Direct Implantation of Ph' Chromosome-Positive Myeloblasts Into Newborn Hamsters

By Isao Miyoshi, Ichiro Kubonishi, Hiroshi Uchida, Shunkichi Hiraki, Hironobu Toki, Toshio Tanaka, Hiroshi Masuji, and Kiyoshi Hiroki

Peripheral leukocytes from a male patient with chronic myelogenous leukemia in blast crisis were implanted directly into the intraperitoneal cavity of seven newborn hamsters treated with antilymphocyte serum. At sacrifice 17-19 days after implantation, three hamsters were found to have disseminated tumors. The other four hamsters were cannibalized. Chromosome analysis of cells from the enlarged lymph nodes revealed the presence of a human male karyotype. Most metaphase cells were pseudodiploid with the Ph' chromosome and isochromosome 17, while few metaphase cells were classically diploid. Identical chromosome constitutions were observed in the original peripheral leukocytes of the patient. The results strongly suggested simultaneous heterotransplantation of Ph'-positive leukemic and normal leukocytes.

EUKOCYTES freshly isolated from patients with various lymphoproliferative diseases1,2 and from normal human subjects3 have been transplanted successfully into newborn hamsters. The transplanted tumors are lymphoid; however, few of these tumors are presumed to have derived from leukemic cells.4 The present paper reports on the successful heterotransplantation of Ph' chromosome-positive myeloblasts from a chronic myelogenous leukemia (CML) patient in blast crisis.

MATERIALS AND METHODS

Case History

The patient was a 32-yr-old male with a definitive diagnosis of CML. On admission, the white cell count was 160 x 10^9/liter. The hemogram indicated 2% myeloblasts, 9% promyelocytes, 10% myelocytes, 13% metamyelocytes, 19% band forms, 11% polymorphonuclear neutrophils, 12% eosinophils, 22% basophils, 1% monocytes, and 1% lymphocytes. The hemoglobin was 11.6 g/dl and the platelet count 1869 x 10^9/liter. The patient was treated with dibromomannitol. A gradual decrease of white cell count and moderate regression of splenomegaly followed treatment. Eight months after admission blast crisis of CML occurred. The response to intensive chemotherapy was poor, and the patient died 2 mo after the blast crisis.

Leukocyte Separation and Heterotransplantation

One day prior to death, 15 ml of peripheral blood was collected into a heparinized syringe. At this time, the white cell count was 176 x 10^9/liter, with 98% myeloblasts, 1% polymorphonuclear neutrophils, and 1% lymphocytes. The myeloblasts were negative for peroxidase and no Auer bodies were seen (Fig. 1). The blood was allowed to sediment in a vertical position at 37°C for 1 hr, and the leukocyte-rich plasma was centrifuged lightly. About half of the collected leukocytes

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Submitted August 8, 1975; accepted September 12, 1975.

Supported by grants from the Ministry of Education and the Ministry of Health and Welfare of Japan.

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Blood, Vol. 47, No. 3 (March), 1976 355
Fig. 1. Smear of peripheral blood used for leukocyte implantation into hamsters. A nearly pure population of myeloblasts is seen. May–Grünsfeld–Giemsa. × 2000.

were used for cytogenetic analysis, long-term culture, or stored in liquid nitrogen. The other half of the leukocytes was resuspended in medium RPMI 1640. A volume of 0.1 ml of this leukocyte suspension (2 × 10⁷ cells) was implanted intraperitoneally into newborn Syrian hamsters within 24 hr of birth. The transplantation was followed by twice weekly intraperitoneal inoculation of 0.1 ml of rabbit antilymphocyte serum against hamster thymocytes. This serum was prepared in our laboratory according to the method of Levey and Medawar.⁵

Chromosome Analysis

The collected leukocytes were resuspended in a concentration of 5 × 10⁶/ml in medium RPMI 1640 supplemented with 20% fetal calf serum and antibiotics. This cell suspension was dispensed into 35-mm petri dishes and incubated at 37°C in a humidified atmosphere of 7.5% CO₂ in air. After 3-day culture, chromosome preparations were made by a modification of the method of Moorhead et al.⁶ Well-spread metaphases were photographed and karyotyped. Furthermore, the enlarged lymph nodes from hamsters implanted with leukocytes were removed and finely minced; the resulting cell suspensions were cultured in the same manner for 3 days, and the chromosomes of the cultured cells were analyzed. No phytohemagglutinin was added in the cultures. For the chromosome banding studies, some of the conventional Giemsa-stained slides were destained after microphotography, treated with trypsin, and stained again with Giemsa, as described by Kajii et al.⁷

Histology

The animals were sacrificed by ether inhalation. Histologic sections were prepared from organs including the liver, kidneys, lungs, spleen, lymph nodes, brain, and lumbar vertebrae. The sections were stained with hematoxylin and eosin and imprint smears of lymph nodes were stained with May–Grünsfeld–Giemsa and for peroxidase.

RESULTS

Four hamsters were lost by cannibalism and could not be examined histologically. The three remaining animals showed retarded growth and signs of weakness. These animals were sacrificed at 17–19 days after transplantation of CML leukocytes to avoid probable further loss by tumor death and cannibalism. All these surviving hamsters had disseminated tumors.
Histopathologic Findings

Gross findings included moderate hepatosplenomegaly, retroperitoneal masses, and generalized lymphadenopathy involving the inguinal, axillary, para-aortic, parathymic, and mesenteric lymph nodes (Fig. 2). The peritoneal surfaces of the abdominal organs were partly covered with grayish white tissue.

