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Successful Graft of HTLV-I-transformed Human T-Cells (MT-2) in Severe Combined Immunodeficiency Mice Treated with Anti-asialo GM-1 Antibody

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To develop an experimental model of adult T-cell leukemia/lymphoma in small animals, severe combined immunodeficiency (SCID) mice treated with anti-asialo GM-1 antibody were inoculated with MT-2 cells, a cell line transformed by the human T-cell leukemia virus (HTLV-I). Three mice injected with 4×10^7 cells subcutaneously or intramuscularly developed tumors at or near inoculation sites. Immunofluorescent antibody (IFA) staining for HTLV-I structural protein, p19, revealed the specific antigen in the cytoplasm of most cells from tumors and the DNA signals of HTLV-I proviral DNA were also positive in cellular DNA by polymerase chain reaction assay with HTLV-I *tax* gene primers, SK43/SK44. The MT-2 cells did not invade in mouse organs.

Key words: SCID mouse — HTLV-I — MT-2 — Asialo GM-1

The C.B-17 severe combined immune deficient (SCID) strain of mice, which lack functional B and T lymphocytes, was first described in 1983 by Bosma *et al.*[1] Because these mice tolerate xenografts, they have been established as a murine model for human tumor growth *in vivo.*[2-4] In addition, they have been used as a model to study lymphocyte function and differentiation at the cellular and molecular level.[5-8]

Adult T-cell leukemia/lymphoma (ATL) cells have not been reported to grow in this strain, except for the observation of growth of lymph node tissue fragments implanted into the subcapsular space of the kidney.[9] SCID-hu mice, in which human fetal liver, thymus, and lymph node cells engraft,[10] can be used for the analysis of human hemato-lymphoid differentiation and function. Such animals have been infected with HIV-I and used as a model of the acute infection phase of AIDS.[11,12] We are interested in application of this system to human T-cell leukemia virus (HTLV-I) infection; however, the use of fetal organs is not permissible in this country.

The effect of the mutation in these mice on chromosome 16[13] is that the genes encoding antigen-specific receptors on B and T cells do not rearrange,[14] as they normally would during differentiation. However, NK cells are functional in SCID mice as demonstrated by the NK activity of spleen cells against YAC lymphoma target cells.[15] This activity may be the major reason why ATL cells can not be grafted in SCID mice.

The treatment of mice with anti-asialo GM-1 antibody is a method used to eliminate NK cell function.[16] In this paper we report that anti-asialo GM-1-treated SCID mice can support the growth of an HTLV-I-transformed human T-cell line (MT-2),[17] as a potential *in vivo* model for ATL.

C.B-17 male mice, homozygous for the SCID gene, 7–10 weeks old, were obtained from Nihon Clea Inc., Tokyo. The mice were kept on trimethoprim and sulfamethoxazole (Bactor) after purchase and aseptic procedures were used routinely.

A total of 6 mice were injected either intraperitoneally, intramuscularly or subcutaneously with MT-2 cells. The animals received $4\text{–}5\times10^7$ cells, which were cultured in RPMI-1640 supplemented with 15% fetal bovine serum in 5% CO_2 in air at 37°C and were in the log phase of growth when injected. Each of the six animals received an intraperitoneal aliquot of 50 μl (500 μg) of anti-asialo GM-1 rabbit gamma-globulin (Wako Pure Chemical Industries, Ltd., Tokyo) daily from three days before until five days after the cell injection, and twice a week thereafter. Three animals, 35 weeks old, were injected with 1×10^7 cells without anti-asialo GM-1 antibody treatment as the control.

Touch smears of the tumors found at autopsy of mice that died were prepared on slides and fixed in acetone at room temperature for 10 min for indirect immunofluorescent antibody staining (IFA). Sections of tumors were also prepared in 10% formalin, and examined histologically following hematoxylin-eosin (HE) staining. Tumor fragments were also stored at −70°C.

