The Circulation of Malignant Lymphoid Cells in Thoracic Duct Lymph of Rats with Lymphosarcoma and Lymphatic Leukemia

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SUMMARY

The circulation of lymphoid cells in thoracic duct lymph of rats with lymphosarcoma (LSA) and leukemia was investigated. Rats were inoculated with Murphy-Sturm lymphosarcoma either by the i.m. route, which yields a localized tumor-simulating LSA, or by the i.v. route, which results in lymphatic leukemia (LL). Thoracic duct fistulae were established in four groups of rats, including untreated normal rats, rats inoculated with normal lymphocytes, and rats inoculated with Murphy-Sturm lymphosarcoma to produce either LSA or LL. The percentage of large lymphocytes in thoracic duct lymph of rats with LSA and LL was higher than that of either control group and was greater in leukemic rats than in those with LSA. Chromosomal studies provided direct evidence for the circulation of malignant lymphoid cells to the thoracic duct of rats with either leukemia or LSA, but this phenomenon appeared to be quantitatively more significant in rats with LL.

INTRODUCTION

It has been established that lymphocytes in normal rats continually recirculate from blood to lymph nodes and then back to blood via the thoracic duct (4, 5, 8). This recirculation of lymphocytes provides a mechanism whereby immunologically competent cells from the total circulating pool may be delivered to any regionally stimulated lymph node. The postcapillary venule within the lymph node is the critical structure through which lymphocytes migrate to leave the circulatory system and enter sinusoids of the lymph node. Galton (3) has suggested that a basic difference between patients with LSA3 and CLL may be in the pattern of circulation of lymphoid cells in these 2 diseases. According to this hypothesis, in CLL, lymphocytes follow the normal recirculation pathway, resulting in a physiological dissemination of cells and the early appearance of generalized lymphadenopathy; in LSA, lymphoid cells do not recirculate normally, and hence LSA may retain its regional nature until the advanced stages of the disease (3).

In this report, the circulation of lymphoid cells in the thoracic duct lymph of rats inoculated with MSL was investigated. The biological behavior of MSL is such that an i.m. inoculation yields a localized tumor which resembles LSA, while i.v. inoculation results in LL (10, 13).

MATERIALS AND METHODS

Wistar albino male rats weighing 100 to 150 g were obtained from the Canadian Breeding Laboratories, St. Constant, La Prairie City, Quebec, Canada. Four groups of rats were investigated, including uninoculated normal rats, rats inoculated i.v. with 2 X 10⁶ splenic lymphocytes, rats inoculated i.m. with MSL (the LSA group), and rats inoculated i.v. with the same tumor (the LL group).

The MSL was obtained from Dr. I. Kline, Microbiological Associates, Bethesda, Md., and has been maintained by serial i.m. transplantation every 10 to 12 days in Wistar male rats. Tumor cell suspensions were prepared from fragments of solid tumor removed aseptically, finely minced, and homogenized in 2 to 3 ml of balanced salt solution. The homogenate was strained through sterile gauze and centrifuged at 800 rpm for 3 min, and the cells were resuspended in Fischer medium to give a final concentration of 10⁷ cells/ml. Trypan blue dye exclusion showed that 98% of the cells were viable. Rats assigned to the LSA group were inoculated i.m. in a lower limb with 500 to 1000 cells, and those in the LL group were given injections of 2 X 10⁶ cells in the lateral tail vein. Rats inoculated i.m. developed a localized 2 to 3 cm tumor 7 to 10 days following the injection which showed the microscopic features of LSA. Approximately 7 to 10 days after an i.v. inoculation, rats developed generalized lymphadenopathy, hepatosplenomegaly, subcutaneous nodules, and a blood picture indicative of LL with lymphocytosis and immature lymphoid cells in the peripheral blood.

Thoracic duct fistulae were established in the 4 groups of rats following the method of Bollum et al. (1) and continuous lymph drainage was maintained. In rats with LSA and LL, fistulae were established 7 to 10 days after tumor transplantation. The rats were restrained in individual cages and fed fox chow and water. Daily total and differential leukocyte counts were performed on both lymph and blood.

