HEREDITARY ASPLENIC-ATHYMIC MICE: TRANSPLANTATION OF HUMAN MYELOGENOUS LEUKEMIC CELLS*

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A mutant strain of mice suffering from hemimelia and asplenia was reported by Searle (1). The abnormalities are inherited as an autosomal dominant gene (Dh). Asplenic mice have normal cellular immunity (2) and a depressed immunoglobulin synthesis (2, 3) owing to a marked impairment of thymus and bone marrow cell cooperation (4, 5) needed for antibody formation.

Another mutant strain of hairless (nude) mice, discovered by Flanagan (6), is characterized by congenital athymia (7) which is inherited as an autosomal recessive gene (nu). In addition to a marked deficiency of thymus-derived lymphocytes and depressed humoral immune response to thymus-dependent antigens, nude mice fail to reject grafts of normal and neoplastic xenogeneic tissues (8).

The availability of these two mutant strains of mice made it possible to develop a unique animal model with agenesis of both the thymus and spleen by crossing nude with asplenic mice. Such an animal would be expected to have a marked impairment of both cellular and humoral immunity, thus making it an ideal model for heterotransplantation as well as a variety of immunological and hematological studies.

Materials and Methods

Mice. The development and maintenance of the nude and asplenic colonies in our laboratory has been described (2, 9). At the start of these experiments the nude and the asplenic colonies were in the fifth and sixth generations of inbreeding, respectively.

To establish the asplenic-athymic colony, a Dh/+ female was mated with a nu/nu male. The asplenic-athymic hybrids (hereafter referred to as "lasat" mice) were obtained by backcrossing females heterozygous for the nu and Dh genes (nu/+ , Dh/+) with their athymic father (nu/nu). This second mating was carried out in a pathogen-free isolator.

Culture of Chronic Myelogenous Leukemic (CML) Cells. The development and maintenance of the CML cell line K-562, which has the Philadelphia (Ph1) chromosome and a translocation 15;17 after more than 4 yr in culture, has been reported (10).

Transplantation of CML Cells. The method of Peters and Hewitt (11) which takes advantage of the Revaz effect (12) to grow transplantable tumor cells was used with slight modifications. Cells were obtained at passages 222 and 225 from suspension cultures and centrifuged at 800 g. The cell pellet was resuspended in fresh culture medium (10) at a concentration of 50 x 10^6/ml and 0.1 ml was mixed with 0.1 ml of solution of human fibrinogen in the same medium (100 mg/ml). The pH

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was adjusted to 7.2 with 1.0 N NaOH and two drops of a freshly prepared bovine thrombin solution (1,000 U/ml) were added. Each clot containing 5 × 10⁶ CML cells was implanted subcutaneously (s.c.) in each of 10 lasat mice.

Cytogenetic Studies. Chromosome studies were routinely made before transplantation. A homogenous cell suspension was prepared from CML tumors and aliquots containing 10⁶–10⁷ cells were cultured for 7 days. Samples were taken at intervals from the cultures and karyotypes made by standard techniques (10).

Antigenicity of CML Cells. The antigenicity of cells from solid CML tumors was tested by use of a primate (Macaca-speciosa) antiserum cytotoxic for the CML cell line K-562. Each injection contained 5 × 10⁶ washed CML cells in 1 ml of saline which was emulsified in 1 ml of complete Freund’s adjuvant. Each immunization schedule consisted of three 1 ml injections given on days 0, 7, and 14. On day 21 the monkey was bled. The serum was collected, treated at 56°C for 30 min to inactivate complement (C), and absorbed on 30 × 10⁶ normal A-B blood or bone marrow cells of human origin. The anti-CML serum used in these experiments was from the second immunization schedule which was given with an interval of 3 wk. The absorbed antisera demonstrated C-dependent cytotoxicity (titer 1:560, 50% cells dead) for cultured CML cells by the trypan blue (13) and ⁵¹Cr-release methods (14). The cytotoxicity from 1 ml of immune serum was completely removed by absorption with 30 × 10⁶ CML cells. Preimmune monkey serum was also used in control assays.

General Procedures. The diameter of the tumor was measured with a caliper with a precision of 0.1 mm. Tumors were removed at intervals after transplantation, weighed, fixed in formaldehyde, and processed by standard techniques for light microscopy. Blood cell counts were made (9) weekly in all mice to determine if the growth of CML cells as a solid tumor was accompanied by an abnormal number of leukemic cells in the circulation.

