarized in Table 4, we detected the elevation of serum-soluble human IL-2Ra chain (Tac) levels and histopathologic findings indicating the tumor growth in mice in 6 of 8 cases. Furthermore, we confirmed the proliferation of ATL cells in SCID mice by the detection of CD4⁺Tac⁺ cells by FACS analysis of tumor cells and the same integration site of HTLV-I provirus as that of the original leukemic cells by Southern blot hybridization analysis of tumor cell DNA in 4 of 8 cases.

DISCUSSION

In this report we have shown the model of in vivo cell proliferation of leukemic cells from ATL patients using SCID mice. In the early studies we either injected PBMC or LNC containing a high proportion of leukemic cells or put a small piece of an LN obtained from an ATL patient on an upper pole of kidney of the mouse. We often observed a tumor growth 2 to 3 months after the inoculation of cells. However, these tumors were found to contain predominantly human B cells but not T cells. We could not detect
IN VIVO CELL GROWTH MODEL OF ATL

Table 3. FACS Analysis of Tumor Cells From SCID Mice Engrafted With ATL Cells

<table>
<thead>
<tr>
<th>Case</th>
<th>Control</th>
<th>CD2</th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
<th>CD20</th>
<th>HLA-DR</th>
<th>α Chain</th>
<th>β Chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. F.S.</td>
<td>0.5</td>
<td>40</td>
<td>53</td>
<td>32</td>
<td>30</td>
<td>14</td>
<td>84</td>
<td>24</td>
<td>NE</td>
</tr>
<tr>
<td>2. Y.K.</td>
<td>0.7</td>
<td>54</td>
<td>53</td>
<td>45</td>
<td>19</td>
<td>3</td>
<td>66</td>
<td>59</td>
<td>76</td>
</tr>
<tr>
<td>3. F.Y.</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>4. K.Ya.</td>
<td>1.2</td>
<td>99</td>
<td>NE</td>
<td>85</td>
<td>54</td>
<td>0</td>
<td>99</td>
<td>66</td>
<td>NE</td>
</tr>
<tr>
<td>5. K.Y.</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>6. K.Yu.</td>
<td>0.6</td>
<td>96</td>
<td>95</td>
<td>94</td>
<td>2</td>
<td>0</td>
<td>95</td>
<td>90</td>
<td>NE</td>
</tr>
</tbody>
</table>

Abbreviation: NE, not examined.

the integration of HTLV-I provirus in tumor DNA by Southern blot analysis using pX of HTLV-I as a probe. In other words, human EBV-infected B cells showed the capability to preferentially grow in vivo as well as in vitro, as previously reported by others.24-33

Therefore, we then used three techniques to prevent B-cell growth and to accelerate T-cell growth in mice. First, the treatment of mice with TM-β1 MoAb markedly diminished the number and almost completely abrogated the function of natural killer (NK) cells,48 which might play a major role in the rejection of implanted tissues or cells in SCID mice.49-52 Several studies have reported that the elimination of NK cells using anti-asialo GM-1 MoAb enhanced the engraftment of human cells into immunodeficient mice.53-54 Taken together with the unsuccessful engraftment of ATL cells without TM-β1 MoAb in our early studies, it appears that the treatment with TM-β1 MoAb facilitates the engraftment of ATL cells, although it remains to be determined whether its use is requisite to the successful engraftment. Secondly, we intensively removed B cells and monocytes by the treatment of PBMC or LNC with a cocktail of MoAbs against human B cell, CD11b, and CD14 antigens followed by the absorption to the magnetic beads. The cell preparations injected into the mice still contained a very small number of human B cells. However, these residual B cells did not form a tumor in 3 to 4 weeks that was enough for the engraftment of ATL cells. The number of B cells may be too small to proliferate and form a tumor in vivo. Alternatively, the residual B cells that were treated with antibodies may be killed in vivo because of the opsonic effect or antibody dependent cell-mediated cytotoxicity-inducing effect by the antibodies. Thirdly, we performed daily injection of human recombinant IL-2 to facilitate or to support the growth of ATL cells in SCID mice. Most of the HTLV-I-infected T-cell lines were immortalized in vitro with the support of exogenous IL-2. However, our studies for the proliferative response of PB leukemic cells from ATL patients to IL-2 disclosed that leukemic cells from only 20% to 30% of patients examined proliferated in response to IL-2.7 Others reported exceptional ATL cases in which leukemic cells proliferated in an IL-2 autocrine manner.55 These controversial reports imply that the role of IL-2 and its receptor system in the neoplastic cell growth in ATL still remains obscure. Our results that the successful engraftment of ATL cells was observed in 4 of 8 ATL cases with the injection of human IL-2 do not necessarily indicate the requirement of

