Jpn. J. Cancer Res. 86, 322–328, March 1995

# Detection of Homing, Proliferation, and Infiltration Sites of Adult T Cell Leukemia Cells in Severe Combined Immunodeficiency Mice Using Radiometric Techniques

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**To clarify the mechanism of *in vivo* proliferation of adult T cell leukemia (ATL) cells, we examined the organ distribution of ATL-43T cell line cells derived from original leukemic cells in severe combined immunodeficiency (SCID) mice using radiometric techniques. First, we injected $^{111}$In-oxine-labeled ATL-43T cells into SCID and CB17 mice. On day 6, significant accumulation of radioactivity was found in the spleen and thymus of SCID mice (33.3±9.4 and 10.0±3.6 % injected dose/g of tissue [%ID/g], respectively) in comparison with that in CB17 mice (19.1±2.5 and 3.7±0.9 %ID/g, respectively). Next, we injected radiolabeled anti-Tac monoclonal antibody (MoAb) recognizing human interleukin-2 receptor (IL-2R) α chain or isotype-matched control MoAb RPC5 in SCID mice bearing ATL-43T cells 4 weeks after cell inoculation. The amounts of radioactivity found in the spleen and thymus of SCID mice injected with $^{125}$I-labeled anti-Tac MoAb (22.5±6.9 and 22.8±9.6 %ID/g, respectively) were significantly higher than those in the corresponding organs of SCID mice injected with $^{125}$I-labeled RPC5 MoAb (12.0±5.1 and 7.5±4.6 %ID/g, respectively). Similar results were obtained with $^{111}$In-labeled anti-Tac MoAb. These results were consistent with the histological findings of SCID mice bearing ATL-43T cells, indicating that ATL-43T cells infiltrated preferentially into the lymphoid organs, such as the spleen and thymus, and proliferated there. Thus, the radiometric techniques employed in this study were very useful to evaluate the proliferation sites of ATL-43T cells in SCID mice. Furthermore, this murine model could give us an opportunity to test the feasibility of therapeutic application of radiolabeled anti-Tac MoAb.**

Key words: Adult T cell leukemia — Human T cell leukemia virus type I — Severe combined immunodeficiency mice — Anti-Tac monoclonal antibody — Radioimmunoscintigraphy

It has been established that human T cell leukemia virus type I (HTLV-I) is an etiologic agent of adult T cell leukemia (ATL)[1,2] and much information regarding its genetic structure and function has been accumulated,[3-5] although the precise mechanisms of the development of ATL and the neoplastic cell growth still remain unclear. One of the characteristic clinical manifestations of ATL is leukemic cell infiltration into various organs including the lymph nodes, spleen, liver, lungs, and skin, depending on the clinical type of ATL. However, we still do not have evidence directly indicating where and how the leukemic cells proliferate in the body of ATL patients. The molecular basis of the preferential infiltration of ATL cells into organs is also of great interest.[6] Recently we have developed an *in vivo* cell proliferation model of fresh leukemic cells from ATL patients[7] and some HTLV-I-infected T cell lines (K. Imada *et al.*, manuscript submitted) using severe combined immunodeficiency (SCID) mice in order to obtain clues to the above questions and to get deeper insights into the mechanism of the neoplastic cell growth of ATL. Histopathological examination and flow-cytometric analysis of the cells proliferating in SCID mice have demonstrated cell infiltration into various organs similar to that seen in ATL patients. One of the HTLV-I-infected T cell line derived from an ATL leukemic cell clone, termed ATL-43T, was found to proliferate and infiltrate into various organs in SCID mice. In an attempt to analyze and determine the target organs and the sites of the cell proliferation of ATL-43T cells more precisely and to trace the time course, we have employed the radiometric techniques using radiolabeled ATL-43T cells and radiolabeled anti-Tac monoclonal antibody (MoAb), which binds to human interleukin 2 receptor (IL-2R) α chain and selectively recognizes ATL-43T cells in SCID mice.

## MATERIALS AND METHODS

**Cells** ATL-43T is an HTLV-I-infected T cell line which was established by Dr. M. Maeda (Chest Disease Institute, Kyoto University) from an ATL patient who showed leukocytosis and gastrointestinal involvement at

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hospitalization.[8] The autopsy revealed the infiltration of ATL cells into various other organs including the lungs, liver and spleen. This cell line has the same phenotype (CD2+, CD3+, CD4−, CD8−, Tac+) and the same integration site of HTLV-I provirus as those of the original leukemic cells.

