Chromosome Studies on Transplanted and Virus-induced Lymphoid Leukemias in Rats

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Summary

The i.p. transplantation of viable leukemic cells from AKR mice to newborn W/Fu rats resulted in 2 manifestations of lymphoid leukemia. An early form was characterized by growth of the transplanted leukemic cells in the abdominal cavity. Late leukemias were thymic lymphosarcomas with or without leukemic blood changes (12). Late leukemias were previously considered viral tumors induced by the leukemia virus of Gross (9). To further substantiate this hypothesis, leukemic cells from female AKR mice were transplanted to male and female newborn W/Fu rats, and the chromosomes of early and late leukemias were analyzed. The chromosomes of leukemic cells from the early leukemias had the telocentric configuration and female sex constitution of the transplanted mouse cells. In contrast, the chromosomes of leukemic cells from the late leukemias were morphologically rat chromosomes, and their sex constitution corresponded with the sex of the recipient. These findings indicated that late, thymic leukemias originated from rat cells probably by virus release from the grafted mouse leukemic cells with infection and malignant transformation of rat thymic cells.

Introduction

"Early" and "late" lymphoid leukemias are elicited in rats when viable leukemic cells from AKR mice are injected i.p. into newborns at birth and 2 weeks later (11-13). Less than half of the recipients die after 4-8 weeks from growth of the transplanted leukemic cells in the peritoneal cavity. Most rats seem to reject the heterologous cells, but some of the survivors develop late leukemias 12-24 weeks after birth. The primary site of tumor development in late leukemias is invariably the thymus from which these lymphosarcomas disseminate to involve the lymphatic system, blood, and other organs. Cell grafts from the late, thymic leukemias are consistently rejected by the original donor strain of AKR mice but grow in adult isologous rats, whereas cells from early leukemias are readily transplantable to AKR mice (12). These findings suggest that early leukemias represent donor cells multiplying in the heterologous host, whereas late leukemias are composed of recipient cells transformed by the leukemogenic virus of Gross (9). If this assumption is correct, leukemic cells from the early leukemias should have the number, configuration, and sex chromosomes of mouse cells, and late leukemias should be identical with rat cells. We now present chromosome analyses of the 2 leukemic manifestations which show that this is indeed the case.

Materials and Methods

Animals

The origin of the inbred AKR mouse colony now in generations F₀ to F₃₀ of continuous brother-sister mating and of the inbred Wistar/Furth (W/Fu) rats has been described (11, 12).

Isologous Tumor Transplants

A cell suspension from the pooled thymus and lymph nodes of 5 female AKR donor mice with spontaneous lymphoid leukemia was injected i.p. into female, 6-week-old AKR mice (11). One group of 10 AKR mice received 30 × 10⁶ viable leukemic cells per mouse, a 2nd group of 10 AKR mice received 10 × 10⁶ leukemic cells per mouse. The 2 groups were killed by cervical dislocation 14 or 28 days later. A cell suspension was prepared of the combined abdominal tumor masses and ascites which was used for inoculation into newborn W/Fu rats and for chromosome studies.

Preparation and Injection of Leukemogenic Cell-free Filtrate

The pooled thymus and lymph nodes from 2 male and 3 female leukemic AKR mice were employed to prepare a cell-free extract by filtration through a Selas 02 filter candle (12). The cell-free filtrate was centrifuged in a Spinco ultracentrifuge, Model L (No. 40 head) at 104,000 × g for 1 hr at 4°C. The pellet was diluted 1:10 in buffered saline and immediately injected into 36 newborn W/Fu rats. The 34 rats surviving for 3 days were given reinjections of the same filtrate preparation on Day 28 post-partum (12). The leukemias resulting from filtrate injections will be referred to as virus-induced leukemias.

Preparation of Chromosomes

Mice and rats with clinical signs of leukemia were given 0.5-0.75 ml of 0.025% colchicine i.p. 1-2 hr before they were killed by cervical dislocation. Thymus and cervical lymph nodes were removed aseptically from AKR mice with spontaneous leukemia and from W/Fu rats with late or virus-induced leukemia. The ascites and intraabdominal tumors were used for chromosome preparations from AKR mice carrying the isologous leukemic transplant. Sections for histologic examination were obtained

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from all animals that were used for chromosomal studies in order to assess the number of nonleukemic cells within the leukemic tissues. The supportive stroma in the leukemic tissues was minimal, and the chance to analyze nonleukemic cells of host origin instead of leukemic cells was therefore considered remote.

The leukemic tissues were finely minced with scissors in Earle's or Tyrode's solution and repeatedly pipetted. Larger fragments either were allowed to settle out or were removed by short centrifugation at 1000 rpm for 5 min. The cloudy supernatant was washed with Earle's solution and again centrifuged at 1000 rpm for 10 min, and the pellet was resuspended in a hypotonic mixture of cold 0.75% sodium citrate for 20 min. The cells were then centrifuged at 3000 rpm for 5 min and fixed in acetic-alcohol (3 parts methyl alcohol to 1 part glacial acetic acid). Airdried chromosome preparations were made according to the method of Moorhead et al. (16). To minimize technical errors inherent in chromosome analyses, only metaphases with intact margins and circular configuration were used for analyses.

