

# *Acta Medica Okayama*

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*Volume 33, Issue 1*

1979

*Article 3*

FEBRUARY 1979

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## Abstract

Human leukemic B-cell (BALL-1), T-cell (TALL-1) and null-cell (NALL-1) lines have been established from three patients with acute lymphoblastic leukemia (ALL). To study the heterotransplantability and in vivo growth characteristics, attempts were made to transplant these ALL cell lines into newborn Syrian hamsters treated with rabbit anti-hamster thymocyte serum. Intraperitoneal implantation of  $1.8-3.5 \times 10^7$  cells gave rise to invasive tumors in all recipients after 15 to 41 days. In addition to a common in vivo feature of mesenteric and retroperitoneal tumors, BALL-1 line was characterized by infiltration of the skin, massive ascites and bone marrow invasion. TALL-1 cells infiltrated various organs including the lymph nodes, liver, gallbladder, spleen, bone marrow, central nervous system and eyes. NALL-1 line grew slowly, producing the least tumors, although there were distant metastases in the lungs. Tumor cells were detected in the blood of 2 of 3 BALL-1-bearing hamsters and in the blood of 4 of 5 TALL-1-bearing hamsters. Thus, these three ALL cell lines were found to exhibit a characteristic biological behavior in hamsters, which might be related to the different cell lineage.

**KEYWORDS:** heterotransplantation, human ALL cell lines

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\*PMID: 155978 [PubMed - indexed for MEDLINE]

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Acta Med. Okayama 33, (1), 15-20 (1979)

## HETEROTRANSPLANTATION OF HUMAN LEUKEMIC B-CELL, T-CELL AND NULL-CELL LINES IN HAMSTERS

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*Received August 24, 1978*

*Abstract.* Human leukemic B-cell (BALL-1), T-cell (TALL-1) and null-cell (NALL-1) lines have been established from three patients with acute lymphoblastic leukemia (ALL). To study the heterotransplantability and *in vivo* growth characteristics, attempts were made to transplant these ALL cell lines into newborn Syrian hamsters treated with rabbit anti-hamster thymocyte serum. Intraperitoneal implantation of  $1.8-3.5 \times 10^7$  cells gave rise to invasive tumors in all recipients after 15 to 41 days. In addition to a common *in vivo* feature of mesenteric and retroperitoneal tumors, BALL-1 line was characterized by infiltration of the skin, massive ascites and bone marrow invasion. TALL-1 cells infiltrated various organs including the lymph nodes, liver, gallbladder, spleen, bone marrow, central nervous system and eyes. NALL-1 line grew slowly, producing the least tumors, although there were distant metastases in the lungs. Tumor cells were detected in the blood of 2 of 3 BALL-1-bearing hamsters and in the blood of 4 of 5 TALL-1-bearing hamsters. Thus, these three ALL cell lines were found to exhibit a characteristic biological behavior in hamsters, which might be related to the different cell lineage.

*Key words:* heterotransplantation, human ALL cell lines

The heterogenous population of lymphocytes in the blood and lymphoid tissue is currently subdivided into T-, B- and null-cells on the basis of the presence or absence of characteristic surface markers. Extensive studies of surface markers in acute lymphoblastic leukemia (ALL) revealed that in 70-80% of cases the cells were null-cell in type, 20-30% were T-cell and cases with B-cell markers were rare (1-3). The correlation between the T-B immunological classification of ALL and the prognosis has been studied by several investigators. T-cell as well as B-cell ALL were associated with poorer prognosis than null-cell ALL and the majority of T-cell ALL were characterized by massive leukemic

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Supported by Grants from the Ministry of Health and Welfare of Japan.

infiltration and frequently a mediastinal mass (2, 3).

Recently, the successful establishment of human leukemic B-cell (BALL-1), T-cell (TALL-1) and null-cell (NALL-1) lines from three patients with ALL has been reported (4-7). To study the heterotransplantability and *in vivo* growth characteristics, these three ALL cell lines were implanted into newborn Syrian hamsters treated with rabbit anti-hamster thymocyte serum (ALS). These cell lines were found to be heterotransplantable. Of particular interest is that each cell line exhibits a characteristic biological behavior in hamsters.

#### MATERIALS AND METHODS

*ALL Cell Lines.* The establishment of BALL-1, TALL-1 and NALL-1 lines has been previously reported (4-7). These ALL cell lines have been maintained for more than 2 years in continuous log-phase suspension cultures in RPMI 1640 medium supplemented with 15% fetal calf serum.

*Hamsters.* Newborn Syrian hamsters were bred in our laboratory by mating males with females obtained from a professional breeder (Kagawa Tsuda Animals, Japan).

*Transplantation.* Newborn hamsters less than 24 h old were used as recipients. The cultured cells were washed once with RPMI 1640 medium and suspended in physiological saline to yield a concentration of  $1.8-3.5 \times 10^8$  cells/ml. The viability of cells was assessed by trypan blue exclusion. Point one ml of cell suspension ( $1.8-3.5 \times 10^7$  cells/hamster) were implanted intraperitoneally using a tuberculin type syringe with a 30-gauge needle. This was followed by twice weekly intraperitoneal inoculation of 0.1 ml of ALS, which was prepared as previously described (8).

*Histology.* Histological sections were taken from all visually abnormal appearing tissues and from almost all normal tissues and stained with hematoxylin and eosin. Smears of blood and ascites were stained with May-Grünwald-Giemsa.

#### RESULTS

*Transplantation of BALL-1 Line.* The BALL-1 cells,  $3.1 \times 10^7$ /hamster, were implanted into 4 ALS-treated newborn hamsters. One hamster was lost through cannibalism and could not be examined histologically. The 3 remaining animals were sacrificed at 23 to 26 days after implantation.