**Mice** SCID (CB17 scid/scid) mice[9] and their strain of origin, CB17 mice, were obtained from Nihon Clea Inc. (Tokyo). The mice were bred and maintained under specific pathogen-free conditions in the animal facility of the Institute for Virus Research, Kyoto University.

**Radiolabeling of MoAbs** Anti-Tac MoAb recognizes human IL-2R $\alpha$ chain, which is expressed on activated T and B cells,[10–12] and also constitutively expressed on ATL cells.[13, 14] RPC5 (Organon Teknika Co., West Chester, PA) is a myeloma protein which was used as an isotype-matched control (IgG2a). MoAbs were radioiodinated using the chloramine-T method as previously described.[15] Briefly, purified MoAb (40 $\mu$g) in 0.3 *M* phosphate buffer, pH 7.5, and $Na^{125}I$ (11.1 MBq) (Dupont, Wilmington, DE) were mixed with 2.5 $\mu$g of chloramine-T (Nacalai Tesque, Kyoto) dissolved in the same buffer. After 5 min of reaction, radiolabeled MoAb was separated from free iodine through PD-10 gel chromatography (Pharmacia LKB Biotechnology, Uppsala, Sweden). The specific activity of $^{125}I$-labeled anti-Tac MoAb was 289 MBq (7.8 mCi)/mg.

MoAb was also labeled with $^{111}In$ using diethylenetriaminepentaacetic acid (DTPA) as a bifunctional chelating agent as previously described.[16] MoAb solution (2 mg/ml) in 0.1 *M* $NaHCO_3$ was mixed with DTPA cyclic anhydride (Dojindo Laboratories, Kumamoto) at a DTPA:MoAb molar ratio of 3 at room temperature for 1 h, and unconjugated DTPA was separated through a PD-10 column using 0.2 *M* citrate buffer as the eluent. DTPA-conjugated MoAb was mixed with $^{111}In$-chloride (Nihon Mediphysics, Takarazuka) at room temperature for 1 h, and radiolabeled MoAb was separated from free $^{111}In$ through a PD-10 column. The specific activity of $^{111}In$-labeled anti-Tac MoAb was about 55.5 MBq (1.5 mCi)/mg.

**Antibody binding assay** Antibody binding assay was performed as previously described.[17] $^{125}I$- and $^{111}In$-labeled anti-Tac MoAb (2 ng/100 $\mu$l) was incubated with increasing concentrations of ATL-43T cells ($5\times10^4$ to $1\times10^7$ cells) in microcentrifuge tubes at 4°C for 1 h. After centrifugation at 10,000*g*, the supernatant was aspirated and the tubes were cut. The radioactivity bound to cells was counted in an auto-well gamma counter.

To calculate the binding affinity constants from Scatchard plot analysis,[18] increasing amounts of $^{125}I$-labeled anti-Tac MoAb were incubated with $5\times10^5$ ATL-43T cells at 4°C for 30 min, and the radioactivity bound to cells was counted. Nonspecific binding was estimated by incubating cells with both radiolabeled anti-Tac MoAb and more than 250-fold excess of unlabeled anti-Tac MoAb.

**Radiolabeling of ATL-43T cells** $^{111}In$-8-Hydroxyquinoline (oxine) was prepared as previously described.[19] Briefly, 74 MBq/1 ml of $^{111}In$ chloride, 1 ml of distilled water, 0.3 *M* acetate buffer, pH 5.0, and 0.02 ml of oxine (Sigma, St. Louis, MO)(4 mg/ml dissolved in ethanol) were mixed and incubated at room temperature for 15 min. The $^{111}In$-oxine complex was extracted with 2.42 ml of chloroform (Nacalai Tesque) and after evaporation of the chloroform, $^{111}In$-oxine deposited on the tube wall was dissolved with 50 $\mu$l of ethanol and diluted with 200 $\mu$l of saline.

Appropriate numbers of ATL-43T cells suspended in 2 ml of RPMI1640 were incubated with 1.37 MBq of $^{111}In$-oxine at 37°C for 30 min. These cells were washed once and resuspended in the medium for the injection into SCID mice.

