The Histogenesis of Gross's Viral Induced Mouse Leukemia*

STEPHEN B. GOODMAN† AND MATTHEW H. BLOCK

(Department of Medicine, University of Colorado Medical Center, Denver, Colorado)

SUMMARY

The histogenesis of mouse leukemia induced in C3H(f)/Bi mice with Gross's passage A virus was studied. The disease began in the thymus. Prior to the development of leukemia the thymus underwent cortical atrophy and then regeneration. The histogenesis of Gross leukemia in the C3H(f)/Bi mouse closely resembled that of certain other forms of mouse leukemia.

Observations were made on the thymus of normal C3H(f)/Bi mice at various ages.

In 1951 Gross reported the development of leukemia in C3H/Bi mice given injections during the newborn period of cell-free extracts of leukemic tissue from the AK mouse (10). Work by Gross and others has established with reasonable certainty that these extracts contain a virus which is associated with the development of leukemia in the recipient animal (13). In 1957 Gross (12) reported the development of a particularly potent strain of this virus which he referred to as "passage A" virus.

Although Gross leukemia has been the subject of intensive investigation, little is known of its histogenesis. There is considerable variation in the histogenesis of other forms of mouse leukemia, including some viral-induced leukemias (3, 9, 15, 17–21, 24, 25).

It was the purpose of this study to investigate the histogenesis of Gross leukemia induced in C3H(f)/Bi mice with passage A virus.

MATERIALS AND METHODS

ANIMALS

Most of the animals used in the present study were derived by recognized standards of inbreeding (25) from a pair of CSH(f)/Bi mice supplied by Dr. L. Gross. A few mice in the experimental series were noninbred, as indicated in Table 2.

Animals were housed in both plastic and metal cages and kept in an air-conditioned animal room.

The diet consisted of Purina Mouse Breeder Chow supplemented once or twice a week with lettuce and oats. Water was given ad libitum.

DESIGN OF STUDY

Animal sacrifice and tissue preparation.—Each animal was weighed, and a white cell count, hematocrit, and blood smears were obtained from tail blood from the unanesthetized animal. The mouse was then killed with chloroform and immediately autopsied. From each animal specimens of the following organs were obtained: spleen, liver, kidney, adrenal, small intestine, lung, mesenteric lymph node, inguinal lymph node, and cervical lymph node attached to the salivary gland. In every animal the thymus was removed in toto and often with attached tracheal nodes. One femur was decalcified and sectioned for study of the bone marrow. Specimens of the heart and gonad were taken from many animals. The spleen was weighed to the nearest 5 mg.

All tissues were fixed in Zenker-formol for approximately 2 hours. They were then embedded in nitrocellulose according to a modified Maximow technic (6). Tissue blocks were sectioned at 4–6 μ and stained with hematoxylin eosin-azure II. Four to six sections were taken from each tissue except for the thymus, which was sectioned at multiple levels in the coronal plane. Sixteen to 32 sections were prepared and examined from each thymus.

Virus used in experimental series.—Approximately 9 cc. of sterile cell-free extract (C3H9G) was prepared, according to the method of Gross (11), from the liver, spleen, and thymus of two mice with lymphosarcoma confined to the thymus. The disease was produced in these two mice with...
tissue extracts prepared in our laboratory from leukemic mice supplied by Dr. Gross. This extract (CSHG9) was used throughout the present study. It was divided into 0.5- to 0.7-cc. amounts and kept in a dry ice chest maintained at —20–50° C. Aliquots of the virus were used as needed over a 6-month period.

**Inoculation of animals.**—Mice were given intraperitoneal injections, when between 8 and 12 days of age, of 0.1 cc. of thawed extract. A variable amount of extract leaked, on occasion. Animals in the experimental series were given the injections as they became available from the breeding colony. At weaning these injected animals were caged in groups of four or five animals. Corresponding groups of control animals were set up. Most groups of four or five animals contained both male and female mice.

**Assignment of dates for sacrifice.**—The first group of experimental mice was sacrificed at age 117 days (107 days post-injection). On the basis of the histologic findings in these five animals, dates of intended sacrifice were assigned to the other experimental groups. These dates were chosen in the hope of obtaining a full spectrum of histologic change in the development of leukemia. As additional histologic material became available, we assigned some of the groups originally designated for early sacrifice to a later date. This was done to insure the development of more advanced disease. Since our sole objective was to obtain histologic material at all stages in the development of the disease, we did not adhere to a rigid, preconceived schedule of sacrifice. No groups were re-assigned a new date of sacrifice because of illness. Actually, only two animals did appear sick prior to sacrifice. One of these mice was sacrificed before the other mice in the same group because of its moribund condition (mouse #16, Table 2). Control groups were matched to the experimental series.

