Leukemic Distribution of a Human Acute Lymphocytic Leukemia Cell Line (Ichikawa Strain) in Nude Mice Conditioned with Whole-Body Irradiation

Shaw Watanabe, Yukio Shimosato, Toru Kameya, Masahito Kuroki, Takeshi Kitahara, Keisuke Minato, and Masanori Shimoyama

Pathology Division, National Cancer Center Research Institute [S. W., Y. S., To. K., M. K.], and Internal Medicine, National Cancer Center Hospital [Ta. K., K. M., M. S.], 5-1-1, Tsukiji, Chuo-ku, Tokyo 104, Japan

ABSTRACT

Induction of leukemia in nude mice (BALB/c nu/nu) was attempted by inoculation with a human acute lymphocytic leukemia cell line (Ichikawa strain, maintained in an ascitic form in our institute). Inoculation of the cells i.v. in normal nude mice failed to produce leukemia. However, conditioning with whole-body irradiation (500 rads) resulted in induction of leukemia after i.v. inoculation, especially when such inoculation was performed 3 days after irradiation. The correlation of survival to inoculum size (10^6 to 10^8) was inversely exponential.

Leukemic infiltration was noted in the spleen, lymph nodes, bone marrow, meninges, liver, kidneys, etc., as seen in human leukemia. These cells retained their original cytological characteristics, ultrastructural features, and surface markers and revealed high terminal deoxynucleotidyltransferase activity as T-derived cells. Chromosome analysis revealed aneuploidy in a hypotetraploid range with a mode of 88 chromosomes.

INTRODUCTION

Transplantation of human neoplasms into athymic "nude" mice has been carried out intensively, and it has been recognized that leukemia and lymphoma are the most difficult to transplant (4, 5, 7, 11, 15, 16, 18, 20). Every attempt thus far in our institute failed to establish serially transplantable tumors from fresh materials of malignant lymphoma or leukemia, except for only 2 cases of ALL^2 (T. Kitahara, S. Watanabe, M. Shimoyama, and K. Minato, unpublished data; Y. Ise, M. Ohira, and I. Muchi, unpublished data).

The nude mice reference center reported that lymphoma and leukemia had been successfully transplanted in only 7 of 52 transplants (18). However, Epstein et al. (4) reported an exceptionally high take ratio of 20:35 by i.c. inoculation. Serial transfers were extremely difficult, and only 1 of the above 27 was maintained. Although many of the cultured cell lines were transplantable by s.c. or i.c. injection, leukemic changes never occurred in these cases (4, 5, 11, 15). There have not yet been reports of successful induction of leukemia in nude mice.

Attempts to induce leukemia in nude mice by i.v. inoculation also failed. For example, a large number of injected cells, up to 10^8 cells, disappeared in a few weeks.

The presence of tumor-associated antigens in leukemic cells has recently been recognized (8). It is likely that the injected cells were rejected by a thymus-independent immune system (2, 3). Natural cytotoxic antitumor antibodies in the nude mice may also play a role (12). This report deals with successful induction of leukemia in nude mice by human ALL cells and their characterization.

MATERIALS AND METHODS

Animals. Male athymic nude mice, 4 to 6 weeks old with a BALB/c genetic background, were supplied by the Central Institute for Experimental Animals, Kawasaki, Japan, where mice were bred and maintained in vinyl isolators under specific-pathogen-free conditions. They were kept in a laminar flow rack (Ishorack; Sanki, Tokyo, Japan) in our laboratory and were given sterilized pellets (CL-2; Clea Japan, Tokyo, Japan) and tap water ad libitum throughout the experiment.

Leukemic Cells. A transplantable leukemia strain has been maintained in our institute by i.p. inoculation of BALB/c nude mice with leukemic cell suspension. This strain was obtained from the peripheral blood of a 7-year-old patient with ALL, successfully transplanted into the peritoneal cavity of nude mice after 10 days of culture by Dr. T. Kitahara, and named the Ichikawa strain. The cells had lymphoblastic features with convoluted or lobulated nuclei and retained their ability to form SRBC rosettes after more than 15 i.p. passages when the experiment had begun.

Irradiation. With a 6-MeV machine at a rate of 320 rads/min, whole-body irradiation, 500 rads, was administered to mice placed in a paper box covered by a paper filter.