Since morphological criteria alone are inadequate to distinguish between malignant lymphoid cells and large lymphocytes in the thoracic duct, chromosomal studies were done on thoracic duct cells collected from both normal and tumor-
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bearing rats and on the tumor homogenate. To stimulate mitosis and thus facilitate chromosomal analysis, normal rats were immunized with two 1.0-ml injections of human blood group AB serum at 2-week intervals; tumor-bearing rats were not immunized. Chromosome preparations were made either on thoracic duct lymphocytes taken directly from normal and tumor-bearing rats 2 hr after an i.v. or i.p. injection of 0.4 ml of 0.1% Colcemid (CIBA Pharmaceutical Co., Summit, N. J.) or on lymphocytes cultured in tissue culture Medium H-597 (Connaught Medical Research Laboratories, Toronto, Ontario, Canada) containing 4 μg/ml Colcemid for 5 hr at 37°. Chromosomal analysis was performed following the method of Yoshida et al. (16).

RESULTS

The maximum total leukocyte counts in the 4 groups of rats are presented in Chart 1. The leukocyte counts of tumor-bearing rats were higher than those in either control group (p < 0.01), and rats with leukemia had significantly higher counts than those with LSA (p < 0.01).

The maximum number of lymphoblasts and prolymphocytes in the blood of the 4 groups of rats are shown in Chart 2; significantly higher counts were observed in leukemic rats than in the other 3 groups (p < 0.01). Rats with LSA had higher immature cell counts than both control groups, but the difference was significant only in comparison with uninoculated normal rats (p < 0.05); no significant difference was noted between the 2 control groups.

The daily output of thoracic duct lymphocytes in control and tumor-bearing rats is illustrated in Chart 3. The output of lymphocytes on the 1st day following fistula was greater in rats with LSA than in the other 3 groups. A more rapid decline in lymphocyte output was observed in rats with LSA, so that after 3 days of lymph drainage output was similar in all 4 groups. The daily volume of lymph flow is also shown in Chart 3; the rate of lymph flow varied widely and was influenced by such factors as age of the rat, thoracic duct caliber, state of hydration, and displacement or kinking of the cannula.

Thoracic duct lymphocytes were classified on the basis of cell diameter into small (7 to 10 μ) or large lymphocytes (>10 μ). On the 1st day of lymph drainage, the output of large lymphocytes was highest in rats with LSA (Chart 4); however, output declined progressively in all rats except those with leukemia, so that by the 3rd day leukemic rats exhibited the highest output.

Since daily output of thoracic duct lymphocytes is dependent on lymph flow, a highly variable function, it was decided a more reliable index of large lymphocyte output could be derived from the percentage of lymphocytes in thoracic duct lymph, a measure independent of lymph flow (Chart 5). Analysis of variance revealed that the percentage of large lymphocytes was higher in leukemic rats than in untreated normal rats (p < 0.001), rats inoculated with splenic lymphocytes (p < 0.01), or rats with LSA (p < 0.01). The percentage of large lymphocytes in the lymph of rats with LSA was also higher than that of normal rats (p < 0.01) or rats inoculated with normal lymphocytes (p < 0.05).
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Chart 3. Daily output of total lymphocytes (top) and volume of lymph flow (bottom) in the thoracic duct of 4 groups of rats: ○, untreated normal rats (mean ± S.E. of 4 rats); ⋄, rats inoculated with 2 x 10⁶ splenic lymphocytes i.v. (mean ± S.E. of 4 rats); ♦, rats inoculated with 3 to 6 x 10⁶ MSL cells i.m. (LSA group); and □, rats inoculated with 2 x 10⁶ MSL cells i.v. (LL group), mean ± S.E. of 7 rats each.

Chart 4. Daily output of large lymphocytes in thoracic duct lymph of the 4 groups of rats shown in Chart 3.

Chart 5. Percentage of large lymphocytes in thoracic duct lymph of the 4 groups of rats shown in Chart 3.

Chromosomal preparations of cells from the thoracic duct of 3 unimmunized normal rats and 3 rats treated with up to 4 weekly injections of phytohemagglutinin showed no mitotic figures, confirming other reports (9, 12). In 4 rats immunized with human AB serum, the mitotic index on 500 to 1000 thoracic duct cells ranged from 0.2 to 1.0%, a result also consistent with previous studies (2, 15). An idiogram of a normal diploid cell from the lymph of an immunized rat is illustrated in Fig. 1. The normal diploid pattern of 42 chromosomes was observed in 13 of 15 cells analyzed, 2 cells had 41 chromosomes, and none of the cells showed chromosomal abnormalities.

