Engraftment of HTLV-I-Transformed Human T-Cell Line into SCID Mice with NK Cell Function

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ABSTRACT. HTLV-I-transformed human T-cell line, MT-2, has been reported to successfully proliferate in only the SCID mice with depletion of NK cell function. However, MT-2 cells could be engrafted into SCID mice possessing normal NK cell function in this study. MT-2 cells ($5-7 \times 10^7$ cells) were intraperitoneally injected into SCID mice without the deletion of NK cell function. The SCID mice developed tumors in the peritoneal cavities 3 months after the inoculation of MT-2 cells. All tumors reacted to anti-HTLV-I p19 and anti-HLA-DR monoclonal antibodies by immunofluorescence assay and were also positive for the HTLV-I gene by PCR assay. DNA obtained from main organs in group of mice with or without tumors showed a high incidence of positive signals for HTLV-I gene by PCR assay.—**KEY WORDS:** HTLV-I, PCR, SCID mouse.

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Human T-cell leukemia virus type I (HTLV-I), the firstly isolated human retrovirus, is a causative agent that induces adult T cell leukemia/lymphoma (ATL), HTLV-I associated myelopathy (HAM) and other HTLV-I associated disorders [3, 9]. To study HTLV-I-induced pathogenesis or the therapeutic agent for HTLV-I associated diseases, it is necessary to establish an animal model. Recently, *in vivo* proliferation model of HTLV-I-transformed human T-cells (MT-2 cell) or leukemic cells from ATL patients in severe combined immunodeficiency (SCID) mice have been reported [2, 4, 5]. These reports have described the growth of MT-2 or ATL cells in the SCID mice require the depletion of NK cell function.

In this study, MT-2 cells could multiply in the SCID mice without the elimination of NK cells by increasing the number of injected MT-2 cells compared with previous reports [2, 4].

Breeding pairs of C.B17-scid/+ and C.B17 mice were kindly provided by Dr. M. J. Bosma, Fox Chase Cancer Center, Philadelphia, U.S.A.; since then homozygotes (scid/scid) have been maintained by brother x sister mating by T. Nomura in his laboratory under the germ-free conditions. Male and female C.B17-scid/scid (SCID) mice which showed undetectable levels of serum IgG and IgM ($<1 \mu\text{g/ml}$) at 12–20 weeks old, were used in this study. Details of the inbreeding of C.B17-scid/scid mice have been reported previously [8].

A total of 9 mice were divided into two groups. The treatment of mice with anti-asialo GM1 antibody was used to eliminate NK cell function [4]. In group 1 (No.1-5), the mice received 500 μg of anti-asialo GM1 antibody (Wako pure Chemical Industries, Ltd., Tokyo) per day intraperitoneally from three days before until five days after the MT-2 cell injection, and three times a week thereafter.

In group 2 (No. 6–9), the mice were not treated with anti-asialo GM1. In both of groups, the mice were injected with $5-7 \times 10^7$ MT-2 cells intraperitoneally. Two mice were controls without injection of MT-2 cells.

After 2 and 24 days, two mice in group 1 died and they had no apparent lesions. After 50 days, the remaining three mice were autopsied, since the animals showed the signs of weakness, weight loss and ruffled fur. One mouse developed a tumor mass in the liver (Table 1). In group 2,

Table 1. Incidence of tumor in SCID mice injected with MT-2 cells intraperitoneally

Mouse No.	Anti-asialo GM1	Survival days	Tumor size (mm)
1	+	2	—
2	+	24	—
3	+	50 ^{a)}	5×5×3, 3×3×3
4	+	50 ^{a)}	—
5	+	50 ^{a)}	—
6	—	67	—
7	—	69	—
8	—	89	1×1×1, 1×1×1, 1×1×1
9	—	97 ^{a)}	2×2×2

a) Autopsied when the mouse showed the signs such as weakness, weight loss or ruffled fur.

two mice died after 67 and 69 days after inoculation, respectively, and they had no significant visceral lesions. After 89 days, mouse No. 8 died and had three small tumors in the peritoneal cavity. Mouse No. 9 was euthanized and autopsied after 97 days. The mouse had a tumor mass in the peritoneal cavity above the right adrenal gland.

For histological examination, the tumors were fixed in 10% formalin. Paraffin sections were cut and stained with hematoxylin and eosin (HE). Immunofluorescence assays were also performed on paraffin sections. These sections were fixed in acetone and they were incubated with anti-HTLV-I p19 monoclonal antibody (Chemicon international, Inc., CA, U.S.A.) or anti-HLA-DR monoclonal antibody (INCSTAR Corporation, MN, U.S.A.) followed by staining with FITC-conjugated anti-mouse IgG (Cappel, NC, U.S.A.).