An additional series of control animals (injected controls) was given injections of a cell-free extract of liver, spleen, and thymus obtained from four normal CSH(f)/Bi mice, 4 months of age. Mice in the injected control series were sacrificed at ages to correspond with the experimental animals, and only their thymuses were removed for histologic study.

**Definition of Terms**

The terms “small,” “medium,” and “large” lymphocyte and “reticular cell” are used in referring to cells of the normal mouse thymus similar to those described by Sainte-Marie and Leblond in the rat thymus (28). This nomenclature also corresponds to that used by Bloom and Fawcett (7). We have used the terms “lymphosarcoma” and “leukemia” interchangeably in referring to those tumors of the mouse composed of cells which appear to belong to the lymphatic series.

In following the genesis of a malignant disease it becomes obvious that a distinction between “malignancy” and “premalignancy” is difficult. We use the terms “malignancy,” “lymphosarcoma,” and “leukemia” only when capsular invasion of the thymus or lesions distant from the thymus were present.

**TABLE 1**

**NUMBER OF MICE AUTOPSYED AT VARIOUS INTERVALS AFTER INJECTION AND NUMBER OF CONTROL MICE OF SIMILAR AGES**

<table>
<thead>
<tr>
<th>DAYS AFTER INJECTION</th>
<th>EXPERIMENTAL SERIES</th>
<th>INJECTED CONTROL SERIES</th>
<th>NONINJECTED CONTROLS OF SIMILAR AGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5 (10 days old)</td>
</tr>
<tr>
<td>18</td>
<td>4</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>29</td>
<td>0</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>28</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>37</td>
<td>5</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>51</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>65</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>72</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>76</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>80</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>98</td>
<td>4</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>100</td>
<td>5</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>107</td>
<td>5</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>114</td>
<td>5</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>135</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>150</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>153</td>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

**RESULTS**

**Control Series**

The control series consisted of 54 normal mice (Table 1). In most respects the thymus of the CSH(f)/Bi mouse conformed to Dunn's general description of mouse thymus (8).

The thickness of the thymic cortex appeared to vary from animal to animal (Figs. 1–5) and at different levels of the same thymus. It is possible that some of this variability was artifactual and depended upon the plane of section of the thymus. In general the ratio of thickness of cortex to medulla appeared to be greater in animals 10–38 days of age (Fig. 3) than in older animals (Fig. 1).

Medium and large lymphocytes were commonly seen in the cortex at all ages (Figs. 4, 5), in contrast to what has been previously described (5).
Their numbers varied in different areas of the same thymus, as well as from animal to animal. They occurred in clusters of three to twenty cells at the outer edge of the cortex, and singly through the remainder of the cortex. The clusters tended to be more abundant in the thymus of 10-day-old animals than in older mice. The difference in frequency, however, was not consistent, nor was it great. Mitoses and degenerating cells were frequent in the cortex (Figs. 4, 5). It was impossible to be certain whether these dividing and dying cells were large or small lymphocytes.

Although it has been claimed that two populations of reticular cells exist in the thymus (7), we were unable to make this distinction (Fig. 6). The cysts and alveoli described in the thymuses of AKR/O and WLO mice (4) were also present in our material. Histologic examination of the other organs studied revealed no unusual findings.

**Injected Control Series**

This series contained 22 mice (Table 1). The thymuses of these mice were similar to the thymuses of the normal control series.

**Experimental Series**

Of the 58 animals in the experimental group, twenty showed a definite histologic abnormality of one or both lobes of the thymus (Table 2). The histopathology of individual lobes of the same thymus often varied greatly. We have divided the observed abnormalities into four categories, but in many cases the abnormalities observed in an individual lobe demonstrated features of more than one category, as indicated in Table 2 by a line extending beyond the boundaries of a single category. Before assigning a lobe to a specific category or categories, a representative slide was examined by each author as an unknown.