Examination of 1000 cells in a tumor homogenate of MSL revealed a mitotic index of 7.2%; chromosomal analysis showed that 9 of 14 cells contained 39 chromosomes, including a single metacentric marker chromosome (Fig. 2). In addition to aneuploidy, occasional near-haploid and tetraploid cells were observed; similar chromosomal abnormalities have been reported in other tumors (11, 14).

The mitotic index on 1000 cells in thoracic duct lymph cultures obtained from 4 leukemic rats and 2 with LSA ranged from 0.4 to 1.2%; cells with chromosomal features identical to those in the tumor homogenate were found. In leukemic rats, 4 of 20 cells contained 39 chromosomes including the marker chromosome (Fig. 3); 1 hypotetraploid cell, 2 near-haploid cells, 1 cell each with 41 and 43 chromosomes, and 11 normal diploid cells were also noted. In rats with LSA, 2 of 8 cells...
examined had 39 chromosomes, including the marker chromosome, 1 cell had 25 chromosomes, and the remaining 5 cells contained a normal complement of 42 chromosomes.

DISCUSSION

The experimental model of Wistar rats inoculated with MSL provides an opportunity of comparing the circulation of malignant lymphoid cells in LL and LSA. MSL is a chemically induced tumor produced by repeated s.c. injections of 1,2,5,6-dibenzanthracene into Wistar rats (10, 13). The site of inoculation, to a large degree, determines the pathological manifestations of the transplanted tumor. Although a direct comparison with CLL or LSA in humans is not justified, interesting information on the circulation of malignant lymphoid cells may be obtained.

This investigation illustrates that malignant lymphoid cells circulate to the thoracic duct of rats with either LL or LSA, but the phenomenon appears to be of greater magnitude in leukemic rats. The percentage of large lymphocytes in thoracic duct lymph of rats with either leukemia or LSA was higher than that of normal rats and was greater in leukemic rats than in those with LSA. Some of the large lymphoid cells circulating in the thoracic duct lymph of rats with leukemia and LSA showed the morphological features of MSL cells. However, strict reliance upon morphological criteria was considered inadequate, and therefore chromosomal studies were undertaken to provide definitive identification of thoracic duct cells.

Chromosomal analysis of thoracic duct cells obtained from rats with leukemia and LSA showed the same chromosomal anomalies observed in MSL cells. Thus, the chromosomal data provide direct evidence for the circulation of malignant lymphoid cells in the thoracic duct of rats with leukemia and LSA.

The reason for the greater output of lymphocytes in thoracic duct lymph of rats with LSA compared to leukemic rats is not entirely clear (Charts 3 and 4). As alluded to previously, this may be an artifact due to variations in the rate of lymph flow. Alternatively, the lymphocytosis may represent an immunological response to tumor-specific antigens comparable to that described for other chemically induced tumors (7); however, further investigation is required to clarify this point.

Although the experimental model used has its limitations, it does allow investigation of LL and LSA in an animal in which the spontaneous occurrence of lymphoproliferative disease is extremely rare (10). The findings of this study indicate that circulation of malignant lymphoid cells via the thoracic duct occurs both in rats with LL and LSA, but this appears to be quantitatively more significant in rats with LL.

Fig. 1. An idiogram of a normal lymphocyte in metaphase arrest (insert) obtained from thoracic duct lymph of a rat immunized with human AB serum, showing a normal diploid pattern of 42 chromosomes. Lymph was collected for a 2-hr period from 2 to 4 hr after an i.p. injection of 0.4 ml of 0.1% Colcemid (CIBA).

Fig. 2. An idiogram of a tumor cell from a tumor homogenate of MSL showing 39 chromosomes including a single metacentric marker chromosome (arrow). The tumor homogenate was incubated in tissue culture Medium H-597 containing 4 μg/ml of Colcemid (CIBA) at 37° for 5 hr.

Fig. 3. Metaphase preparation of a tumor cell in the thoracic duct lymph of a leukemic rat showing 39 chromosomes with a metacentric marker chromosome (arrow), identical to the chromosomal changes found in the tumor homogenate (compare with Fig. 2). Lymph was collected for a 2-hr period from 2 to 4 hr after an i.p. injection of 0.4 ml of 0.1% Colcemid (CIBA).

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