Development of Murine Leukemia after Inoculation of Human Lymphomas

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SUMMARY

Biopsy and/or bone marrow specimens from human lymphomas were inoculated into the spleen of young BALB mice followed by a blind intraperitoneal passage. Five inocula: 2 hemocytoblastomas, 1 fibrosarcoma, 1 Hodgkin’s disease, and a sediment of Burkitt’s lymphoma cells led to the development of short-latency leukemias within 50 days. The incidence of long-latency leukemias, averaging 20 months, was also significantly increased to 34% in 955 mice receiving inocula from 51 cases of human lymphomas compared to 26% in 223 mice inoculated with 15 human control specimens. Of the latter, 4 normal bone marrow and one tonsil inocula gave a significant increase in leukemia compared to the 15% spontaneous incidence of the BALB strain. In the human lymphoma groups, a significantly high incidence of leukemia was obtained in the surviving animals of cases which gave rise to short-latency leukemias in some cases of Hodgkin’s disease, acute leukemia, lymphoadenosarcoma, hypernephroma, and eosinophilic granuloma.

Tumors developing in some of the group included fibrosarcomas, and lung, mammary, parotid, ovarian, testicular, and adrenal tumors.

Both short-latency and long-latency leukemias seem to respond to the same mechanism; this suggests the presence of a leukemogenic factor in human lymphomas and, possibly, in normal bone marrow capable of triggering a preexisting mechanism in the mouse.

INTRODUCTION

Viruses have been proved responsible for the development of leukemia in mice, but no direct evidence has been presented for their participation in the human disease. As reviewed by Southam (9), several authors have inoculated human neoplastic tissues in mice without any definite positive results; more recently, Moore and Cuba (6) and Chowdbury (3), using newborn mice, have obtained an increase in the incidence of leukemia and tumors.

The search for an etiologic agent in human lymphomas led us to the inoculation of these processes into mice (8). In a previous publication (7), it was reported that, under special experimental conditions, short-latency leukemia developed in young adult mice receiving human lymphoma material by the intrasplenic route. Three cases led to positive results, and it was deemed interesting to follow the surviving animals of both the positive and negative cases. A significant increase in the incidence of long-latency leukemia was obtained. These results, along with two other specimens leading to short-latency leukemia, are the object of this paper.

MATERIALS AND METHODS

All the experiments were carried out in BALB mice of either sex, 1 to 2 months old. Human lymph node material was obtained by surgery. Bone marrow was obtained by sternal puncture. All inoculations were carried out according to the technic described previously and consisting of: Inoculation 1 (I1). Insertion of either a solid piece of biopsy material or a clot of bone marrow into the spleen of 10 to 20 anesthetized mice by means of a trocar. Inoculation 2a (I2a). Ten days later, the mice from I1 were splenectomized and a piece of their spleens was inserted into the spleen of another group of 10 to 20 mice. Inoculation 2b (I2b). Simultaneously with I2a, the remaining pieces of spleen were homogenized and injected intraperitoneally into 10 to 20 mice. Inoculation 3 (I3). Ten days later, the mice from I2a were splenectomized; the spleens were homogenized and injected intraperitoneally into 10 to 20 mice.

All mice, including the splenectomized ones, were kept under observation until spontaneous death or until obvious signs of leukemia were detected; the spleen and mesenteric lymph nodes were usually palpable a few weeks before death. The experiment was concluded when the animals reached 2 years of age, at which time the survivors were sacrificed. The diagnosis of leukemia was based on autopsy data as well as histologic examination of spleen, lymph nodes, thymus, liver, kidney, heart, and lung. The term leukemia is used in a broad sense, including leukemias as such, reticulo sarcomas, and lymphosarcomas, according to Dunn’s classification (4).

As murine controls, 500 stock mice were kept under observation for 2 years in order to determine the spontaneous incidence...
of leukemia in our BALB colony. Furthermore, a group of mice received normal BALB spleen instead of human material.

Tonsils were selected as human control tissue because of their hyperplastic lymphoid nature and because they are not neo-plastic. Since they are also known to harbor viruses, muscle was obtained from normal individuals in the operating room. A total of 6 specimens of tonsils and 5 of muscle were inoculated. A third control group consisted of bone marrow obtained by sternal puncture from 4 normal persons.

Tissue culture cells from a Burkitt lymphoma line, EB1 (2), were sedimented by centrifugation and mixed with fibrinogen and thrombin in order to obtain a clot which could be loaded into a trocar; 30 mice were inoculated intrasplenically on 3 different occasions; each time followed by an intraperitoneal blind passage, I2n.

