Characterization of an Influence Affecting Growth of Transplantable Leukemias in Mice*

L. W. Law, Ph.D.

(From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.)

(Received for publication August 18, 1943)

It has been shown in previous communications (1, 2) that an influence transmitted in the milk of certain lactating female mice was effective in influencing the growth of lymphoid leukemias (lines LL449 and P1543) in mice normally refractory to their growth. This "susceptibility influence" was found to be present during the first 10 days of the lactation period. The influence was found to be transmitted to second generation mice obtained by mating littermates of refractory foster-nursed mice. Total deprivation of "susceptible" milk in mice of the susceptible strain did not affect the response of these mice to inoculations of leukemic cells. A change in the growth capacity of the malignant cells of lymphoid leukemias, lines LL449 and P1534, whereby leukemic cells could be grown by serial transfer in totally refractory hosts was observed. This change occurred following initial growth of the malignant cells in refractory mice that were foster-nursed by mothers of the susceptible strain. Such serial transfers have subsequently been carried through 10 generations. These data suggested that the type of reaction provoked in refractory foster-nursed mice depended upon the quantity of "susceptibility influence" obtained.

In an attempt to learn if other leukemias as well as other types of tumors were affected by the same or similar influences a total of 14 transplantable malignant tumors were tested (Table I). Previous tests have been made on a few transplantable leukemias (1, 2). Malignant tumors arising in one inbred strain were inoculated into other inbred strains. The inbred strains of mice used throughout were A, C, L, C3H, C57 black, C57 brown, and dba. Several sublines of some of the inbred strains were used, particularly within the dba and C57 black strains. At least 50 refractory control mice and 50 refractory foster-nursed mice were inoculated with each tumor type. Refractory mice were foster-nursed by lactating mothers of the strain of origin of the malignant process, as described in a previous communication (1). The greatest percentage of takes occurred in foster-nursed mice of sublines of the strain of origin of the neoplasm but was not limited to these.1

Malignant tumors induced by carcinogens as well as those arising spontaneously were used. Transplantable tumors carried by serial transfer for many generations and tumors of recent origin also were tested. Myeloid leukemia, line C1498, was found to grow

![Table I: Types of Malignant Tumors Tested for Presence of "Susceptibility Influence"](https://example.com/table.png)

* (S)= spontaneous tumor; (I)= tumor induced by carcinogen.
† = See text.

---

1 Pronounced physiological differences are found to exist between sublines within inbred strains of mice particularly within the dba and C57 black strains.
in refractory hosts following foster nursing of refractory mice by lactating females of the susceptible strain.

The growth capacities of two other leukemias, lines LL593 and P1679, both lymphoid in origin, were possibly affected in a similar manner by an influence transmitted through the milk of susceptible mice. The numbers of experimental mice used here were smaller than in other series. Leukemia, line P1679, produced 100 per cent takes in subline 2 of the dba strain (subline of origin) and failed to grow in 50 subline 1 mice. On the other hand 35 per cent takes resulted following inoculations in dba, subline 1 mice foster nursed by dba subline 2 mothers (35 mice). Leukemia, line LL593, grew only in dba, subline 1 mice and failed completely to grow in mice of subline 2. Foster nursing of subline 2 mice by subline 1 mothers produced 25 per cent susceptibility (25 mice). Mice of other inbred strains, A, C, L, C3H, C57 black and C57 brown foster nursed by susceptible mothers remained refractory to inoculations of both lines of leukemia, LL593 and P1679.

No such influences transmitted in the milk were found to affect the growth capacities of 2 fibrosarcomas (line LL552 and line C252), a melanoma (line S91), a mammary adenocarcinoma (line dbrB), a monocytic leukemia (line LL336), 2 lymphoid leukemias (line P1682 and line P1592) and 2 myeloid leukemias (line P1591 and line LL493).

Leukemia C1498 arose in a female mouse, 10 months of age, of the B subline, C57 black inbred strain. The liver was greatly enlarged, gray, friable, but not nodular. The spleen was enlarged, pale, and nodular. All lymph nodes were found negative upon gross examination. Ascites was present. No blood count was obtained on this animal. The first 7 generations of serial transplant in B subline mice were carried by Dr. A. M. Cloudman, of this laboratory. This leukemia has now been carried through 25 transplant generations and shows the same typical growth characteristics.

Following the subcutaneous inoculation of leukemic cells from the liver or spleen a definite growth appears at the site of inoculation in 1 or 2 weeks. The liver and spleen show pronounced infiltration of cells myeloid in origin. In the gross these organs are extremely swollen, friable, marked with huge grayish nodules, and contain numerous hemorrhagic areas. There occurs at first an infiltration in the mesenteric lymph nodes, followed usually by infiltration in the lumbar and renal nodes and frequently by infiltration in the subcutaneous nodes. Considerable invasion has been seen in the lungs, kidneys, adrenals, and ovaries. Peripheral white blood cell counts were from 20,000 to 150,000 with approximately 25 per cent of the cells myeloid in origin, either myeloblasts or myelocytes, which give a positive peroxidase reaction.