Results

At present, the colony is being inbred by mating athymic males (nu/nu, +/+ ) with asplenic sisters (nu/+, Dh/+). The offspring of these matings follow the typical Mendelian ratio of 25% normal (nu/+, +/+), 25% athymic (nu/nu, +/+), 25% asplenic (nu/+, Dh/+), and 25% asplenic-athymic mice (nu/nu, Dh/+). Perinatal deaths occurred in 7.5% of the offspring and 30 lasat mice have had 100% survival thereafter.

Two lasat mice are shown in Fig. 1 A. Like the nude mice, the lasat mice are hairless and have prominent skeletal anomalies of the hind legs, a characteristic of the Dh gene. Autopsies of 20 lasat mice confirmed the agenesis of both the thymus and the spleen. All lymph nodes from lasat mice were hypertrophic and were devoid of thymus-dependent areas. The hyperplastic Peyer’s patches were largely populated by well-differentiated lymphocytes and occupy one-third of the cross-section of the small intestine. Several small collections of lymphoblasts were observed among these mature lymphocytes. The small intestine showed no other alterations. Large accumulations of lymphocytes in the cecum and a marked hyperplasia of Kupffer’s cells and other liver reticuloendothelial sinusoidal lining cells were also seen in lasat mice.

The leukocyte counts were similar in 20 nude and 10 lasat mice (7,000 ± 800/mm³). The lasat mice had an intermediate lymphocyte to granulocyte ratio (53:44) as compared to that of asplenic mice (70:28) or that of athymic mice (40:58). Bone marrow cell counts revealed that lasat mice had only 8.9 ± 0.6 × 10⁶ cells per femur as compared with 15.6 ± 0.8 × 10⁶ cells for asplenic mice and 17.0 ± 1.2 × 10⁶ cells for athymic mice. The diminished number of total bone marrow cells was due to a hypoplasia of lymphocytic and erythrocytic series. The latter, in turn, was the cause of the normocytic anemia observed in lasat
mice. The anemia was more severe in lasat (6.7 ± 0.3 × 10^6 erythrocytes/mm^3) than in nude mice (7.8 ± 0.2 × 10^6 erythrocytes/mm^3), whereas a similar hypoplasia of bone marrow lymphocytes was observed in both nude and lasat mice. The total serum protein concentration (6.0 ± 0.3 g %) was similar in all three groups even though lasat mice had 50% less γ-globulin than nude or asplenic mice.

One of the most important observations made in lasat mice has been the transplantation and growth of myelogenous leukemic cells with the Ph^1 chromosome. The incidence of "takes" was high and 9 out of 10 lasat mice given the cells in a fibrin clot developed similar tumor masses. The transplanted cells grow out from the clot after a latent period of 4–5 days. The macroscopic appearance of a solid tumor at 31 days is illustrated in Fig. 1 B. At this time, CML tumors were round or lobulated vascularized masses which reached a diameter up to 2.5 cm and a weight of 2–3 g. In all mice the whole tumor was excised.

Microscopic sections (Fig. 2) revealed that CML cells proliferated into the s.c. tissue by growing along the blood vessels. As a result of this infiltrative growth, many capillaries surrounded by the leukemic cells formed a well-developed intratumoral vascular network. Only a few tumors contained small hyaline areas which most likely derived from the partial involution of the original fibrin implant during the latent phase preceding the cell growth in vivo. Although leukemic cells formed a solid nodular mass, they did not develop cell-to-cell attachments. This structural arrangement created an extensive intercellular space connected with the perivascular areas. The random distribution of mitotic activity in the tumor cells suggests that the intercellular space may have constituted a suitable channel for the diffusion of nutrients and metabolites.

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1 Bamberger, E. G., E. A. Machado, and B. B. Lozzio. 1975. Hematopoiesis in hereditary athymic mice. Manuscript submitted for publication.
FIG. 2. Section of the CML cell tumor shown in 1B. Thin-walled capillary vessels (C) can be seen amidst the proliferation of malignant myelogenous cells. Hematoxylin and eosin, × 200.

from the vascular network, which in turn would have accounted for the absence of ischemic necrotic areas in all growing CML tumors for at least 3 wk. Secondary local growth in the surrounding tissues or distant metastatic proliferations were not observed. Imprints, stained with Giemsa, revealed the two types of CML cells described previously (10).