*Cortical atrophy.*—Cortical atrophy is defined as the disappearance of small lymphocytes from the circumference of the thymus, with consequent thinning or loss of the cortex (Figs. 7, 8, 12, and 14). A minor degree of cortical thinning, such as occasionally seen in control mice (Fig. 2), was not considered "cortical atrophy" for the purposes of this study. This abnormality was observed in one or both lobes of nine animals. Cortical atrophy was the earliest change noted in the experimental

---

**Table 2**

HISTOLOGY OF EACH THYMIC LOBE OF MICE IN EXPERIMENTAL SERIES. DOTTED LINE INDICATES ONE LOBE, AND DASHED LINE INDICATES OTHER LOBE. LENGTH OF LINE CORRESPONDS TO SIZE OF LOBE. ONE LOBE OFTEN SHOWED FEATURES OF MORE THAN ONE HISTOLOGIC CATEGORY

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>Sex</th>
<th>Interval after injection</th>
<th>Histologic categories</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>100 days</td>
<td>..</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>100</td>
<td>..</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>100</td>
<td>..</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>100</td>
<td>..</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>100</td>
<td>..</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>107</td>
<td>..</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>107</td>
<td>..</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>107</td>
<td>..</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>107</td>
<td>..</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>107</td>
<td>..</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>114</td>
<td>..</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>114</td>
<td>..</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>114</td>
<td>..</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>114</td>
<td>..</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>114</td>
<td>..</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>110</td>
<td>..</td>
</tr>
<tr>
<td>17†</td>
<td>M</td>
<td>125</td>
<td>..</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>135</td>
<td>..</td>
</tr>
<tr>
<td>19‡</td>
<td>M</td>
<td>135</td>
<td>..</td>
</tr>
<tr>
<td>20</td>
<td>M</td>
<td>135</td>
<td>..</td>
</tr>
<tr>
<td>21</td>
<td>F</td>
<td>135</td>
<td>..</td>
</tr>
<tr>
<td>22‡§</td>
<td>F</td>
<td>135</td>
<td>..</td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>135</td>
<td>..</td>
</tr>
<tr>
<td>24</td>
<td>M</td>
<td>135</td>
<td>..</td>
</tr>
</tbody>
</table>

* Focal areas fulfill morphologic criteria of LSA in situ.
† Noninbred.
‡ Cells in this lobe resemble reticular cells.
§ Regenerative lobe shows focal LSA, and there is invasion of the capsule.
series, occurring first in an animal 72 days post-injection, but being found as late as 135 days post-injection (Table 2).

A mild degree of cortical atrophy involving one lobule of one thymic lobe is shown in Figure 7. A more severe degree of cortical atrophy involving an entire lobe is shown in Figures 8, 12, 14. These markedly atrophic lobes consisted primarily of reticular cells in a loose irregular arrangement, with only a few scattered, normal-appearing, small, medium, and large lymphocytes (Figs. 11, 13, 15). These lobes were small, suggesting that the increased concentration of reticular cells was due to the disappearance of small lymphocytes and collapse of reticular cells rather than to their proliferation. Many of the cells in atrophic areas had morphologic features common to both reticular cells and large lymphocytes (Figs. 11, 13).

Regeneration.—"Regeneration" is defined as a focal or generalized increase of cells morphologically similar to normal medium and large lymphocytes. Ten animals showed this type of abnormality (Table 2). Varying degrees of regeneration were seen, and the milder degrees always occurred in an otherwise atrophic area (Figs. 14, 15). It was seen as early as 100 days and as late as 153 days post-injection. "Regeneration" was never observed in an otherwise normal lobe.

The finer morphologic details of focal regenerative changes in an atrophic lobe are illustrated in Figure 16. There were small collections of medium and large lymphocytes scattered throughout this lobe. Many cells in this lobe had features intermediate between a reticular cell and medium and large lymphocytes. These transitional forms were always present in the most markedly atrophic areas or in those areas showing early regenerative changes.

An advanced stage of regeneration was seen in the other lobe of the same thymus. This lobe was packed with cells morphologically similar to normal medium and large lymphocytes (Figs. 14, 15). However, the monomorphous growth pattern of these cells was distinctly different from the clusters of medium and large lymphocytes frequently found in normal adult mouse thymus and illustrated in Figures 4 and 5. The advanced stage of regeneration illustrated in Figure 15 differs from the histologic picture of lymphosarcoma in two ways: (a) the syncytial arrangement of cells characteristic of lymphosarcoma is not present; and (b) the amount of karyorrhexis and phagocytosis of nuclear debris is similar to what may be seen in the normal thymus. Multiple sections of this lobe showed no evidence of capsular invasion. At some levels of sectioning a medulla was present. An intermediate stage of regeneration is shown in Figures 8–10. One lobe of this thymus showed a well defined cortex and medulla (Fig. 8). The cortex was composed entirely of cells morphologically similar to the normal medium and large lymphocyte. Islands of these cells arising in the medulla, and presumably proliferating there, are shown in Figure 10.

In one lobe of one thymus (mouse #19, Table 2) the general features of advanced regeneration were present, but the cytology was atypical (Fig. 17). These cells resembled the transitional forms that are seen in lobes showing mild regenerative changes —i.e., they showed morphologic features intermediate between reticular cells and large lymphocytes.