Transplants of leukemic tissue into mice of inbred strains dba, C57 brown, A, C, L, C3H, and C and other sublines of C57 black failed to grow. Following foster nursing, however, some refractory mice of the C57 brown and dba strains showed a local growth of leukemic cells of leukemia, line C1498, with a subsequent regression in most cases. Mice of subline W of the C57 black strain that were refractory (3.5 per cent positive) proved to be 66.7 per cent susceptible following foster nursing by mothers of the B subline, C57 black strain (Table II). Sixteen of the 44 mice showed only a local, but progressive, subcutaneous growth of leukemic tissue, followed in late stages by invasion into surrounding subcutaneous lymph nodes and a moderate escape of myeloid cells into the blood stream. Regressions were infrequent. Most foster-nursed W subline mice developed the typical systemic leukemia.

The susceptibility influence has been found to be transferred by B subline mothers to suckling young of the W subline as late as the 18th day of lactation, indicating the presence of the influence throughout the lactation period.

Following transfer of the susceptibility influence to refractory mice by foster nursing, this influence was maintained in normally refractory mice by breeding. Three generations of W subline mice grew leukemic tissue of line C1498 myeloid leukemia without resort to subsequent foster nursing by B subline mothers.

In an attempt to identify this influence transferred through the milk of susceptible mothers, various tissue extracts were administered to young refractory mice by both feeding and inoculation. Leukemia, line C1498, was used. The data obtained are only preliminary because of interruption of this work.

It was found that liver, spleen, or mammary gland, homogenized and extracted with physiological saline, when fed to or inoculated into refractory mice from 10 days to 3 weeks of age resulted in the development of a susceptibility to growth of the leukemic cells of line C1498. The most potent extract was obtained from lactating mammary glands (Table III).

In order to standardize the test procedure 0.25 gram equivalent of tissue in physiological saline (0.25 cc.) was used throughout all tests. Refractory mice 3 weeks of age were used as test animals and the inoculations of tissue extract were made subcutaneously into the right axillary region. A single inoculation of tissue extract was given each test animal. In all cases 7 days following the inoculation of homogenized tissue extract the refractory mice were inoculated with leukemic cells of myeloid leukemia, line C1498. Transfer
of the influence by inoculation of 0.25 gram equivalent homogenized mammary gland extract resulted in 17 of 34 refractory subline W mice growing the leukemic cells of line C1498. In contrast, only 23.1 per cent W subline mice grew the leukemia following inoculations with homogenized splenic extract of male or female susceptible mice.

A series of tests were made using as a source of susceptibility influence mammary gland extract. Each separate test was controlled by uninoculated subline W mice, which in most cases were litter mates to animals showed complete systemic growth of leukemic cells, a certain heat stability is indicated.

4. The Seitz filter failed to remove all the susceptibility influence in mammary gland extract. Two separate tests were made and in each the filtered extract produced susceptibility to transplanted leukemic cells.

5. Desiccation of mammary gland and of leukemic tissue was accomplished under vacuum for 4 hours at room temperature. Extracts of desiccated mammary gland produced susceptibility in only 1 of 10

<table>
<thead>
<tr>
<th>Donor of leukemia cells</th>
<th>Recipient</th>
<th>Number of mice</th>
<th>Number positive</th>
<th>Per cent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57 black, B subline</td>
<td>C57 black, B subline</td>
<td>131</td>
<td>130+++; 1--</td>
<td>99.2</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>C57 &quot;&quot;, W &quot;&quot; fostered by C57 black, B subline</td>
<td>86</td>
<td>1+++; 3++; 82--</td>
<td>9.5</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td>66</td>
<td>28+++; 16++; 22--</td>
<td>66.7</td>
</tr>
</tbody>
</table>

* (++++) = systemic infiltration of leukemic cells; (+) = local progressive growth of leukemic cells; (--) = no growth.

