Studies on leukemia developing spontaneously in an inbred family of rats

(spontaneous rat leukemia/genetic transmission)

LUDWIK GROSS AND YOLANDE DREYFUSS
Cancer Research Unit, Veterans Affairs Medical Center, Bronx, NY 10468
Contributed by Ludwik Gross, March 8, 1993

ABSTRACT This study is a continuation of our recently reported observations on leukemia and lymphomas developing spontaneously in a subline of Sprague–Dawley rats bred by brother-to-sister mating in our laboratory. The previous preliminary report described our observations made in the course of the initial 12 generations of our leukemic subline. The current study reviews the data collected during 8 additional generations and results of experimental and morphologic studies. There was no problem in transmitting the spontaneously developing leukemia by inoculation of suspensions of leukemic cells into newborn or very young suckling Sprague–Dawley rats. Attempts to transmit the disease by inoculation of cell-free, filtered leukemic extracts gave thus far positive results only in one experiment in which two of six inoculated rats developed leukemia and a third one developed an angiosarcoma on the neck. In six additional experiments, of a total of 37 rats inoculated with leukemic filtrates, none developed leukemia and 9 females developed mammary fibroadenomas. Reviews of microscopic slides of blood and of sections of lymphoid tumors, livers, spleens, kidneys, and fragments of bone marrow of the leukemic animals are discussed as well as electron microscopic studies.

From a nucleus of random-bred Sprague–Dawley rats received in 1960 from the National Institutes of Health, a colony of rats has been raised in our laboratory by brother-to-sister mating. Unexpectedly, about 5 years ago, we observed a relatively high incidence of leukemia and lymphomas developing spontaneously in successive generations of untreated male and female descendants of an untreated female (1). The incidence of leukemia or lymphomas developing spontaneously continued to be high in several additional generations thus far observed beyond the level previously reported (Table 1) and was substantially higher than that observed in rats of our healthy colony of Sprague–Dawley rats. Many of the animals reported in this study are still relatively young at the time of the preparation of this manuscript, and some of them that are reported to be healthy in our Table 1 may be expected to develop leukemia.

MATERIALS AND METHODS
Sprague–Dawley rats, bred by brother-to-sister mating in our laboratory and descendants of female no. 1 described in our previous publication (1), were used in this study. No animals from outside sources were added. These animals were not treated. They were observed for symptoms of weakness or anemia or development of tumors or leukemia. Those that remained healthy were observed until they reached ~19 months of age. They received Purina Lab Chow ad lib and water.

RESULTS
The relatively high incidence of leukemia and/or lymphomas developing spontaneously in descendants of our leukemic subline of Sprague–Dawley rats continued in successive generations beyond the level indicated in our previous publication (1). This incidence varied (Table 1), was slightly lower than that observed in the initial 12 generations, but was still substantially higher than that observed in our regular colony of Sprague–Dawley rats, which was 1.6% in females and 1.2% in males (2).

The great majority of leukemias developing in rats of our leukemic subline continued to be of the myelogenous form, with high or very high peripheral blood cell counts (frequently as high as 300,000 to 400,000 cells per cc, including many myeloblasts, promyelocytes, and myelocytes), very advanced anemia, and the presence of nucleated red cells. Less frequently, leukemia developing spontaneously in the rats was lymphatic, with the replacement of the thymus and peripheral lymph nodes by large lymphoid tumors and the development of large mesenteric tumors.

Slides of organs removed from a group of 43 leukemic rats (22 females and 21 males) were checked recently by T. Faraggiana (previously at Mount Sinai School of Medicine, City University of New York; presently at the Department of Experimental Medicine, University of Rome). The organs reviewed were livers, spleens, kidneys, thymuses, and mesenteric lymph nodes. All of them had diffuse, heavy infiltrations with leukemia cells. Microscopic slides of bone marrows were also examined and were found to be infiltrated with leukemia cells and their precursors.

Leukemia or lymphomas that developed spontaneously in rats of our leukemic subline could be readily transmitted by cell suspensions inoculated subcutaneously into newborn or suckling (less than 7 days old) Sprague–Dawley rats.

Of 14 inoculated rats, 10 developed lymphoid tumors at the site of inoculation after latency varying from 25 to 43 days. The tumors grew progressively and induced generalized disease. Four inoculated rats remained negative during the observation time of 9 months.

Attempts to transmit the spontaneously developing leukemia or lymphomas by inoculation of cell-free, filtered (Selas 02), extracts into newborn or suckling, <7-day-old Sprague–Dawley rats of our non-leukemic colony gave negative results, except for one experiment described below.

Leukemia that developed spontaneously in a rat of our leukemic subline was transplanted by cell graft two consecutive times. From leukemic organs of the animal that developed lymphomas resulting from the second successive leukemia cell transplantation, a cell-free, filtered (Selas 02) extract was prepared and inoculated into six suckling, 4-day-old Sprague–Dawley rats; among the inoculated animals, two females developed leukemia after a latency of 9 and 16 months, respectively; one female developed an angiosarcoma on the neck at 11 months of age; the remaining three
Table 1. High incidence of leukemia developing spontaneously in rats of an inbred Sprague-Dawley subline