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Table I. Size of MT-2 Cell Tumor in Anti-asialo GM-1-treated SCID Mice

Group	Injection route[a]	Number of injected cells	Number of mice	Observation period (days)	Tumor size (mm)	Remarks
1	i.p.	5×10^7	2	5	ND[c]	died
				7	ND	died
2	i.m.	4×10^7	2	7	ND	died
				33	$4\times4\times4$	palsy[d]
3	s.c.	4×10^7	2	61	$6\times4\times4$	palsy
				61	$15\times10\times7$	skin ulcer[e]
4[b]	i.p.	1×10^7	3	245	ND	none
				252	ND	none
				56	ND	none

a) "i.p." represents intraperitoneal, "i.m." intramuscular, and "s.c." subcutaneous injection.
b) No anti-asialo GM-1 antibody treatment.
c) Not detected.
d) Unilateral palsy of the hind leg on the injection side.
e) Ulcer formation of the skin on the tumor.

The IFA was performed by incubation with anti-HTLV-I p19 monoclonal antibody (Cellular Products Inc., USA) at 1:500 dilution in phosphate-buffered saline followed by staining with FITC anti-mouse IgG antibody (TAGO Inc., USA).

Chromosomal DNA was isolated from the frozen tumor fragments by sodium dodecyl sulfate-proteinase K digestions at 56°C and then by phenol-chloroform extractions followed by ethanol precipitation. One μg of the DNA was subjected to polymerase chain reaction (PCR) assays[18] using the HTLV-I *tax* gene primers, SK43/SK44. HUT 102 cells and Molt-4 cells were used as positive and negative controls, respectively.

Table I summarizes the results of the experiments. Two mice injected intraperitoneally with 5×10^7 MT-2 cells died on the 5th or 7th day of injection, respectively. No definite tumor mass was found at autopsy. One of two animals injected intramuscularly with 4×10^7 cells developed a tumor mass. The mass was located in the peritoneal cavity adjacent to the inoculation site and was $4\times4\times4$ mm in size. The animal showed paresis of the left hind leg and ascites 23 days after injection. The other of this group died on the 7th day by accident. The two animals injected subcutaneously with 4×10^7 cells both developed tumor masses at the injection site. One had a mass of $15\times10\times7$ mm and had an ulcer at the site of the tumor. All surviving mice initially lost weight for two to three weeks after the cell injection. None of the tumor masses invaded mouse organs. No tumor masses have been observed in the three control SCID mice.

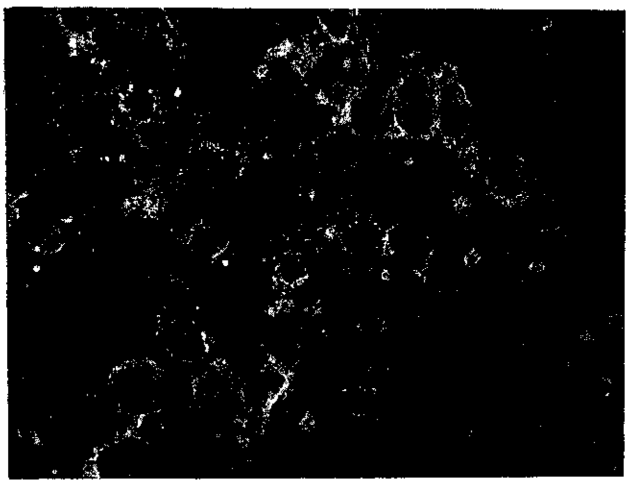

Fig. 1. Tumor cells on touch smear stained by the IFA method with mouse monoclonal antibody for HTLV-I structural protein p19. (Magnification is 1:400).

Cells on the touch smears from all three tumors were positive for HTLV-I *gag* protein, p19, in their cytoplasm by the IFA method (Fig. 1). Histological examination of the HE-stained sections showed a diffuse distribution of cells of various sizes (Fig. 2). HTLV-I proviral DNA signals were detected in DNA samples from the tumors by the PCR method (Fig. 3).