At autopsy, besides the diffuse infiltration in the ventral subcutaneous tissue, the abdominal cavity was filled with massive tumors in all the animals (Fig. 1). In two animals there were 1.0 to 4.0 ml of milky abdominal ascites containing  $0.9-4.8 \times 10^8$  tumor cells/ml. The ascites tumor cells were morphologically indistinguishable from BALL-1 cells. The abdominal tumors were soft, white and substantially free of necrosis and invaded the peritoneum, mesentery, retroperitoneum and diaphragm. There was neither visible lymph node enlargement nor hepatosplenomegaly.

Microscopically, infiltrations of tumor cells were observed in the bone marrow and the capsules of the kidneys and liver as well as in the above-mentioned sites. Circulating tumor cells (2-4%) were detected in 2 animals, whose peripheral leukocyte counts were 2,300 and 3,350/mm<sup>3</sup>.



Fig. 1. BALL-1-implanted hamster. Note the presence of massive subcutaneous and intraabdominal tumors. The milky ascites in the syringe was removed from this hamster and contained numerous BALL-1 cells.

Fig. 2. TALL-1-implanted hamster. Intraabdominal massive tumors are seen. Note enlargement of axillary and para-thymic lymph nodes and a small tumor at gallbladder.

Fig. 3. NALL-1-implanted hamster. Tumors in porta hepatis, mesentery and retroperitoneum are seen. Tumors are much smaller than tumors produced with BALL-1 and TALL-1 lines.

*Transplantation of TALL-1 Line.* The TALL-1 cells,  $1.8-2.9 \times 10^7$ /hamster, were implanted into 8 ALS-treated newborn hamsters. One hamster was cannibalized and of the 7 remaining animals, 5 were killed and 2 died with tumors at 26 to 41 days post-implantation.

Macroscopically, large white tumor masses were observed in the mesentery and retroperitoneum of all the animals (Fig. 2). A small tumor mass was also seen at the gallbladder as a result of massive infiltration or total replacement of the gallbladder in 6 animals (Fig. 2). There were 0.5 to 1.8 ml of slight milky ascites containing  $0.8-5.8 \times 10^7$  tumor cells/ml in 5 animals, which were similar in morphology of TALL-1 cells. Besides the intraabdominal tumors, the thymus was invariably surrounded by enlarged para-thymic lymph nodes, which frequently extended into the thymus. Hepatomegaly was frequently found but

splenomegaly was not seen. There were occasionally enlarged axillary and inguinal lymph nodes.

Microscopically, in addition to the above-mentioned organs, tumor cells frequently infiltrated into the bone marrow, the portal and sinusoidal space of the liver and the renal capsule with extension into the pelvis and cortex. Furthermore, metastases to the brain, meninges and eyes were found in 2 animals. In the eyes, the ciliary body and choroid were massively infiltrated by tumor cells. Tumor cells were detected in the peripheral blood (13–25%) of 4 of 5 animals examined. The peripheral leukocyte counts of these 4 animals ranged from 1,400 to 17,200/mm<sup>3</sup>.

*Transplantation of NALL-1 Line.* The NALL-1 cells,  $2.5\text{--}3.5 \times 10^7$ /hamster, were implanted into 5 ALS-treated newborn hamsters. All of them were killed at 15 to 39 days after implantation. All these surviving animals had tumors in the porta hepatis, mesentery and retroperitoneum (Fig. 3). However, the intra-abdominal tumors were small in comparison with tumors produced with BALL-1 and TALL-1 lines. There was no ascites in any of the animals.

Microscopically, besides the direct extension into the capsules of the kidneys and liver, distant metastases to the lungs were observed in 4 animals and to the meninges in 2 animals. In the lungs, tumor cells infiltrated around the small vessels. No circulating tumor cells were detected in any of the animals.

#### DISCUSSION

In 1977, three human ALL cell lines with B-cell, T-cell and null-cell surface markers have been successfully established (4–7). These cell lines are considered to have originated from the respective donor's leukemic cells, on the basis of their cytogenetic, morphologic and functional features. The results described here demonstrated that these ALL cell lines can be transplanted into immunosuppressed newborn hamsters. The development of invasive tumors in the heterologous hosts was further evidence that these cell lines were of leukemic cell origin.

The *in vivo* biological features of human leukemic B-cells, T-cells and null-cells are not yet clear. In the present *in vivo* experiments, the BALL-1 line was characterized by infiltration of the skin and the bone marrow, massive ascites and rapid tumor growth. The TALL-1 line infiltrated other organs including the gallbladder, central nervous system and eyes. In contrast to the BALL-1 and TALL-1 lines, the NALL-1 line produced the least tumors and their growth was slow. In view of a poor prognosis of B-cell and T-cell ALL (2, 3), it is important and of interest that each of these cell lines exhibits a different characteristic biological behavior and the tumors produced with the BALL-1 and TALL-1 lines grew rapidly in hamsters. Furthermore, it is worthy to note that

the donor of the TALL-1 line had widespread leukemic involvement including the meninges and eyes during his clinical course.

The heterotransplantation of human leukemic cells has been reported by several investigators. Adams *et al.* (9, 10) reported that T-cell ALL cells produced tumors in hamsters, which infiltrated into various organs including the central nervous system like our TALL-1 line. Miyoshi *et al.* (11) and Lozzio *et al.* (12) reported that Ph<sup>1</sup> chromosome-positive myeloblasts could be implanted into hamsters or hereditary asplenic-athymic mice. However, there is no report of heterotransplantation of human leukemic B-cells or null-cells thus far.

The *in vivo* system of ALL described in this paper would be useful as an experimental model of human B-cell, T-cell and null-cell ALL and might be especially applicable in the evaluation of antileukemic agents which are active *in vivo* but inactive *in vitro*.

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