New Experimental Model of Meningeal Leukemia

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ABSTRACT

Meningeal leukemia is a common, often fatal complication of leukemia. Efforts to devise better prophylactic or therapeutic regimens have been limited by large gaps in knowledge regarding the pathogenesis of the process. This report describes a new experimental model of meningeal leukemia that should facilitate its study and fill some of the gaps. T-cell lymphomas, originally induced in C3H mice by Gross murine leukemia virus, were established as transplantable tumor lines in syngeneic mice. Three of ten tumor lines produced meningeal leukemia when injected i.v. into normal recipient animals. The others produced predominantly visceral lymphomas.

INTRODUCTION

The invasion of the CNS by malignant cells has become a major clinical problem since the introduction of life-prolonging chemotherapeutic drugs. The situation is especially common in leukemia (6, 27). Patients now often survive the acute phase of this disease only to succumb later from the consequences of meningeal leukemia. Infiltration of the leptomeninges by leukemic cells and their proliferation lead to obstructive and ischemic processes incompatible with normal brain function (19, 24). Efforts to prevent or treat this process with CNS radiation and i.t. methotrexate have been quite successful, but not so successful or safe that they can be considered adequate (1, 11, 12, 15, 23).

Numerous studies conducted both in humans and in experimental animals have revealed several important events in the pathogenesis of meningeal leukemia. Malignant cells do, in fact, invade the leptomeninges rather than develop there de novo (10, 14, 16, 25). The route they take is probably via the walls of the arachnoid veins (16, 19). The concentrations within spinal fluid of most systemically administered chemotherapeutic drugs do not reach levels cytocidal for leukemic cells (3, 20). Finally, 2 studies performed in humans indicate that the proliferative rates of leukemic cells within spinal fluid are lower than those for leukemic cells outside the CNS (13, 26).

Many critical aspects of this problem remain unexamined, however, because both human and experimental animal studies have been constrained by unavoidable circumstances. Knowledge of the histopathology of human meningeal leukemia is based primarily on autopsy examinations. These observations are static, miss important early events, and are often influenced by preceding treatment regimens and the agonal events. In addition, they often are a compilation of examinations of people who have suffered from a variety of types of leukemia. The cellular kinetic studies in humans have been very limited by the often strenuous character of the methodology. The experimental animal models have been principally of 2 types, both restricted by necessary manipulations of the animals. In order to produce meningeal leukemia, the malignant cells must be injected directly into the brain (4, 7) or the animals must be treated with systemic chemotherapy (16, 25).

The purpose of this report is to describe a new experimental animal model of meningeal leukemia that does not require any manipulation of the animal other than the i.v. injection of leukemic cells. It appears ideal for addressing issues such as the distribution of leukemic cells, selective trafficking to the CNS, selective proliferative advantages in various body sites, and the role of chemotherapy in potentiating, preventing, or treating meningeal leukemia.

MATERIALS AND METHODS

Inbred Mice. C3H/BL mice originally obtained from Dr. L. Gross (Bronx Veterans Administration Hospital, Bronx, N. Y.) were bred and maintained in our laboratory. This genetic background is critical for leukemia induction by the murine leukemia virus (8). Other strains of C3H are not susceptible.

Murine Leukemia Virus. Passage A filtrate, originally obtained from Dr. L. Gross (Bronx Veterans Administration Hospital, Bronx, N. Y.), was prepared and utilized as described by him (9). The preparation is a cell-free tumor filtrate containing the murine leukemia virus. It is kept in sealed vials in liquid nitrogen until used.

Production of Primary T-Cell Lymphomas. Three-day-old C3H mice were given i.p. injections of 0.2 ml of tumor filtrate. Essentially, 100% of animals thus treated developed lymphomas at 8 to 12 weeks of age. Most animals had thymomas.

Tumor Cell Lines. Thymomas were removed, minced with knives into 2-3 mm pieces, and implanted s.c. into the right flank of 2- to 3-month-old syngeneic recipient mice. Tumors were routinely transferred at 14-day intervals. Fourteen lines derived from 14 thymomas were established. All were Thy-1 positive and surface immunoglobulin negative and possessed normal karyotypes (18). Ten of the 14 were analyzed for their capacity to produce meningeal leukemia.