Pathophysiological and immunological studies of ATL have been limited by the lack of small animal models,

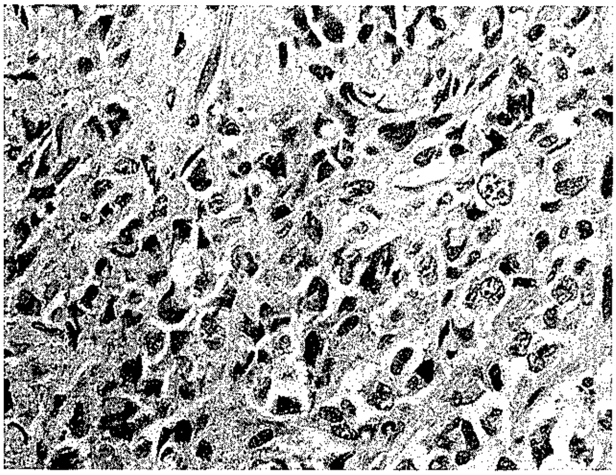

Fig. 2. Histology of the tumor. HE-stained section 5 μm thick. (Magnification is 1:400).

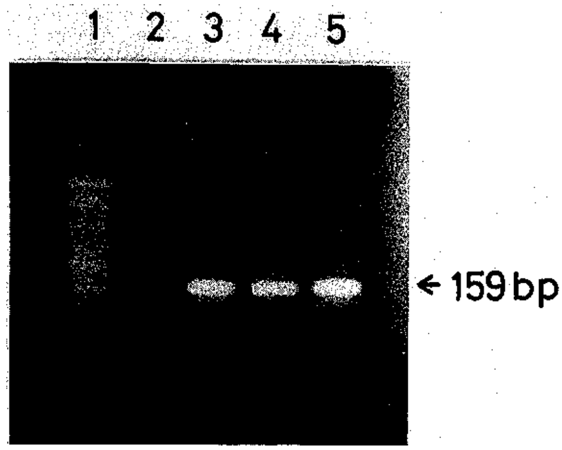

Fig. 3. Agarose gel electrophoresis of PCR products of DNA samples from the tumors in SCID mice. Molecular weight markers (lane 1), no DNA (lane 2), DNA from tumor of SCID mouse inoculated intramuscularly or subcutaneously with MT-2 cells (lanes 3 and 4), and HUT 102 DNA as the positive control (lane 5).

other than rabbits[19] or rats.[20] Several characteristics of the SCID mouse make it attractive as a model to study human cancer and to evaluate new therapeutic approaches.[8] Engraftment of ATL cells has not been successful in the murine system, probably due to the presence of NK activity[15] and the sensitivity of the tumor cell. Continuing treatment of SCID mice with anti-asialo GM-1 antiserum has promoted human thymus engraftment.[21]

In this study, MT-2 cells grew in animals treated with anti-asialo GM-1 antibody, when observed for 33 days or longer. Twice the dose of the antibody was administered more frequently than that reported[21] in order to abrogate NK activity completely.

The use of MT-2 cells, a representative T-cell line derived from human cord blood lymphocytes by co-cultivation with ATL cell lines and 100% positive for ATLA,[17] facilitated the identification of tumor cell growth in the mouse organs by staining for HTLV-I antigen. The tumor cells were also positive by PCR assay for HTLV-I gene.[18]

No graft survived in the untreated control SCID mice in the present study. Irradiation of SCID mice before engraftment did not improve the survival of human T-cells (data not shown).

Our demonstration of the growth of an HTLV-I-transformed T-cell line in the SCID mouse suggests that this model can be used to study the pathophysiology of ATL cells and to evaluate the effect of treatment *in vivo*, which cannot be achieved by using other HTLV-I-infected animal models.

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