Biopsy material and/or bone marrow from 51 human lymphomas were inoculated intrasplenically, followed by the corresponding blind passages, I2a, I2b, and I3. These included the 3 cases described previously (7): 2 hemocytoblastomas and 1 fibrosarcoma. The remaining cases were diagnosed as: 13, Hodgkin's disease; 11, acute leukemia; 3, chronic lymphoid leukemia; 5, lymphosarcoma; 3, reticulosarcoma; 7, lymphoadenocarcinosis (nonspecific generalized lymphadenitis); 1, hypernephroma with hyperplastic lymph nodes; 2, metastatic lymph node infiltrations from mammary adenocarcinomas; 2, nonspecific granulomas; and 1, eosinophilic granuloma. A specimen of tonsils from the latter case was also inoculated.

RESULTS

Leukemias of very short latency developed in mice inoculated with human lymphoma in 5 instances; this result was so outstanding that the first three cases were the object of a previous publication (7). These were 2 hemocytoblastomas and 1 fibrosarcoma. The remaining cases were diagnosed as: 13, Hodgkin's disease; 11, acute leukemia; 3, chronic lymphoid leukemia; 5, lymphosarcoma; 3, reticulosarcoma; 7, lymphoadenocarcinosis (nonspecific generalized lymphadenitis); 1, hypernephroma with hyperplastic lymph nodes; 2, metastatic lymph node infiltrations from mammary adenocarcinomas; 2, nonspecific granulomas; and 1, eosinophilic granuloma. A specimen of tonsils from the latter case was also inoculated.

Table 1

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>Diagnosis</th>
<th>No. of mice</th>
<th>Leukemias</th>
<th>%</th>
<th>Latency (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hemocytoblastoma</td>
<td>22</td>
<td>10</td>
<td>45</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>Hemocytoblastoma</td>
<td>10</td>
<td>10</td>
<td>100</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>Fibrosarcoma</td>
<td>17</td>
<td>15</td>
<td>88</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>Hodgkin's disease</td>
<td>5</td>
<td>4</td>
<td>80</td>
<td>35</td>
</tr>
<tr>
<td>5</td>
<td>Burkitt cells (EB1)</td>
<td>10</td>
<td>3</td>
<td>30</td>
<td>17</td>
</tr>
</tbody>
</table>

Short-latency leukemias in mice inoculated with human lymphomas.

Table 2

<table>
<thead>
<tr>
<th>No. of specimens</th>
<th>Inocula</th>
<th>No. of mice</th>
<th>Leukemias</th>
<th>%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Murine controls</td>
<td>518</td>
<td>78</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>Human lymphomas</td>
<td>985</td>
<td>320</td>
<td>34</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Long-latency leukemias in mice inoculated with human lymphomas: overall results.

* Calculated by the x² test.
subgroup of 18 since, when the experiment was initiated, the human nonmalignant inocula were to be the controls. Since the values obtained both with this inoculation of isologous spleen cells and with the muscle and tonsil inocula, with one exception, were not different from the spontaneous incidence of leukemia in the stock mice, it was felt that the latter 500 mice could be included in the murine control group.

In Group II, human controls, the 5 specimens of muscle and 5 of the tonsils, gave results comparable to the murine controls: 13% and 17%, respectively. However, one specimen of tonsils gave rise to a high incidence of leukemia, 67%; this differed significantly from the other tonsils. The 4 inocula of normal bone marrow gave rise to 27% leukemia, which is significantly different from the murine control value of 15%.

Group III through Group XIII include the 51 cases of human lymphomas. Group III is made up of the 2 cases of hemocytoblastomas and 1 of fibrosarcoma which had led to a high incidence of short-latency leukemia (Table 1). The surviving 150 mice led to a 47% incidence of leukemia. The average latency period was 19–20 months; this was not different from the other groups, but the earliest leukemia, 11 months, was observed in this group.

Group IV is that of Hodgkin’s disease; the 13 cases studied gave such heterogeneous results that they were separated into 3 subgroups significantly different from one another, at the $P < 0.001$ level. Thus, 4 cases gave rise to no leukemias at all, the mice remaining in a state of caquexia to the end of the 2-year term. The second subgroup of 6 cases gave rise to an 18% incidence of leukemia, not different from the murine control value but containing the second earliest leukemia, 12 months. The third subgroup of 3 cases led to a significantly high incidence of leukemia, 44%. In Group V, 11 cases of acute
leukemia were also separated into 2 subgroups, heterogeneous and different from one another at the P < 0.001 level; one led to 19% and the other to 41%, a significantly high incidence of leukemia. Group VI consisted of 3 cases of chronic lymphoid leukemia; VII had 3 reticulosarcomas; VIII had 5 lymphosarcomas; and XI, with 2 metastasis, gave rise to an incidence (26%) of leukemia not significantly different from that of Group II, the human controls. Group IX had 7 cases of lymphosarcoma and gave rise to a 35% value, just significantly different from the 26% control value of Group II. Group X, a case of hypernephroma, surprisingly gave the highest of values, 70% leukemia with the same average latency of 20 months. Group XII, with 2 cases of nonspecific granulomas, gave a nonsignificant value of 23%. Group XIII, a case of eosinophilic granuloma in a child, gave a significant value of 38%; when the patient’s tonsils were removed and inoculated into mice, a high incidence of leukemia, 59%, occurred.