**Biodistribution study** $^{111}In$-Oxine-labeled ATL-43T cells ($2.9\times10^7$ cells) were injected intraperitoneally into SCID and CB17 mice. CB17 mice used as control mice were not engrafted with ATL-43T cells. At 2 days and 6 days after the inoculation of cells, the mice were killed, their organs were removed and weighed, and the radioactivities were counted. The results were expressed as the percentage of injected dose per gram of tissue (%ID/g).

ATL-43T cells ($7.7\times10^7$ cells) were inoculated intraperitoneally into SCID mice for the study using radiolabeled anti-Tac MoAb. Potassium iodide solution was administered to mice 1 day before the injection of radioiodinated MoAbs to inhibit the uptake of released radioiodine into the thyroid. We injected 37 kBq of radiolabeled anti-Tac and RPC5 MoAbs into SCID mice bearing ATL-43T cells through the tail vein 4 weeks after the inoculation of cells. We also injected radiolabeled anti-Tac MoAb into other groups of SCID mice before, and 2, 3, and 4 weeks after the inoculation of cells. The dose of MoAb was adjusted to 10 $\mu$g per mouse by the addition of the corresponding unlabeled MoAb. Each mouse was killed at 48 h after the injection of radiolabeled MoAbs, its organs were removed and weighed, and the radioactivities were counted, because our previous observation (M. Hosono *et al.*, unpublished data) showed that the specific accumulation of radiolabeled anti-Tac MoAb was highest at this time. The results of radiolabeled MoAbs biodistribution were expressed as the percentage of injected dose per gram of tissue (%ID/g).

**Statistical analysis** Analysis of variance was used to determine the statistical significance of differences. The criterion of significance was $P<0.05$. All values are reported as mean±standard deviation (SD).

**Serial histological study of SCID mice bearing ATL-43T cells** We also injected $7.7–10\times10^7$ ATL-43T cells into

SCID mice intraperitoneally for the histological study. Mice were killed 2, 3, and 4 weeks after the inoculation of cells, and the tumors and other tissues of interest were fixed in 10% formalin, processed to 4-$\mu$m paraffin wax-embedded sections and stained with hematoxylin and eosin.

## RESULTS

***In vitro* reactivity of anti-Tac MoAb** The bindable fraction of both $^{125}$I- and $^{111}$In-labeled anti-Tac MoAb was more than 70% when determined by measuring the binding of radiolabeled anti-Tac MoAb to an excess number of cells. Scatchard plot analysis revealed that the association constant of the MoAb to ATL-43T cells was $6.6\times10^{9}\ M^{-1}$ (data not shown).

**Biodistribution of $^{111}$In-oxine-labeled ATL-43T cells in SCID and CB17 mice** First, we studied the homing and proliferation of injected cells in SCID mice within a week after the inoculation of cells. We injected $^{111}$In-oxine-labeled ATL-43T cells into SCID or CB17 mice intraperitoneally and examined the radioactivity of their organs 2 and 6 days after the inoculation of cells. On day 2, we could not detect significant accumulation of radioactivity in any organ of SCID mice ($P<0.05$), although slight elevation of radioactivity in the spleen and thymus of SCID mice was detectable (Fig. la). On day 6, significant accumulation of radioactivity was detectable in the spleen and thymus of SCID mice ($33.3\pm9.4$ and $10.0\pm3.6$ %ID/g, respectively) as compared with that in the corresponding organs of CB17 mice ($19.1\pm2.5$ and $3.7\pm0.9$ %ID/g, respectively) ($P=0.012$ and 0.005, respectively) (Fig. 1b). There was no detectable difference between the radioactivity of other organs of SCID mice and that of control CB17 mice ($P<0.05$).