<table>
<thead>
<tr>
<th>Generation</th>
<th>Total no. of rats</th>
<th>Rats developing leukemia</th>
<th>Rats developing tumors</th>
<th>Negative rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>Age, months</td>
<td>No.</td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td>3</td>
<td>50</td>
<td>9.5</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>5</td>
<td>38</td>
<td>11.4</td>
</tr>
<tr>
<td>13</td>
<td>6</td>
<td>5</td>
<td>45</td>
<td>1.5</td>
</tr>
<tr>
<td>14</td>
<td>17</td>
<td>8</td>
<td>47</td>
<td>17</td>
</tr>
<tr>
<td>15</td>
<td>14</td>
<td>2</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>16</td>
<td>17</td>
<td>2</td>
<td>12</td>
<td>6.5</td>
</tr>
<tr>
<td>17</td>
<td>19</td>
<td>13</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>18</td>
<td>18</td>
<td>8</td>
<td>25</td>
<td>7</td>
</tr>
<tr>
<td>19</td>
<td>18</td>
<td>9</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>20</td>
<td>12</td>
<td>3</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>118</td>
<td>33</td>
<td>30</td>
<td>3.5</td>
</tr>
</tbody>
</table>

The initial 12 generations were reviewed in a previously published table (1). Generations 11 and 12 were repeated here because additional animals developed leukemia. Figures here reported do not always represent the actual number of animals in each litter. Some of the animals were discarded at the time of weaning.

In six additional experiments with six individually prepared filtered extracts from spontaneously developing leukemia, a total of 31 suckling, 4- to 7-day-old Sprague-Dawley rats were inoculated. Among the inoculated animals, none developed leukemia during the time of observation varying from 8.5 to 21.5 months (average 15.5 months); 8 females developed mammary fibroadenomas at an average age of 16 months. Results of these experiments, although interesting, are not conclusive and require additional studies.

Extensive electron microscopic studies of leukemic tumors as well as fragments of spleens, livers, and bone marrows, carried out by Dorothy Feldman (previously for over 20 years a member of our staff; presently at Hoffmann-La Roche, Nutley, NJ), did not reveal the presence of virus particles. Among other unpublished studies carried out by Ray Price (personal communication) and his coworkers at the Laboratory of Tumor Biology and Connective Tissue Research at this medical center, bone marrow cell cultures have been established from both leukemic and normal control rats.

Cultures of healthy rats were characterized by rapid proliferation of stromal cells and lasted 3-4 months. However, bone marrow cells from leukemic rats have been in continuous culture for >6 months, some of them producing leukocytes at a constant, slow rate. No culture has reacted to recombinant colony-stimulating factors (IL-3 or GM-CSF).

Karyotyping of cells from bone marrow (3 leukemia and 1 normal, but from the high-leukemic rat subline) has not detected any difference between normal and leukemia cells.

**DISCUSSION**

As a working hypothesis, we assume that leukemia developing spontaneously in our subline of Sprague-Dawley rats is of viral origin. The fact that thus far we have not been able to identify the virus either by cell-free transmission experiments or by electron microscopic studies is disappointing but does not exclude the assumption that this disease is of viral origin.

As an example we refer to bovine leukosis, which is known to be of viral origin; yet the viral etiology was difficult to establish, since attempts to detect virus particles in blood and organs of cattle suffering from leukemia did not give positive results until Janice Miller and her coworkers (3) noticed unexpectedly that blood cells, removed from leukemic cattle and kept in vitro for a few days, produced in abundance characteristic virus particles representing presumably the causative bovine leukosis virus. In another study, we have tried for several years with Robert Marshak, J. Ferrer, and coworkers (Department of Veterinary Medicine, University of Pennsylvania) to transmit bovine leukemia by cell-free extracts to newborn calves with disappointing, negative results. An unexpected accidental transmission of bovine leukosis to sheep was reported by K. Enke (4), who inoculated a flock of 560 sheep with a vaccine against piroplasmosis consisting of defibrinated blood removed from a calf with piroplasmosis, whose mother died from leukemia. Before long, 40 of the 560 inoculated sheep unexpectedly developed leukemia, a disease not very common in that species. Who would have anticipated that sheep may be susceptible to inoculation of the bovine leukosis virus (5)?

We have spent several years trying with Dorothy Feldman to visualize in the electron microscope the parotid tumor—i.e., polyoma virus. Our attempts, as well as those of several other investigators, did not give positive results until this small DNA virus was recognized in electron microscopic studies almost simultaneously and independently in 1959 in several laboratories (6). It was soon realized that innumerable polyoma virus particles can be observed in certain organs, such as the kidneys, following virus inoculation into suckling hamsters, which are highly susceptible to the inoculation of the polyoma virus; however, as soon as tumors, predominantly sarcomas, develop in the infected kidneys or other
susceptible virus-infected tissues, no virus particles can be detected anymore (6).

It is very possible, although it still remains to be proven, that the leukemia-causing virus in rats (and possibly also in several other species, including humans) may often, but not always, be integrated in the genome, become in this way an integral part of the DNA sequences, and thus be transmitted from one generation to another.

The search for the causative etiological factors (including viruses) in the study of tumors, leukemias, and certain other pathologic conditions that have a tendency to develop in successive generations of affected families in animals and in man is full of surprises and unexpected observations; it requires persistence and a prepared mind, ready to accept unexpected, revealing observations. Experimental transmission experiments, electron microscopy, and molecular biology are only some of the tools. Serological methods may also recognize the viral etiology of leukemia, a method now routinely used in cattle (5) and also used more recently by Poiesz, Gallo, and their associates, who demonstrated the viral etiology of a form of leukemia in humans (7).

We thank Dr. Dorothy Feldman for the electron microscopic studies of leukemic tissues, and Prof. Dr. Tullio Faraggiana for review and interpretation of pathologic slides. This study was supported by the American Cancer Society grant RD-337-1, the Cancer Research Institute, and the Veterans Affairs Medical Research Service.