Experimental Groups. Three mice each were given i.v. injections of 3 x 10^7 Ichikawa cells at 4 hr, 3 days, and 1 week after whole-body irradiation to examine the effect of radiation concerning the induction of a leukemic state. Sixteen irradiated and 18 nonirradiated mice were inoculated i.v. with 1 to 3 x 10^7 Ichikawa cells 3 days after irradiation. Blood samples were taken from the tail vein at regular intervals, and the number of WBC and differentials were counted on a Bürker-Türk plate after staining with Türk solution. Differentials were reevaluated on Giemsa-stained slides. Groups of 2 or 3 irradiated mice each were inoculated with 10^8, 10^7, 10^6, 10^5, 10^4 Ichikawa cells,

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2 The abbreviations used are: ALL, acute lymphocytic leukemia; i.c., intracerebrally; SRBC, sheep erythrocyte; TDT, terminal deoxynucleotidyltransferase.
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and their survival was recorded. All these animals were observed closely and weighed daily.

Histology and Cytology. In a study of the sequence of pathological changes after the inoculation, 1 or 2 animals were sacrificed 3 days and 1, 2, and 3 weeks after inoculation in the second group. All other animals were autopsied when they became moribund to observe the distribution of the leukemic cells. All the organs were examined histologically, some by an electron microscope. Imprints of the various organs were stained with Giemsa solution, and some were stained for β-glucuronidase and acid phosphatase activities. Details of the staining method were described in a previous paper (9). Sudan III, oil red O, periodic acid-Schiff reaction, and methyl green-pyronin stains were also used.

Analysis of Chromosome and Functional Markers. Cell suspension in Roswell Park Memorial Institute Medium 1640 were made from some enlarged spleens with leukemic infiltration and used for chromosome analysis, surface markers, and the activity of TDT. For chromosome analysis cells were expanded with 0.075 M KCl, fixed 4 times with methyl alcohol:acetic acid (3:1), and dropped onto cold slides. The slides were quickly flame dried and stained with Giemsa solution. Chromosome counts and karyotypes were analyzed on photomicrographs.

Incubation with SRBC or neuraminidase-treated SRBC for SRBC receptors; with SRBC activated by IgG or SRBC activated by IgM for Fc receptors; and with SRBC activated by human complement C3b, SRBC activated by mouse complement C3d, or yeast particles coated with mouse complement C3d for C3 receptors was carried out by the methods described in the previous paper (13). Giemsa-stained preparations were made for confirmation of rosette formation. Immunoglobulin-bearing cells were detected by staining with the use of fluorescein isothiocyanate-labeled rabbit antiserum against human immunoglobulin (Behringwerke AG, Marburg, West Germany) (2).

TDT was assayed by the method of Sarin et al. (17) in a standard reaction mixture (50 μl) containing 50 mM Tris-HCl, pH 7.5; 50 mM KCl; 0.5 mM MgCl₂; 5 mM dithiothreitol; 10 μl [3H]dGTP (specific activity, 9.0 Ci/mol); 20 μl polydeoxyadenylate primer; and 10 μl of a crude cell extract (1.0 ml of 0.25 M Tris-HCl, pH 7.5, containing 0.2% Triton X-100 and 0.25 M KCI for 10⁸ leukemic cells). The net TDT activity was determined by subtracting the endogenous activity counted from the reaction solution, omitting the primer polydeoxyadenylate from the primed activity.

RESULTS

Body Weight and Leukemic Change in Irradiated Mice. The mice lost an average of 2 g of body weight for the first 3 to 4 days after whole-body irradiation and gained weight over the following 2 weeks after inoculation of 3 × 10⁸ cells. Paralysis of the hind legs was noted, without exception, 2 to 3 weeks after inoculation (Fig. 1). Then the mice became progressively emaciated and died of cachexia 4 weeks after inoculation. Nonirradiated mice showed no significant changes during more than 12 weeks of observation.

All irradiated animals revealed meningeal metastasis, while only a few nonirradiated mice (2 of 18) retained injected cells in the meninges, none developing paralysis at the time of sacrifice in the 6th week. Intervals between irradiation and inoculation influenced the distribution of neoplastic cells and the frequency of leukemic change (Table 1). Inoculation of the cells 3 days after irradiation was most effective; therefore subsequent experiments were performed under this condition.

Changes in the Peripheral Blood. Injected Ichikawa cells remained 7 days in the peripheral blood of both irradiated and control mice. They disappeared completely in nonirradiated mice 2 weeks after inoculation, while leukemic cells continued to increase in the blood of irradiated mice until the terminal stage [WBC, 14,667 ± 7,203; leukemic cells, 24.5 ± 11.0% (S.D)] (Fig. 2a).