By histological examination of HE stained sections, it is revealed the tumor mass consisted of fibrosis tissues and diffuse distribution of lymphoid cells of various size (data not shown). These findings were similar to those reported previously [4]. All three tumors reacted to anti-HTLV-I p19 and anti-HLA-DR monoclonal antibodies by immunofluorescence assays (Table 2).

To assess for the presence of HTLV-I genomic sequences, 0.5 μg of the DNA from a tumor was subjected to

Table 2. Characterization of tumors in SCID mice

Mouse No.	Anti-asialo GM1	IFA ^{a)}			PCR			
		HTLV-I p19	HLA-DR		gag	env	tax	HLA-DQ β
3	+	+	+		+	+	+	+
8	-	+	+		+	+	+	+
9	-	+	+		+	+	+	+
MT-2 cells		+	+		+	+	+	+
F647a cells ^{b)}		+	-		+	+	+	-

a) Immunofluorescence assay.

b) HTLV-I-transformed rabbit T-cell line.

Table 3. Detection of HTLV-I specific sequence in DNA obtained from various organs by PCR assay

Mouse No.	Tumor	Kidney	Liver	Lung	MLN ^{a)}	Spleen	Blood
1		-	+	-	N.T. ^{b)}	+	N.T.
2		+	+	-	N.T.	-	N.T.
3	+	-	-	-	-	-	N.T.
4		+	+	-	-	-	N.T.
5		-	-	-	-	-	N.T.
6		+	+	+	+	+	N.T.
7		+	+	+	+	+	N.T.
8	+	+	+	+	N.T.	-	N.T.
9	+	+	-	-	-	N.T.	+
control 1		-	-	-	-	-	N.T.
control 2		-	-	-	-	-	-

a) Mesenteric lymph node.

b) Not tested.

polymerase chain reaction (PCR) assay [6]. The oligonucleotides primers for gag gene (upstream [5'-TCCCTATGTTGAGCCTACGG-3'] and downstream [5'-GGCCTCTGATATAAGGCTATCT-3']), env gene (upstream [5'-GTCCTATACTCTCCCAACGT-3'] and downstream [5'-TTTCCTGAGGCGAGGGACAT-3']) and tax gene [7] (upstream SK 43 [5'-CGGATACCCAGTCTACGTGT-3'] and downstream SK44 [5'-GAGCCGATAACGCGTCCATCG-3']) were used. In addition, primers for HLA-DQ β [10] (upstream GH28 [5'-CTCGGATTCG-CATGTGCTACTTCACCAAGC-3'] and downstream GH29 [5'-GAGCTGCAGGTAGTTGTGTCTGCACA-C-3']) were also used. The gag, env and tax gene specific sequences of HTLV-I provirus were detected in all three tumors (Table 2). Furthermore, all tumors were also positive for the HLA-DQ β gene.

The results of immunofluorescence and PCR assays suggested that tumors in the SCID mice were of injected MT-2 cells origin.

The DNA extracted from the various organs in SCID mice exhibited HTLV-I specific sequences by PCR assay (Table 3). These HTLV-I specific sequences were found not only in the organs of the tumor bearing SCID mice but also those of the SCID mice without tumors. No positive signals were observed in the organs of the control mice.

These findings suggest that the injected MT-2 cells have invaded various SCID mice organs.

NK cells are present in SCID mice as demonstrated by the NK activity of spleen cells against YAC lymphoma target cells [1]. Therefore, for the growth of HTLV-I-transformed human T cells, MT-2 or ATL cells, in the SCID mice, it is considered that depletion of the NK cell function is essential [4, 5]. However, in the present study, we demonstrated that HTLV-I-transformed human T cells, MT-2 cells, could proliferate in the SCID mice without the elimination of NK cell function. The discrepancy is probably due to the fact that the number of injected cells in our study is about 5–7 times more than that used in the previous studies [2, 4]. In fact, no tumors were observed in the SCID mice inoculated $1-2 \times 10^7$ cells in another experiment (data not shown).

Furthermore, in our study, HTLV-I specific sequence was detected by PCR assay from DNA of the various organs in the MT-2 cells inoculated SCID mice with or without tumors. These findings indicated that the injected MT-2 cells could infiltrate into various organs in SCID mice. The incidence of detection of HTLV-I specific sequence in the organs of the SCID mice treated anti-asialo GM1 serum was lower than that in the SCID mice untreated with the serum. This may be due to the survival time of the SCID mice. Probably, the longer in the survival time, the higher in the incidence of detection of HTLV-I specific sequence occurred. We consider that the SCID mice inoculated MT-2 cells intraperitoneally is a good animal model for analysis of infiltration of ATL cells into various organs.

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