Assay of Meningeal Leukemia-inducing Capacity. Tumors s.c. were minced and pressed through a 60 mesh stainless steel screen, and the resulting cell suspension was washed 3 times in Hanks' balanced salt solution. The cells were then centrifuged on a Ficoll-Hypaque cushion (2), and the cells remaining at the interface were removed, washed, and assayed for viability by trypan blue exclusion. Viability always exceeded 85%. Cell concentration was adjusted to 10⁶ cells/ml Hanks' balanced salt solution. Aliquots (0.1 ml) containing 10⁶ tumor cells were injected into the tail veins of 2- to 3-month-old syngeneic recipient mice. Each tumor line was injected into 3 to 13 recipients. Two different passages of lines 2 and 11 were tested; the others were tested once. Animals were examined...
RESULTS

All animals given injections of all tumor lines became engrafted and eventually showed signs of leukemia. Most animals were weak and debilitated by 14 to 21 days postinjection. They manifested weight loss, ruffled hair, lymphadenopathy, and hepatosplenomegaly. Although no attempt was made to quantitate the visceral metastases at autopsy, it was clear that all tumor lines produced metastases in the lung, spleen, and liver.

Three lines produced CNS disease. Signs of CNS disease typically occurred suddenly in healthy-appearing animals 10 to 21 days post-tumor cell injection. Many animals died within min of the onset of symptoms; essentially all died within hr. Severe ataxia, paralysis, and seizures preceded death (Table 1).

Lines 2, 6, and 11 all produced CNS disease. Lines 2 and 11 were especially effective, as assessed by both the incidence and the rapid onset of the disease. There was no statistically significant difference, however, in the incidence among the 3 lines. The other 7 lines did not produce CNS disease, a difference that was statistically significant for lines 1, 4, 7, 13, and 14 when they were compared with line 11 using Fisher’s exact probability test (20). Although no statistical evaluation was performed, the time between leukemia cell injection and death or debilitating disease also varied considerably among the lines. For example, all line 11-injected animals were dead by 14 days postinjection while recipients of many other lines were free of CNS symptoms at the time of sacrifice as late as 26 days postinjection.

Most animals with CNS symptomatology displayed leptomeningeal leukemic infiltrates which involved only the leptomeninges without extension into the brain. The infiltrates usually involved both the superficial and deep leptomeninges. Three animals showed focal extension of the leukemic infiltrates along the Virchow-Robin spaces, and in one there was limited focal extension into the brain parenchyma. In one animal, no leptomeningeal infiltrates could be seen in the examined section. Fig. 1 illustrates some of these pathological findings.

Parenchymal changes of the brain were seen in all animals with meningeal leukemia. They included neuronal necrosis of the Somner’s sector in the hippocampus and neuronal necrosis of the Purkinje cells and foci, usually symmetrical, of necrosis in the colliculi. Most striking lesions were seen in the basilar portion of the pons. They consisted of bilateral symmetrical areas of necrosis of the brain parenchyma. The most severely affected brains showed areas of total tissue rarefaction with loss of all cellular elements and no inflammatory infiltrates (Fig. 2). There was no apparent relationship between leptomeningeal infiltration and parenchymal lesions. We interpreted the necrotic areas to be secondary to ischemia and anoxia.

Table 1

<table>
<thead>
<tr>
<th>Line</th>
<th>Time (days) postinjection at death or sacrifice</th>
<th>No. with CNS disease</th>
<th>% with CNS disease</th>
<th>p (compared with line 11)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>0/10</td>
<td>0</td>
<td>0.01</td>
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<tr>
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<td>15–19</td>
<td>6/9</td>
<td>0</td>
<td>0.46</td>
</tr>
<tr>
<td>2cr</td>
<td>14</td>
<td>0/5</td>
<td>0</td>
<td>0.06</td>
</tr>
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<td>4</td>
<td>21</td>
<td>0/8</td>
<td>0</td>
<td>0.02</td>
</tr>
<tr>
<td>6</td>
<td>21</td>
<td>1/4</td>
<td>25</td>
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</tr>
<tr>
<td>7</td>
<td>26</td>
<td>0/12</td>
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</tr>
<tr>
<td>9</td>
<td>14</td>
<td>0/4</td>
<td>0</td>
<td>0.07</td>
</tr>
<tr>
<td>11</td>
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<td>14/16</td>
<td>88</td>
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<tr>
<td>13</td>
<td>18–22</td>
<td>0/14</td>
<td>0</td>
<td>0.002</td>
</tr>
<tr>
<td>14</td>
<td>25</td>
<td>0/9</td>
<td>0</td>
<td>0.01</td>
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* Calculated by Fisher’s exact probabilities test (21).