<table>
<thead>
<tr>
<th>Material inoculated</th>
<th>Number of mice</th>
<th>Number positive</th>
<th>Per cent positive reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammary gland extract (0.25 gm. equiv.)</td>
<td>34</td>
<td>15+++; 2+</td>
<td>50.0</td>
</tr>
<tr>
<td>Splenic extract</td>
<td>13</td>
<td>3+++</td>
<td>23.1</td>
</tr>
<tr>
<td>Desiccated extract (C1498 leukemic tissue)</td>
<td>20</td>
<td>2+++; 3+</td>
<td>25.0</td>
</tr>
<tr>
<td>40 hour dialysate</td>
<td>17</td>
<td>8+++</td>
<td>47.0</td>
</tr>
<tr>
<td>&quot; &quot; non-dialysate</td>
<td>13</td>
<td>7+++</td>
<td>53.8</td>
</tr>
<tr>
<td>Glycerolated mammary gland</td>
<td>21</td>
<td>13+++; 3+</td>
<td>76.2</td>
</tr>
<tr>
<td>Heated mammary gland extract</td>
<td>25</td>
<td>4++; 1+</td>
<td>20.0</td>
</tr>
<tr>
<td>Desiccated extract (mammary gland)</td>
<td>10</td>
<td>1++</td>
<td>10.0</td>
</tr>
<tr>
<td>Partially digested milk (gastric content)</td>
<td>10</td>
<td>7+++</td>
<td>70.0</td>
</tr>
<tr>
<td>Seitz filtrate</td>
<td>6</td>
<td>2+++</td>
<td>33.3</td>
</tr>
<tr>
<td>Controls †</td>
<td>86</td>
<td>1+++; 3+</td>
<td>3.5</td>
</tr>
</tbody>
</table>

* (++++) = systemic infiltration of leukemic cells; (+) = local progressive growth leukemic cells.
† Litter mates to mice used in experimental series.

the experimental group. The following observations were made:

1. Dialysis of mammary gland extract against physiological saline was accomplished by use of parchment paper (Will Corporation—Grade A). Dialysis was carried on for 40 hours at −4°C. Refractory test animals were rendered susceptible by both the dialysate and non-dialysate (Table III). Five separate tests were made for each of the extracts.

2. Homogenized mammary gland, stored in 50 per cent glycerol for 30 days at −4°C, then extracted with physiological saline, proved to contain the susceptibility influence. Four separate series of tests were made and, in total, 16 of 21 inoculated mice proved susceptible to transplanted leukemic cells.

3. Mammary gland extract heated for 20 minutes at 85°C in a water bath produced susceptibility in 5 of 25 animals. One animal in each of 5 separate tests proved susceptible. Since 4 out of 5 experimental mice. On the other hand, extracts of desiccated leukemic cells produced susceptibility in 5 of 20 mice, with 1 susceptible animal in each of 5 test series.

6. In another attempt to obtain a source of material for characterization tests of the susceptibility influence, partially digested milk obtained from stomachs of subline B (susceptible) mice was extracted and inoculated into refractory subline W mice. Gastric contents were removed from suckling mice 1 hour after suckling. Seven of 10 mice proved susceptible to the growth of transplanted myeloid leukemia cells. As a control experiment milk obtained from stomachs of strain A and subline W, C57 black mice was extracted and inoculated into test mice. These mice remained refractory to inoculations of leukemic cells. It has been found, in contrast, that digestion or partial digestion for 30 minutes will inactivate the mammary tumor inciter.
SUMMARY AND CONCLUSIONS

1. An influence transmitted in the milk of certain lactating female mice was found to be effective in promoting the growth of two transplantable lymphoid leukemias and a transplantable myeloid leukemia in normally refractory mice. Preliminary data indicate the probability that such an influence, or influences, affects the growth potentialities in refractory mice of two other lymphoid leukemias.

2. The growth in mice of refractory strains of 2 fibrosarcomas, a melanoma, a mammary adenocarcinoma, a monocytic leukemia, and 2 other lines of myeloid leukemia were found not to be affected by an influence or influences transmitted in the milk.

3. The susceptibility influence was found to be present throughout the period of lactation in susceptible lactating mice.

4. Following transfer of the susceptibility influence to refractory mice by foster nursing, this influence was found to be maintained through three generations, by breeding mice of the normally refractory strain.

5. Saline extracts prepared from homogenized liver, spleen, or mammary gland were found to contain the susceptibility influence of myeloid leukemia, line C1498.

6. The following characteristics of the susceptibility influence affecting the growth of this myeloid leukemia were found: (a) It will apparently dialyze through parchment paper at $-4^\circ$ C. Five separate tests were made and in each the dialysate contained the influence. (b) It remains stable in 50 per cent glycerol for 30 days at $-4^\circ$ C. (c) A certain heat stability is indicated. The extract heated for 20 minutes at $85^\circ$ C. rendered refractory mice susceptible to the growth of leukemia cells. (d) The Seitz filter apparently failed to remove all the influence from an extract. (e) Desiccation in a vacuum for 4 hours at room temperature inactivated the influence present in mammary gland extract. Desiccation under similar conditions failed to inactivate completely the influence in leukemia cells. (f) Digestion apparently failed to inactivate the influence. Four separate tests were made and in each test refractory animals were rendered susceptible by partially digested milk. This does not conclusively prove that the influence is unaffected by digestion. Gastric contents obtained 1 hour after suckling contained the susceptibility influence. This is in contrast to the effect of digestion on the mammary tumor inciter.

REFERENCES


Characterization of an Influence Affecting Growth of Transplantable Leukemias in Mice

L. W. Law


Updated version  Access the most recent version of this article at:  
http://cancerres.aacrjournals.org/content/4/4/257.citation

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.