Results

Transplantation and Viral Induction of Leukemia in W/Fu Rats

Leukemic cells from the isologous transplant in AKR mice were injected i.p. into each of 32 W/Fu rats at birth and 14 days later (Table 1). Ten of the 31 surviving rats died from early leukemias after 4-5 weeks, whereas 8 rats died from late leukemias after 13-18 weeks. Morphologically, early leukemias were characterized by growth of the grafted cells in the abdominal cavity, and late leukemias were typical thymic lymphosarcomas. The injection of a leukemogenic cell-free filtrate resulted in deaths of 7 of 34 rats from thymic lymphosarcomas, with or without leukemic blood changes, after 7-16 weeks postpartum (Table 1). These findings confirmed previous results obtained in this laboratory (11-13).

Chromosome Studies

It was the purpose of this study to identify the numerical distribution, species-specific morphology, and sex chromosomes of early and late leukemias in W/Fu rats. No attempts were made to characterize chromosomal abnormalities in individual cells of the donors. Detailed karyotypes of primary and transplanted leukemias in AKR mice have been presented by others (4, 24). Cells from 7 rats with early leukemia, 7 rats with late leukemias, and 6 rats with leukemia induced by cell-free filtrates were analyzed.

Most leukemic cells from the primary (67%) or isologously transplanted (86%) leukemias apparently had the normal diploid mode (2n = 40) and the telocentric configuration characteristic for mice (Table 2). The commonest aneuploid value was 41 chromosomes, which was most frequently obtained from thymic leukemic cells. The sex chromosomes in leukemic cells of spontaneous and transplanted AKR mouse leukemias were determined according to the differences in the number of small chromosomes described for female and male mouse cells (21). Female

<table>
<thead>
<tr>
<th>TYPE OF LEUKEMIAS AND HOST</th>
<th>TISSUES</th>
<th>NO. OF ANIMALS USED</th>
<th>NO. OF CELLS COUNTED</th>
<th>PERCENTAGE OF CELLS WITH INDICATED CHROMOSOME NUMBER*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Spontaneous leukemia in AKR mice</td>
<td>Thymus, lymph nodes</td>
<td>4</td>
<td>54</td>
<td>5</td>
</tr>
<tr>
<td>Isologously transplanted leukemia in AKR mice</td>
<td>Ascites, solid tumors</td>
<td>6</td>
<td>49</td>
<td>2</td>
</tr>
<tr>
<td>Early leukemias in W/Fu rats</td>
<td>Ascites, solid tumors</td>
<td>4</td>
<td>3</td>
<td>53</td>
</tr>
<tr>
<td>Late leukemia in W/Fu rats</td>
<td>Thymus, lymph nodes</td>
<td>3</td>
<td>4</td>
<td>116</td>
</tr>
<tr>
<td>Virus-induced leukemia in W/Fu rats</td>
<td>Thymus, lymph nodes</td>
<td>3</td>
<td>3</td>
<td>83</td>
</tr>
<tr>
<td>Nonleukemic W/Fu rats</td>
<td>Thymus, lymph nodes</td>
<td>2</td>
<td>3</td>
<td>64</td>
</tr>
</tbody>
</table>

* The numbers in parentheses refer to the actual number of cells found with the chromosome set indicated.

TABLE 1

<table>
<thead>
<tr>
<th>INOCULUM</th>
<th>NO. OF NEW-BORN RATS INOCULATED</th>
<th>EARLY LEUKEMIAS</th>
<th>LATE LEUKEMIAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Isologous leukemic transplant in AKR mice</td>
<td>36</td>
<td>7/34</td>
<td>4</td>
</tr>
<tr>
<td>Leukemogenic cell-free filtrate from AKR mice</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* L.P., latent period from virus injection to death from leukemia, in weeks.

a A cell suspension from the ascites and solid tumors of an isologous leukemic cell transplant in AKR mice was inoculated into newborn W/Fu rats. Each newborn received approximately 30 X 10^6 viable cells i.p. at birth and on Day 14 after birth. The donor cells for the 1st and 2nd injections were derived from the same transplant.

b A cell-free filtrate was prepared from leukemic AKR mice and injected into newborn W/Fu rats (0.1 ml/rat).
mouse cells are characterized by 2 short chromosomes of equal size which are significantly shorter than the 2nd smallest homologous pair, whereas male cells of mice contain 3 small chromosomes. Fifty-three leukemic cells from the ascitic fluid and abdominal tumors of 4 female and 3 male rats with early leukemia were examined. The prevailing chromosome number of these leukemic cells was 40, and the chromosomes had a typical telocentric configuration. Moreover, all 43 diploid leukemic cells had the female sex constitution as evidenced by the presence of 2 short chromosomes (Fig. 1). Thus, early leukemias in rats were composed of tumor cells which were cytogenetically mouse cells.