**Biodistribution of $^{125}$I- and $^{111}$In-labeled anti-Tac MoAb in SCID mice bearing ATL-43T cells** Second, we studied the proliferation and infiltration of cells in SCID mice by

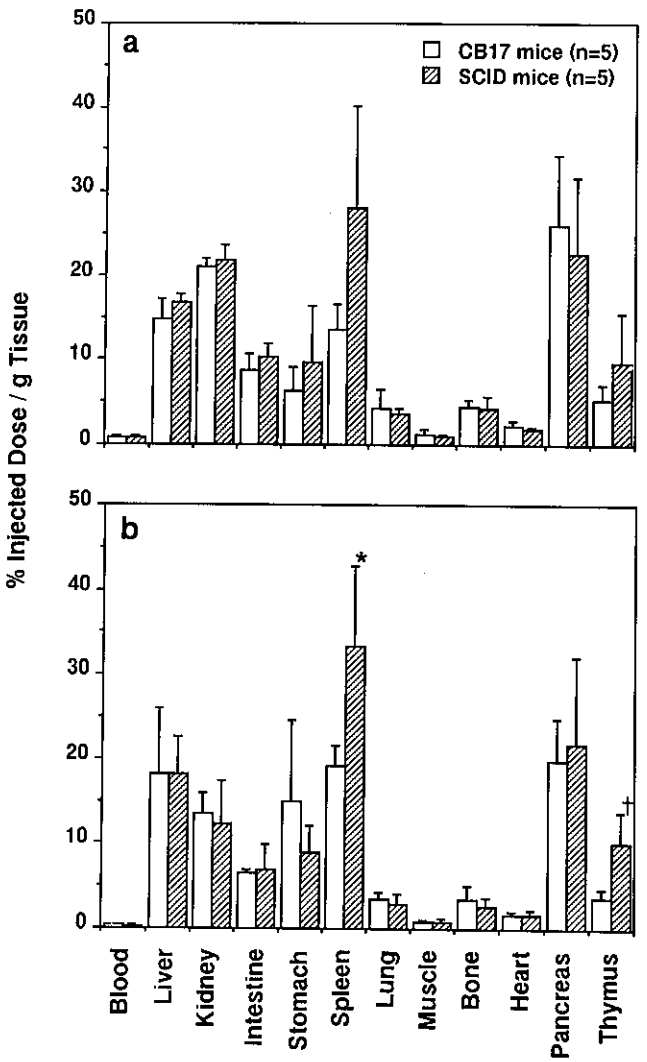


Fig. 1. Biodistribution of $^{111}$In-oxine-labeled ATL-43T cells in CB17 and SCID mice 2 (a) and 6 days (b) after the inoculation of cells. On day 6, significant accumulation was found in the spleen and thymus of SCID mice as compared with that of CB17 mice ($*$ $P=0.012$ and $\dagger$ $P=0.005$).

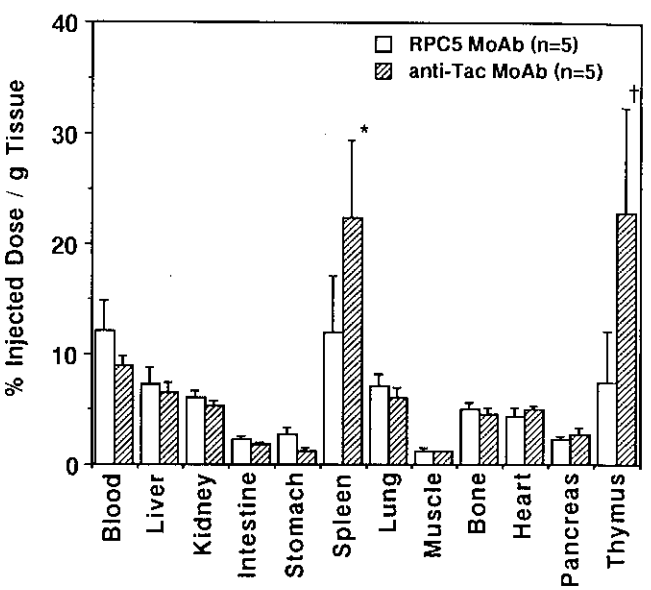


Fig. 2. Biodistribution of $^{125}$I-labeled anti-Tac and RPC5 MoAbs in SCID mice bearing ATL-43T cells 4 weeks after the inoculation of cells. Significantly higher amounts of $^{125}$I-labeled anti-Tac MoAb were found in the spleen and thymus of SCID mice, as compared with those of $^{125}$I-labeled RPC5 MoAb in the corresponding organs ($*$ $P=0.026$ and $\dagger$ $P=0.012$).