Effect of the Number of Cells Injected. All the irradiated mice, receiving 10⁵ to 10⁸ cells, died due to proliferation of neoplastic cells. Their survival periods corresponded well to the inoculum size between 10⁵ and 10⁸ (Chart 1). Smaller inoculum size gave longer survival and less leukemic change: 11.8% in the peripheral blood of a mouse inoculated with 10⁸ cells and 0.9 to 1.1% in mice inoculated with 10⁵ cells. Neoplastic cells proliferated about 10 times/week, the doubling time in the mice being estimated to be 48 hr, which was slightly longer than those in the in vitro system (43.3 hr; K. Minato and M. Shimoyama, unpublished data).

Histology. Sacrifice 1 day after inoculation revealed numerous cell debris in the lymph node and spleen. Tumor thrombi were not detected in the lung, brain, or other tissues. One week later neoplastic cell colonies were found only in the subcapsular region and the lymphatic sheath of the spleen of irradiated mice.

Table 1

<table>
<thead>
<tr>
<th>Sites</th>
<th>Inoculated 4 hr after irradiation</th>
<th>Inoculated 3 days after irradiation</th>
<th>Inoculated 7 days after irradiation</th>
<th>Nonirradiated</th>
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<tr>
<td>Peripheral blood</td>
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<td>10-25%</td>
<td>0-3%</td>
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<td>+ + + + + + + + + + + + + + + + + +</td>
<td></td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Bone marrow</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Meninges (brain)</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
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* a = no infiltration; +, slight infiltration; ++, moderate infiltration; +++, marked infiltration.

Chart 1. Relationship between survival after i.v. inoculation of Ichikawa cells following whole-body irradiation and the size of inoculum. Inverse correlation was obtained between survival and inoculum size of 10⁴ to 10⁸. Correlation coefficient was −0.948 within this range, W, weeks.
At death the leukemic animals revealed marked splenomegaly (250 to 500 mg), systemic lymphadenopathy, hepatomegaly of varying degrees, and clouded meninges with frequent bleeding (Fig. 3). Microscopically, the spleen, lymph nodes, and bone marrow were filled with diffusely proliferated monotonous Ichikawa cells (Figs. 4a and 5). These histological features were identical with those of ALL and diffuse lymphoblastic lymphoma in humans. Meninges of the brain and spinal cord were also diffusely infiltrated by leukemic cells (Fig. 4b). The portal area and sinusoids of the liver and the kidney revealed a moderate degree of leukemic infiltration (Fig. 4, c and d). There were no ascites, pleural effusion, or extranodal tumors.

**Cytology.** Imprints of the various organs contained variable numbers of leukemic cells, corresponding with the degree of infiltration. Most nuclei revealed atypical lobulation and had small distinct nucleoli, and scanty basophilic cytoplasm contained a few vacuoles, and cytoplasmic processes were rarely present. Granular reaction products of acid phosphatase and \( \beta \)-glucuronidase were usually present in the cytoplasm at the concave side of the nuclei. The periodic acid-Schiff reaction was very faint or negative. Occasional lipid droplets were also present.

**Electron Microscopy.** Leukemic cells in various organs had features characteristic of ALL cells, i.e., indented nuclei, with a few distinct nucleoli, and scanty cytoplasm containing polysomes, lipid droplets, aggregation of mitochondria, Golgi complexes, lysosome granules, and bundles of fine fibrils. Annulate lamellae were occasionally found. No viral particles were observed.

**Surface Markers.** SRBC receptor was retained through passages in the ascitic form. Leukemic cells in the spleen also kept SRBC and neuraminidase-treated SRBC rosette-forming ability (91 and 94%, respectively). The presence of Fc receptor was uncertain because of a cross-reaction of SRBC receptors with immunoglobulin-coated SRBC. C3 receptors were detected in 17% of cells by yeast particles coated with mouse complement C3d. There was no surface immunoglobulin on the leukemic cells.

**Chromosome Analysis.** Chromosomes of most of the cells infiltrating in the spleen were in the hypotetraploid range with a modal number of 88 (34.3%). An increase in number was noted in Groups A, C, D, E, F, and G. There was no contamination of the mouse-derived chromosomes.