The chromosome number in normal diploid cells of W/Fu rats was 42 (Table 2) (8, 22). Between 50 and 71% of the leukemic cells from the late and virus-induced leukemias were apparently diploid; the remainder had chromosome numbers which ranged from hypodiploid to polyploid. Aneuploidy in lymphoid leukemic cells of a virus-induced lymphoma in mice was previously described by Tsuchida and Rich (23). Only diploid leukemic cells were used for karyotype analyses. The karyotypes were arranged according to the position of the centromere as proposed by Tjio and Levan (22). The y chromosome was identified as one of the smallest chromosomes with a terminal centromere (8, 22). The x chromosome belonged to the group of large chromosomes with a terminal centromere (8, 22). The chromosome morphology in 20 metaphase plates from diploid leukemic cells of late leukemias was typical of rats. In addition, the sex chromosome constitution corresponded in each case with the sex of the rat from which the chromosome preparation was obtained. For example, the leukemic cells of 4 male rats which had received an inoculation of female leukemic mouse cells had the xy sex constitution (Fig. 3). Conversely, the leukemic cells of 3 female rats with late leukemia were all female (Fig. 4). The leukemic cells of virus-induced leukemias in rats showed variations in numerical chromosome distribution similar to those of the late leukemias (Table 2). No consistent karyotypic abnormalities have been found in the late and virus-induced leukemic cells as have been reported for a viral, granulocytic murine leukemia (25).

Discussion

In the experiments reported here the species-specific differences between mouse and rat chromosomes were used to further substantiate our contention that early leukemias represented tumor grafts whereas late leukemias were induced by the leukemia virus of Gross (11-13). Only female leukemic mouse cells were employed as donor cells. The chromosomes of leukemic cells from all early leukemias were found to have the telocentric configuration, female sex, and modal characteristic of the transplanted mouse cells. In contrast, the chromosomes of cells from late leukemias were morphologically rat chromosomes and the sex constitution of late-leukemic cells corresponded with the sex of the recipient rather than the donor. This indicated that late leukemias were virus induced and not the result of proliferation of the injected cells.

It is now well established by electron microscopic, bioassay, and tissue culture studies that virus-induced murine leukemia cells can release virus for prolonged periods of time (5-7, 9, 10, 12, 14, 15, 17, 19, 20). The inoculation of a large number of viable leukemic cells into the newborn rat provides a favorable environment for the multiplication of the grafted cells. Even under optimal experimental conditions only less than half of the rats die within 1-2 months from the intraabdominal tumor grafts. The transplanted cells presumably release leukemogenic virus which infects and transforms host cells into leukemic cells. Some of the rats that reject the leukemic mouse cells consequently develop late leukemias. However, it is conceivable that late leukemias are caused by leukemia virus already present extracellularly in the cell suspensions rather than by virus released from the grafted cells. The present experiments do not provide evidence for or against this explanation. Earlier findings suggest that the frequency of late leukemias depends in part on the dosage of the cells inoculated, because late leukemias fail to occur when low concentrations of leukemic mouse cells are injected twice into rats or when a single inoculation of a large number of leukemic cells is given to the newborn (11, 12).

Sex chromosomes have been used as markers in homologous transplants of tumor cells induced by the virus of avian myeloblastosis and Rous sarcoma virus (1, 18). The results resemble ours in that the donor cells are rejected in a homograft reaction but virus released from the transplanted cells induces tumors which originate from recipient cells. Bergs and Groupe (2) have reported that during isologous and homologous transfer of Rous sarcoma cells in turkeys only transplants carrying appreciable amounts of infectious virus produced tumors in all birds inoculated. Spread of the neoplastic process by viral infection and subsequent neoplastic transformation of new target cells in the host appears to be a general characteristic of tumors induced by oncogenic RNA viruses (3). The role of this phenomenon in murine leukemogenesis in the natural host has yet to be elucidated.

Acknowledgment

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References

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FIG. 1.—Leukemic cell obtained from the abdominal tumor of a male rat with early leukemia. At birth and 14 days later the rat was given an injection of leukemic cells from female AKR mice. The chromosomes of this leukemic cell are telocentric and the sex chromosomes (indicated by arrows) are female.

FIG. 2.—Leukemic cell from the thymus of a male rat with late leukemia. At birth and 14 days later the rat was given an injection of leukemic cells from female AKR mice. The chromosomes of the leukemic cell are rat chromosomes by virtue of the 3 groups with terminal, subterminal, and median-submedian centromeres. The sex chromosomes are male (indicated by arrows).
Fig. 3.—Karyotype of a diploid leukemic cell from the thymus of a male W/Fu rat with late leukemia. At birth and 14 days later the rat had been given an injection of leukemic cells from female AKR mice. The leukemic cell has the diploid mode (2n = 42) and chromosome configuration of rats; the sex chromosomes correspond with the sex of the recipient.

Fig. 4.—Karyotype of a diploid leukemic cell from the thymus of a female W/Fu rat with late leukemia. The sex constitution of this cell is female.
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