Fig 3. FACS analysis of tumor cells from SCID mice engrafted with ATL cells. Tumor cells from SCID mice engrafted with ATL cells from patients 1, 2, and 5 (cases 1-, 2-, and 5-mouse, respectively) were stained with FITC-conjugated anti-Tac MoAb and PE-conjugated antihuman CD4 MoAb and analyzed on FACScan.
IL-2 for the cell growth of ATL cells in SCID mice because we could not examine in vivo cell growth of ATL cells without human IL-2 because of the paucity of leukemic cells available for the study. In addition, HTLV-I-infected T-cell lines that are growing in an IL-2-dependent manner in vitro can proliferate in SCID mice without the supply of human IL-2 (Imada et al., unpublished, August 1993), indicating that the yet undetermined factors in vivo, instead of IL-2, could drive the IL-2-dependent cell line cells to continuously proliferate. Further studies will be needed to determine whether leukemic cells from ATL patients indeed require exogenous human IL-2 for their cell proliferation in SCID mice.

In this study, we used serum-soluble human IL-2Rα chain (Tac) levels as indicators of proliferation of ATL cells in vivo. Serum-soluble IL-2Rα chains are released from the activated T and B cells and neoplastic cells expressing IL-2Rα chains. Their levels in ATL patients are much higher (more than 10 to 100 times) than those in other diseases such as autoimmune diseases in which activated T cells contribute to the elevation of their levels. Therefore, it appears that the markedly elevated levels of serum-soluble human IL-2Rα chain detected in mice engrafted with ATL cells are mainly caused by ATL cells, although the contribution of activated HTLV-I uninfected T cells cannot be excluded. At any rate, the measurement of serum-soluble human IL-2Rα chain level was found to be useful to monitor the cell growth of ATL cells in SCID mice.

We examined the HTLV-I provirus integration site and the human TCR β chain gene rearrangement to confirm that the cells proliferating in vivo were derived from the leukemic cell clone, taking advantage of a random integration of HTLV-I provirus into cellular DNA. Southern blot hybridization analysis showed the in vivo cell growth of the same T-cell clone as the leukemic T-cell clone in 4 of 4 cases examined. However, it is interesting and noteworthy that human T cells other than leukemic cells were also proliferating in SCID mice injected with cells from patient 5 that was coincident with the detection of cells expressing human CD3 and CD8 by flow cytometric analysis. One of the likely interpretations of the oligoclonal cell proliferation in vivo is that human IL-2 administered to the mice supported the cell growth of HTLV-I-infected nonleukemic cells from ATL patients that probably expressed IL-2 receptor constitutively and were capable of responding to human IL-2. The events occurring in the selection of certain HTLV-I-infected T-cell clone(s) in SCID mice might reflect some key changes during the early stages of the development of ATL. Monoclonally expanded HTLV-I-infected T cells have been detected in a very small proportion of HTLV-I-infected individuals who did not show symptoms or signs re-
quired to make a diagnosis of ATL. The minority of them finally develop an overt ATL.99,66 The model using SCID mice may display such processes to us in a short period by condensing some stages of the natural course of leukemogenesis that is initiated by HTLV-I infection.

One of the characteristic clinical features of ATL is the infiltration of leukemic cells into a variety of organs including the spleen, liver, lungs, and skin. The mice engrafted with ATL cells showed a similar cell infiltration pattern although the pattern of the organ infiltration did not completely coincide with that of each patient. Factors determining the pattern of the organ infiltration by leukemic cells might include the interaction between cell surface molecules expressed on ATL cells and endothelial cells of the vessels of each organ. The model we present here would be useful for the studies to examine such factors.

One of the unexpected findings in this study is that leukemic cells from PB as well as LN could grow and form tumors or become leukemic in the mice. We considered that the LN but not PB of an ATL patient was one of the organs where leukemic cells were actively proliferating because the spontaneous 3H-thymidine incorporation by fresh PB leukemic cells were low in most of the ATL cases and cell-proliferation-associated molecules were more commonly expressed in LNC. The dissociation between the results of in vitro proliferation assay and those of in vivo tumorigenicity study in SCID mice not only indicate the importance of in vivo studies but also implies the complex mechanisms underlying the abnormal cell growth of ATL cells in vivo.

Finally, in vivo proliferation model of ATL cells would provide a good opportunity for us to assess the effectiveness and toxicity of the agents in vivo that is found to be potent in killing ATL cells in vitro and to develop a new strategy to overcome the disease.

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A model of in vivo cell proliferation of adult T-cell leukemia

A Kondo, K Imada, T Hattori, H Yamabe, T Tanaka, M Miyasaka, M Okuma and T Uchiyama