**TDT.** TDT activity of the leukemic cells was always very high, ranging from 0.2 to 0.5 nm dGTP uptake per hr per 10⁷ cells. (TDT activities of B-cell neoplasms in our system were less than 0.05 nm/hr/10⁷ cells.)

**DISCUSSION**

Direct transplantation of human hematopoietic tumor cells from the patient to nude mice was tried intensively, but only a few successful cases were reported (4, 18). A previous report from our institute dealt also with trials of heterotransplantation to nude mice, in which cultured Molt and Burkitt cell lines produced s.c. tumors, while every attempt to transplant 15 fresh malignant lymphoma or leukemia materials failed (11). Epstein at al. (4) reported a high take rate (90%) and a short mean latent period by i.c. inoculation. They did not report serial transplantation. The distribution of neoplastic cells was limited within the i.c. and/or spinal space. Therefore, the usefulness of their system appears limited.

By i.p. injection of 10⁷ to 10⁸ Ichikawa cells, BALB/c nude mice accumulated ascites and pleural effusion and usually died of cachexia and respiratory distress in 2 to 3 weeks (T. Kitahara, S. Watanabe, M. Shimoyama, and K. Minato, unpublished data). Peritoneal and retroperitoneal tumors and metastases to the anterior mediastinal lymph nodes were common findings, but diffuse leukemic infiltration in the spleen, bone marrow, or other organs has not occurred. In unconditioned mice all the i.v.-inoculated cells disappeared completely after 2 weeks, and no changes were observed over more than 12 weeks except for 2 cases with slight meningeal involvement.

These findings suggested the presence of some kind of rejection mechanism in nude mice against the xenogeneic tumor, probably by B-cell immunity and/or the presence of natural antibodies against the heterologous hematopoietic cells. It also prevented the spread of neoplastic cells from the transplant site to cause systemic leukemia, although the tumor grew locally.

Sublethal irradiation can reduce the immunity of animals and has been used for heterologous transplantation of hematopoietic cell lines by Toolan (19), Imamura et al. (10), and others. Imamura et al. reported high s.c. transplantability of cells of human origin in conventional non-"nude" DBA/2 mice. Campanile et al. (2), however, reported that the radioresistant inhibition of murine lymphoma growth was noticed in nude mice if the inoculated lymphoma cells had a different H-2 locus and concluded that the nude mice were stronger responders to lymphoma cells than were conventional mice. Cudkowicz (3) also reported that the bone marrow allografts were rejected by irradiated nude mice. They thought that such rejection was due to B-cell immunity or to phagocytosis by macrophages.

In this experiment, the tumor cells seemed to have proliferated before the recovery of an inhibitory immune response following whole-body irradiation to such an extent that progressive leukemia was the result. Inoculation 4 hr after irradiation was not sufficiently effective, probably because the immune system still kept some function, and inoculation 1 week after irradiation was less effective than after 3 days, probably because the immune system began to recover. Topological problems, however, should be considered as well, in that the damage to the splenic and bone marrow structures might have made it easier for the inoculated cells to settle.

Nilsson et al. (15) examined the transplantability from the aspect of the cellular character. They compared tumorigenicity of newly established and old human hematopoietic cell lines in nude mice and found that only old cell lines were transplantable. All the chromosome patterns of these transplantable cell lines were aneuploid. Imamura et al. (10) reported on the correlation of heterotransplantability with abnormal chromosome and higher cloning efficiencies. Zamecnic and Long (20) also reported that only aneuploid Hodgkin’s cultured lines were transplantable. The Ichikawa strain in this experiment was also aneuploid. The question of whether or not the leukemic cells were a selected clone in the conditioned mice was answered by the preliminary
result of our additional experiment, which showed that the i.v.-inoculated leukemic spleen cells did not cause leukemia in nonirradiated nude mice. Therefore, the leukemic condition depends upon the altered reactivity of hosts and not upon the cell character itself.

Convoluted nuclear appearance and other cellular characteristics, including enzyme histochemical and ultrastructural features, all coincided with those of human ALL cells with SRBC receptors (6, 9, 14). High cell TDT activities also indicated their T-cell nature (17).

A long-term preservation of surface and other markers in nude mice was convenient for further functional investigation. Inverse exponential correlation between survival and inoculum size indicates that this human leukemia-nude mice system is a good experimental model for the treatment of human leukemia. This leukemic model may provide an important new resource for laboratory investigations of several aspects of the biology of human malignant lymphomas and leukemias.

REFERENCES


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