The development of leukemia in these animals was by far the most outstanding feature, but several other tumors were also observed. The most common was an alveolar carcinoma of the lung; it appeared in approximately 35% of our stock mice; 13 (6%) were observed in Group II, human controls, and a total of 25 (3%) in human lymphoma, Groups III to XIII. These tumors are often very small and may have been missed in some animals. Other tumors, such as fibrosarcomas, and parotid, mammary, ovarian, testicular, and adrenal tumors, which are exceptional in stock mice (less than 1%), have appeared in the experimental groups and are included in Table 3. Thus, in the human control Group II, tumors were observed only in the one tonsil and 4 bone marrow specimens which gave a high incidence of leukemia; these were 2 mammary adenocarcinomas, 2 adrenal carcinomas, and 1 testicular tumor. In the human lymphoma series, in Group III there were 2 fibrosarcomas, 1 of which is being transplanted serially, and 2 mammary adenocarcinomas. In Group IV, in which there were 4 cases of Hodgkin’s disease, there were 6 tumors, 2 fibrosarcomas, 2 luteomas, 1 parotid tumor, and 1 testicular tumor; the last 4 tumors all appeared in one case.

DISCUSSION

The outstanding result obtained with the inoculation of human lymphomas into the spleen of BALB mice followed by a blind intraperitoneal passage, is the development of leukemia within 50 days in 5 instances. The first three patients died soon after the specimens were taken, but the fourth, with a diagnosis of Hodgkin’s disease, is still alive; the fifth specimen was a sediment of Burkitt’s lymphoma cells, EB1. The explanation of these results is not easier now than at the time of the previous publication (7), but the first hypothesis put forward at the time and discussed by Adams et al. (1), namely, the implantation of human cells, can be discarded. Cytogenetic studies have been carried out on all five of those leukemias which are maintained in cellular passages, and the karyotype is characteristically that of the mouse; moreover, the search for human antigens by immunofluorescence has been negative up to now. The same experimental model has been used with AKR spontaneous leukemia instead of human lymphomas; out of 38 trials, 4 led to a high incidence of short-latency BALB leukemias (results to be published). These results, so similar to those obtained with human lymphomas, where 5 out of 51 cases gave positive results, suggest that the latter may contain an agent similar to that present in spontaneous AKR leukemia; in both cases a certain threshold of activity would have to be reached in order to induce leukemia by this method. This short-latency leukemia apparently belongs to the same type of murine disease as the long-latency leukemias, since it has been impossible to differentiate them in any way except for the different incubation periods, which are impossible to explain for the time being.

As for the long-latency leukemias, the fact that a significant overall increase in their incidence is obtained with human lymphoma inocula, irrespective of the diagnosis, points to the existence in these tissues of some factor which helps to develop the murine leukemia, probably triggering a preexisting mechanism. The fact that the three cases which led to short-latency leukemia also gave a very high incidence of long-latency leukemia along with the difference in response between cases with the same diagnosis, such as Hodgkin’s disease and acute leukemia, again suggest a quantitative factor, an effective level below which no leukemogenic effect is obtained.

Normal bone marrow inocula and one tonsil specimen gave rise to a significantly high incidence of leukemia, contrasting with the lack of effect of the 5 muscle and remaining 5 tonsil specimens; this would suggest that normal bone marrow and occasionally tonsils might harbor the leukemogenic factor. It is interesting to note that Moore and Cuba (6) observed that the human lymphoma group was the only one in their experiments which caused a significant increase in mouse tumors; they also observed some increase in incidence with a laryngeal polyp.

Abundant viruses have been found in these human lymphoma-induced leukemias (7), their size and structure being the same as that observed in 32P-induced leukemias (5). Both these leukemias have been transplanted successfully cellurally and acellurally and, up to now, have proved indistinguishable from each other and from the spontaneous ones. It can be concluded that, though these experiments do not afford any direct evidence for the presence of human leukemogenic viruses, they do not by any means exclude their existence.

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REFERENCES

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