The clone of the CML cell line K-562 used for these experiments showed significant aneuploidy before the inoculation in lasat mice. The proportion of cells with various chromosome markers was as follows: 6% hypodiploid cells with 40–45 chromosomes, 18% hyperdiploid with 48–54, 16% with 55–60, 12% with 61–62, and 48% near triploid (26% with 68–69 and 22% with 70–72 chromosomes). All the cells showed one Ph1 chromosome and one translocation 15;17. Chromosome studies of cells obtained from the tumors (Fig. 3) showed persistence of one Ph1 chromosome and one translocation 15;17 identified by the Giemsa-banding technique as del(22)(q12) and t(15;17)(q21;q24), respectively. The great majority (78%) of the cells were nearly triploid (62% with 65–69 chromosomes and 17% with 70–77) and a minority (21%) were hyperdiploid with 50–60 chromosomes. Most of the nearly triploid cells analyzed had an extra chromosome of each pair. All 100 cells counted had the translocation 15;17 and the Ph1 chromosome, and many of them showed several extra chromosomes number 16 and an isochromosome for the long arm of one chromosome number 7.

Cells from solid CML tumors shared common antigenic determinants with CML cells from the line K-562. Thus, the primate immune sera developed
FIG. 3. Karyotype of a near triploid cell showing a chromosome translocation t(15;17) and the Ph' chromosome. These chromosome markers were observed in cells from the patient, in the long-term cultures, and in all cells analyzed from solid CML tumors. Another abnormal chromosome number 7 (arrows) was found in the cells cultured before and after transplantation in lasat mice. Giemsa-binding technique.

against cultured CML cells were cytotoxic for 100% of the cells taken from all solid CML tumors tested.

The number of blasts in the blood did not increase although the mean leukocyte count was elevated (11,000 ± 800/mm³) in tumor-bearing mice. The leukocyte ratio remained unchanged and no evidence of a peripheral leukemia was observed up to 1 mo after transplantation of CML cells.

Discussion

The development of lasat mice provides a useful model for studies in tumor immunology. The growth of CML tumors in lasat mice for more than 30 days without evidence of rejection confirms the lack of cellular immunity in these animals. Since nonimmunized lasat mice have a very low serum level of γ-globulin it would suggest that lasat mice, as expected, have a marked impairment of immunoglobulin synthesis as well.

It appears that the source of leukemic cells and the transplantation procedures were also essential for the successful outcome of these experiments. We have observed that in suspension cultures, cell clones containing two cell types were overgrown by the cell type with the largest chromosome number (10). Therefore, we transplanted a clone of cultured CML K-562 cells with a nearly triploid modal number of chromosomes. Also, the transplantation of CML cells in a fibrin clot containing the medium used to grow the cells in suspension appears to be beneficial since the cells could not migrate from the site of implantation, and had the necessary nutrients.

It remains open to question whether or not other chromosome abnormalities
were the crucial factors leading to the proliferation of CML cells in mice. We have suggested (10) that certain chromosomal rearrangements might be another cause of the increased growth rate in cultures. The fact that the great majority of cells from solid CML tumors were nearly triploid indicate that there was a clonal selection of CML cells in lasat mice, and those with the largest chromosome number overgrew other leukemic cells. This finding, in turn, lends further support to our previous observations on the rate of proliferation of CML cells K-562 in cultures (10).

In addition to the heterotransplantation of human malignancies, lasat mice may prove useful for other immunological investigations. Thus, the lasat mouse may yield new information on the development of the lymphoid system and offers a new possibility for investigating the existence of structural and functional bursal equivalents in mammals and the influence of the spleen on immunocompetence.

Summary

A new animal model characterized by hereditary athymia and asplenia was used as a recipient of chronic myelogenous leukemic (CML) cells with the Philadelphia (Phi+) chromosome. Transplanted CML cells form solid vascularized tumors containing cells similar to those seen in the patient in a long-term culture. Cells taken from the tumors were nearly triploid, retained all human chromosome markers, and had the same antigenic determinant(s) as cells in culture.

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