the intravenous injection of $^{125}$I- and $^{111}$In-labeled anti-Tac and RPC5 MoAbs into SCID mice bearing ATL-43T cells 4 weeks after the inoculation of cells. Significantly larger amounts of $^{125}$I-labeled anti-Tac MoAb were found in the spleen and thymus of SCID mice (22.5±6.9 and 22.8±9.6 %ID/g, respectively) as compared with those of $^{125}$I-labeled RPC5 in the corresponding organs (12.0±5.1 and 7.5±4.6 %ID/g, respectively) ($P$=0.026 and 0.012, respectively) (Fig. 2). $^{111}$In-Labeled anti-Tac MoAb was also significantly accumulated in the spleen and thymus of SCID mice (30.5±7.0 and 16.9±2.3 %ID/g, respectively) as compared with $^{111}$In-labeled RPC5 in the corresponding organs (12.4±4.2 and 5.3±3.0 %ID/g, respectively) ($P$=0.001 and 0.0001, respectively) (Fig. 3).

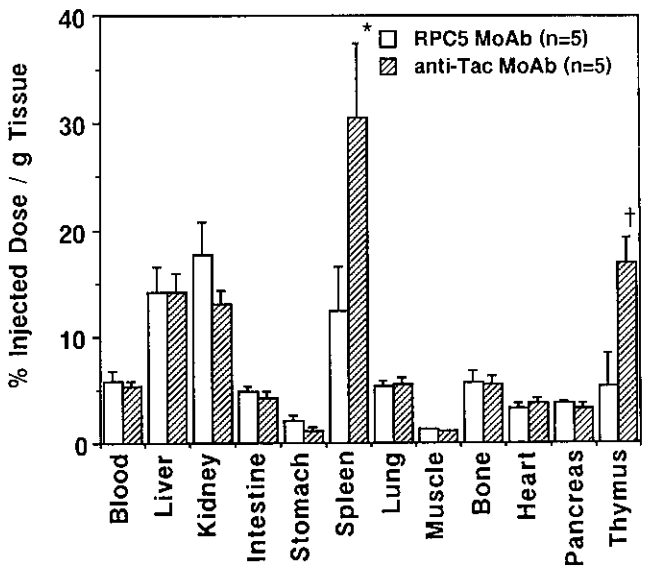


Fig. 3. Biodistribution of $^{111}$In-labeled anti-Tac and RPC5 MoAbs in SCID mice bearing ATL-43T cells 4 weeks after the inoculation of cells. Significantly higher amounts of $^{111}$In-labeled anti-Tac MoAb were found in the spleen and thymus of SCID mice, as compared with those of $^{111}$In-labeled RPC5 MoAb in the corresponding organs (* $P$=0.001 and † $P$=0.0001).

Furthermore, we injected $^{125}$I- and $^{111}$In-labeled anti-Tac MoAb into SCID mice bearing ATL-43T cells before, and 2, 3, and 4 weeks after the inoculation of cells in order to estimate the changes in their biodistribution. We observed the accumulation of $^{125}$I- and $^{111}$In-labeled anti-Tac in the spleen and thymus in a time-dependent manner, suggesting the preferential proliferation of ATL-43T cells in these organs. We detected significant accumulation of both $^{125}$I- and $^{111}$In-labeled anti-Tac MoAb in the thymus 4 weeks after the inoculation of cells. On the other hand, in the spleen, significant accumulation of $^{125}$I-labeled anti-Tac MoAb was detectable as early as 2 weeks after the inoculation of cells. However, significant accumulation of $^{111}$In-labeled anti-Tac MoAb was not detected throughout the entire course of the study (Tables I and II).

**Serial histological examinations of SCID mice engrafted with ATL-43T cells** Serial histological examinations of SCID mice inoculated with ATL-43T cells revealed infiltration of the cells into the thymus 4 weeks after the inoculation. However, histological changes indicating the infiltration of ATL-43T cells into other organs, except for paraaortic lymph nodes, could not be demonstrated before about 6 weeks (Table III).