Microscopically, cellular infiltrations were present in the liver, gallbladder, kidneys, lungs, diaphragm, spleen, lymph nodes, stomach, intestine, and retroperitoneum. The infiltrates were mostly primitive cells with scanty cytoplasm and a round or oval nucleus (Fig. 3). One or two nucleoli were evident in many cells. Immature eosinophils and mature granulocytes were scattered among these primitive cells. Mitotic cells were frequent and even a few eosinophils were observed in mitosis. Imprint smears of enlarged lymph nodes showed many myeloblasts and promyelocytes (Fig. 4). Some of these cells were positive for peroxidase.

Cytogenetic Studies of Original Peripheral Leukocytes

The 3-day cultures of peripheral leukocytes from the CML patient yielded 14 analyzable metaphases: 13 were pseudodiploid and one normal diploid (Table 1). The pseudodiploid cells all contained the Ph1 chromosome and one metacentric marker chromosome of group C size (Fig. 5). The Giemsa banding technique revealed a deletion of the long arm of chromosome 22 (22q−) that gave rise to the Ph1 chromosome and an additional faint band at the long arm of chromosome 9 (9q+). The amount of material added to chromosome 9 was approximately equal to the amount of material missing from the Ph1 chromo-
Fig. 3. Histologic section of a retroperitoneal tumor, showing sheets of tumor cells with scattered mitoses. Immature eosinophils are not apparent in this monochrome micrograph. Hematoxylin and eosin. x 750.

Fig. 4. Imprint smear of an enlarged mesenteric lymph node from the hamster shown in Fig. 2. The cells are predominantly promyelocytes with abundant azurophilic granules. May–Grünwald–Giemsa. x 2000.
Fig. 5. Karyotype from 3-day cultures of peripheral leukocytes shown in Fig. 1. Note the additional faint band at the end of chromosome 9 (9q+), the similarity of bands in the upper and lower arms of the metacentric marker chromosome i(17q) paired with the normal chromosome 17, and the Ph¹ chromosome (22q−). Before (upper row) and after (lower row) trypsin treatment.
Table 1. Karyotype Analysis of Human CML Peripheral Leukocytes and Their Heterotransplants in Hamsters

<table>
<thead>
<tr>
<th>No. of Cells Analyzed</th>
<th>No. of Cells With Karyotype</th>
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<tbody>
<tr>
<td></td>
<td>46, XY, 9q+, i(17q), 22q−</td>
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<tr>
<td>Peripheral leukocytes</td>
<td>14, 13, 1, 0</td>
</tr>
<tr>
<td>Hamster No. 1</td>
<td>18, 16, 2, 0</td>
</tr>
<tr>
<td>Hamster No. 2</td>
<td>14, 8, 1, 5</td>
</tr>
<tr>
<td>Hamster No. 3</td>
<td>22, 15, 0, 7</td>
</tr>
</tbody>
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some. One arm of the metacentric marker chromosome was homologous to the long arm of chromosome 17 and this marker was considered an isochromosome for the long arm of chromosome 17 i(17q).

Cytogenetic Studies of Heterotransplanted Tumor Cells

The 3-day cultures of lymph node cells (inguinal and axillary) from three tumor-bearing hamsters were cytogenetically analyzed. All these hamsters were shown to have tumor cells with chromosome constitutions identical to those of the original peripheral leukocytes (Table 1). Most leukocytes had the Ph1 chromosome and one isochromosome 17 i(17q) (Fig. 6). The deleted portion of the Ph1 chromosome appeared to be translocated onto the long arm of chromosome 9 (9q+). Normal diploid karyotypes were seen in two cells from hamster No. 1 and in one cell from hamster No. 2. Furthermore, there were several metaphases with 44 chromosomes and a hamster karyotype in cells from hamsters No. 2 and No. 3.

DISCUSSION

The development of invasive tumors in hamsters with the Ph1 chromosome would provide unequivocal evidence that such tumors arise from the human leukemic cell population. These tumors histologically resemble myeloblastic tumors in patients with myelogenous leukemia. The persistent admixture of promyelocytes and immature eosinophils in the hamster tumors is indicative of the in vivo maturational potential of Ph1-positive myeloblasts. This finding is consistent with the observation of Whang-Peng et al. that immature CML cells are capable of maturation in vitro. The present experiment suggests that it is
not impossible to obtain a serially transplantable myeloblastic tumor in hamsters.

The original leukocytes and the heterotransplanted hamster tumors both showed the presence of the Ph\(^1\) chromosome (22q\(^-\)) with a translocation to chromosome 9 (9q\(^+\)) and one metacentric marker chromosome i(17q). Such a translocation has been suggested for the genesis of the Ph\(^1\) (22q\(^-\)) and 9q\(^+\) chromosomes.\(^{10,11}\) The occurrence of isochromosome 17 has been described in a number of CML patients entering blast crisis.\(^{10,12}\) It is interesting to note that a few normal diploid cells were present in the original peripheral leukocytes and in the hamster transplants. It is not certain whether the peripheral diploid cell is of lymphoid or myeloid origin; however, the diploid cells in the hamster transplants probably represent lymphoid cells coexisting among the leukemic cells, as normal human lymphocytes have been successfully transplanted into hamsters.\(^3\)

REFERENCES

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