Fig. 1. Infiltration of the cerebral leptomeninges by leukemic cells. Such infiltrates were present in all but one of the animals with neurological symptoms. They were often accompanied by focal subarachnoid hemorrhages and, in a few cases, extended into the Virchow-Robin spaces. H & E, × 160 (negative), × 480 (final).
DISCUSSION

The experimental model of meningeal leukemia described in this communication has several features that warrant discussion. First, the fact that i.v.-injected leukemic cells invade the leptomeninges and produce CNS disease is itself noteworthy. Why this has not been the case with most other experimental models is unclear. Following the injection of the L1210 mouse leukemia lines (25) or the L2C guinea pig cell line (16), animals die of systemic disease rather than meningeal leukemia. Meningeal leukemia will occur only if these cells are injected directly into the CNS or if the animals are treated with chemotherapeutic drugs. Seven of the T-cell lines described in the present study also failed to produce CNS disease. It is not yet known whether they would do so if injected into the CNS or if the recipient animals were treated with chemotherapy. The fact that only 3 of 10 T-cell lines produced meningeal leukemia, coupled with the experience with L1210 and L2C just noted, suggests that only selected cell lines can naturally invade or proliferate in the CNS. Evidence supporting this possibility has been presented previously. Southam et al. (22) analyzed the consequences of injecting 22 human cell lines into newborn rats. Six of the lines produced a CNS disease characterized by the proliferation of cells in the leptomeninges and the brain parenchyma. The other lines became engrafted elsewhere in the recipient animals. Although the experimental conditions of these studies were very different from those of our animal model, the selective propensity of some cell lines to traffic or proliferate in the CNS is a feature of both situations.

If there are unique characteristics that determine meningeal leukemia-inducing capacity, they are unknown. All 10 leukemia lines utilized in the present study were T-cell lymphomas with normal karyotypes, but beyond that they exhibited considerable individuality. Features such as growth rates, hormone sensitivities, ability to bind sheep erythrocytes, cholesterol content, and the expression of Ig antigens were distinctive and stable for each line (5, 17, 18). No combination of characteristics was associated with CNS disease-inducing potential.

The histopathology of the CNS disease produced by the 3 cell lines appeared identical. Leptomeningeal infiltrates of leukemic cells dominated the picture coupled with localized areas of brain necrosis. These latter lesions were suspected of being secondary to ischemia, but further study is necessary to elucidate their pathogenesis. The histopathology of this model is similar to most other experimental models and to human meningeal leukemia (19, 24). Studies have now been initiated to fully elucidate the early as well as late histopathology of this model disease. We also intend to search the cerebrospinal fluid for leukemic cells to further strengthen the parallelism of this model with the human disease.

In theory, the development of meningeal leukemia depends on 2 factors: (a) the ability of the leukemic cells to invade the leptomeninges; and (b) the capacity of the leukemic cells to proliferate within the CNS. The model just described should enable these 2 factors to be individually assessed. It should also permit an analysis of the role systemic chemotherapeutic agents play in facilitating meningeal leukemia. The possibility that they may promote its development, at least under some circumstances, has never before been testable. Additional experiments directed at identifying the earliest events, cellular traffic and distribution patterns, and the kinetic aspects of the disease produced by the T-cell lines just described may reveal significant differences among the lines. It would be surprising to these authors if all meningeal leukemias, induced by a great variety of cell types and under widely disparate conditions, were all the consequence of the same pathobiological event(s).

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REFERENCES

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