## DISCUSSION

In the present study, using radiometric techniques and serial histological examinations, we examined the sites of

Table I. Biodistribution of $^{125}$I-Labeled Anti-Tac MoAb in SCID Mice Bearing ATL-43T Cells

| | % Injected dose/g (mean±SD, n=5) at time postinjection | | | |
|---|---|---|---|---|
| | Before | 2 wk | 3 wk | 4 wk |
| Blood | 13.35±2.50 | 7.52±0.90 | 7.24±0.91 | 8.87±0.92 |
| Liver | 4.83±0.71 | 4.79±0.37 | 4.92±0.53 | 6.49±0.90 |
| Kidney | 5.3±0.66 | 3.82±0.32 | 3.59±0.37 | 5.23±0.48 |
| Intestine | 2.16±0.28 | 1.65±0.16 | 1.49±0.25 | 1.78±0.19 |
| Stomach | 1.61±0.24 | 1.73±0.13 | 1.16±0.14 | 1.24±0.25 |
| Spleen | 7.32±2.54 | 17.9±2.25[a)] | 15.77±1.70[a)] | 22.45±6.93[a)] |
| Lung | 7.73±0.98 | 4.45±1.18 | 4.62±0.87 | 6.05±0.86 |
| Muscle | 1.41±0.23 | 0.69±0.09 | 0.77±0.13 | 1.18±0.10 |
| Bone | 5.09±1.57 | 2.26±0.52 | 2.6±0.44 | 4.52±0.64 |
| Heart | 5.19±0.83 | 3.42±0.29 | 3.34±0.37 | 4.94±0.42 |
| Pancreas | 2.42±0.25 | 1.79±0.25 | 1.57±0.37 | 2.71±0.55 |
| Thymus | 3.41±1.06 | 7.23±3.30 | 7.32±2.51 | 22.83±9.56[a)] |

*a*) Significant difference ($P$<0.05) from the values of mice before the inoculation of cells.

Table II. Biodistribution of $^{111}$In-Labeled Anti-Tac MoAb in SCID Mice Bearing ATL-43T Cells

| | % Injected dose/g (mean±SD, n=5) at time postinjection | | | |
|---|---|---|---|---|
| | Before | 2 wk | 3 wk | 4 wk |
| Blood | 6.78±1.08 | 5.27±0.61 | 5.52±0.78 | 5.28±0.47 |
| Liver | 10.76±1.63 | 14.29±1.85 | 14.44±1.68 | 14.16±1.73 |
| Kidney | 16.12±2.28 | 13.65±0.72 | 15.76±1.74 | 12.98±1.28 |
| Intestine | 3.74±0.19 | 4.11±0.41 | 3.52±0.44 | 4.24±0.56 |
| Stomach | 1.23±0.15 | 2.21±0.51 | 1.09±0.12 | 1.16±0.23 |
| Spleen | 23.23±6.32 | 31.69±2.79 | 29.38±2.94 | 30.49±7.00 |
| Lung | 5.86±1.00 | 5.07±1.07 | 5.3±0.90 | 5.51±0.62 |
| Muscle | 1.12±0.15 | 0.96±0.12 | 1.07±0.16 | 1.08±0.10 |
| Bone | 6.59±2.23 | 5.35±1.46 | 5.26±0.44 | 5.48±0.83 |
| Heart | 3.29±0.48 | 3.35±0.31 | 3.61±0.28 | 3.69±0.41 |
| Pancreas | 2.68±0.21 | 3.17±0.69 | 2.93±0.48 | 3.28±0.43 |
| Thymus | 7.32±2.35 | 8.43±3.45 | 11.19±5.67 | 16.94±2.34[a] |

*a*) Significant difference ($P<0.05$) from the values of mice before the inoculation of cells.

Table III. Serial Histological Findings of SCID Mice Inoculated with ATL-43T Cells

| | Degree of cell infiltration at time postinjection[a] | | | |
|---|---|---|---|---|
| | 2 wk | 3 wk | 4 wk | at autopsy (about 6 wk) |
| Spleen | − | − | − | ++ |
| Thymus | − | ± | + | ++ |
| Lung | − | − | − | + |
| Lymphnode | − | − | +[b] | + |

*a*) −, negative; ±, questionable; +, moderate; ++, marked.
*b*) Paraaortic lymphnode involvement was detectable.

homing and proliferation of an HTLV-I-infected T cell line, ATL-43T, at various times and demonstrated that ATL-43T proliferated preferentially in the lymphoid organs of SCID mice, such as the spleen and thymus. ATL patients present characteristic clinical features including lymphadenopathy, hepatosplenomegaly, skin and pulmonary involvement.[1,2] However, it has been unclear where and how ATL cells proliferate *in vivo*, although the high expression of T cell activation-associated antigens on ATL cells in lymph nodes suggested that ATL cells preferentially proliferated in the lymph nodes, as previously reported.[20] Clinical manifestations also suggested that the skin and lung are candidate sites of the proliferation of ATL cells.[21,22] In this study, we have tried to demonstrate directly the preferential proliferation sites of ATL cells *in vivo* using a murine model that we have recently established. This murine model for proliferation of ATL cells has enabled us to survey the whole body periodically from the cell inoculation to the death of the mouse and to trace the leukemic cells *in vivo* more specifically with the use of radiolabeled cells or radiolabeled anti-Tac MoAb reacting with ATL-43T cells.

We employed two kinds of radiometric techniques in this study. One utilized $^{111}$In-oxine-labeled ATL-43T cells, which revealed the distribution of ATL-43T cells in SCID mice within a week after the inoculation of cells. But we could not follow the distribution of $^{111}$In-oxine-labeled ATL-43T cells beyond several weeks because of the short half life of $^{111}$In. We, therefore, employed another method using $^{125}$I- and $^{111}$In-labeled anti-Tac MoAb, to follow the late-phase distribution of ATL-43T cells by reaction of the MoAb with human IL-2R $\alpha$ chain on the cell surface after injection into SCID mice. In addition, this method enabled us to evaluate roughly the change in the total amount of ATL-43T cells in SCID mice during the course of the study. The reason why we used two different kinds of radionuclides for labeling MoAbs was that MoAbs labeled with different radionuclides might show drastically different biodistributions.[23] Previous studies have reported that $^{111}$In-labeled MoAb is superior to radioiodinated MoAb in the immunodetection of the lesion of T cell lymphoma, because $^{111}$In is retained within lymphoma cells even when $^{111}$In-labeled MoAb becomes internalized, which results in the prolonged retention of $^{111}$In in the whole body and excellent uptake in the lesion, including the lymph nodes and skin.[24] However, the ability of the liver and spleen to accumulate $^{111}$In was a distinct disadvantage, and may have been the reason why we could not detect significant accumulation of $^{111}$In-labeled anti-Tac MoAb in the spleen.

Serial histological studies also suggested that ATL-43T cells preferentially proliferated in the thymus of SCID mice. As we have previously reported, ATL-43T cells showed infiltration into various organs including the spleen, thymus, mesenteric lymph nodes, and lungs in SCID mice at autopsy, which was very similar to that seen in ATL patients. But the radiometric techniques

seemed to be superior to the histological examinations because they were more sensitive in detecting the lesions and could evaluate the quantitative change of the amount of ATL-43T cells proliferating in SCID mice. Indeed, we could not demonstrate histological changes indicating cell infiltration into any organ other than the thymus before autopsy. Taking these data together with the radiometric analysis data, it appears that the spleen and thymus are the primary sites of the cell proliferation *in vivo* and that the lungs may be the infiltrated organs.

Finally, we would like to emphasize that the use of radiolabeled anti-Tac MoAb in the present study to trace ATL-43T cells *in vivo* afforded results in agreement with those obtained using radiolabeled ATL-43T cells and serial histological examinations, and was found to have potential for studying the targeting of ATL lesions. Many attempts have been made to develop a new, effective treatment for ATL, which still has a very poor prognosis. One promising approach is an IL-2 receptor-targeted therapy taking advantage of the constitutive expression of IL-2R $\alpha$ chain on ATL cells as compared with no or minimal expression on unstimulated normal T cells. It has been reported that anti-Tac MoAb alone or conjugated with toxins or radionuclides, or IL-2-toxin fusion protein could selectively and effectively kill HTLV-I-infected T cell lines *in vitro*.[25–27] Waldmann *et al.* reported that the administration of anti-Tac MoAb was effective in 7 of 19 ATL patients.[28] They further extended their studies by utilizing $^{90}$Y-labeled anti-Tac MoAb for clinical trials and obtained promising results.[29] Our model for *in vivo* proliferation of ATL cells could allow us to test the effect of radiolabeled anti-Tac MoAb and provide a solid experimental basis for its clinical application.

ACKNOWLEDGMENTS

We are very grateful to Dr. M. Maeda (Chest Disease Institute, Kyoto University) for providing ATL-43T. This study was supported in part by grants from the Ministry of Education, Science and Culture, Japan.

*(Received September 19, 1994/Accepted December 28, 1994)*

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