Lymphosarcoma in situ.—Lymphosarcoma in situ is characterized by the replacement of thymic architecture by large monomorphous cells packed into a syncytial arrangement (Figs. 18, 19). In those areas of the tumor where the cells are not so closely packed, lymphosarcoma cells are morphologically indistinguishable from normal, large lymphocytes (Fig. 19 vs. Fig. 4). There is no invasion of the capsule and no spread of lymphosarcoma cells to other organs. The great amount of karyorrhexis seen in lymphosarcoma is also present in lymphosarcoma in situ. Mitotic figures are abundant. Four animals showed this change in one thymic lobe (Table 2). The gross dimensions of the lobes showing lymphosarcoma in situ were either within normal limits or moderately enlarged. The histopathology was identical to that of invasive lymphosarcoma (Fig. 19 vs. Fig. 20), except for the lack of capsular invasion.

Lymphosarcoma.—"Lymphosarcoma" is distinguished from lymphosarcoma in situ by invasion of the thymic capsule or spread to other organs by lymphosarcoma cells. Six animals had lymphosarcoma at the time of sacrifice (Table 2). It occurred as early as 107 days post-inoculation. In two animals only local invasion of the capsule had occurred. One thymic lobe of one mouse (§22, Table 2) was unusual in that it showed focal lymphosarcoma with capsular invasion (Figs. 20, 21). The remainder of the lobe appeared to be in the regenerative phase. Four animals showed widespread invasion of distant organs with lymphosarcoma cells.

Miscellaneous observations.—Two animals (#16, 11, Table 2) had leukemic involvement of the peripheral blood with white counts of 21,000 and 25,000, respectively. Smears of the peripheral blood of these animals showed an increase in the number of lymphocytes, and most of these cells were larger than the small lymphocytes ordinarily

Downloaded from cancerres.aacrjournals.org on April 13, 2017. © 1963 American Association for Cancer Research.
seen in normal mouse blood. However, there were no features which served to distinguish the leukemic cells from cells occasionally seen in mouse blood in small numbers.

The femoral marrow of mice with frank leukemia was replaced by lymphocytes. In the marrow of mouse #16 (Table 2) typical lymphosarcoma cells (large lymphocytes) were present, but the femoral marrow of the other leukemic mouse (#11, Table 2) was composed of small lymphocytes. Typical lymphosarcomatous infiltrates were present in other organs of this mouse.

In no animal did we find evidence of lymphosarcoma in any tissue studied until marked abnormality was present in the thymus. Splenic weight did not increase until the spleen was involved with the lymphosarcomatous process. The spleen of mouse #5 (Table 2) weighed 170 mg., and histologic examination showed many atypical large lymphocytes, interpreted as probably malignant cells, concentrated just beneath the splenic capsule (Fig. 28). The basic architecture of the spleen was intact. The thymus of this animal showed marked "regeneration" with some areas of lymphosarcoma in situ. The spleen of mouse #16 (Table 2) weighed 670 mg., and showed virtually total replacement of normal white pulp by lymphosarcoma cells. Nevertheless, the distinction between "white pulp" and red pulp was maintained.

In only one lymph node did we see evidence of localized sarcomatous involvement. This was a tracheal node, lying adjacent to the sarcomatous thymus of mouse #22 (Table 2), in which LSA cells were present only in the hilus of the node and in the perinodal connective tissue. We saw no examples of early bone marrow involvement. The phagocytosis of leukocytes by megakaryocytes reported by Dunn and Moloney (9) was not present in our material.

In those animals showing widespread organ involvement with lymphosarcoma the following organs were involved in at least one animal: lungs, liver, kidney, ovary, small intestine, lymph nodes, spleen, and marrow. The tumor cells tended to collect around blood vessels, but no clear-cut example of vessel wall invasion was seen. No salivary gland carcinomas occurred in our experimental animals.

DISCUSSION

HISTOGENESIS

It is apparent from our results (Table 2) and from mortality statistics obtained by others (5) that Gross leukemia develops with varying rapidity in individual mice despite attempted standardization of experimental conditions including use of inbred mice of similar age, use of a standard dose of one virus lot, and maintenance of animals under similar conditions. This variability makes it difficult to understand the pathogenesis of Gross leukemia by autopsy of animals at varying intervals following injection with Passage A virus.

Arnesen (3) encountered a similar problem in his study of spontaneous AK mouse leukemia. The various preleukemic changes which he observed in the thymus were similar to those observed by us. Arnesen arranged his observations on individual mice into the following sequence: An initial period of cortical thinning and "fragmentation" with eventual loss of the thymic cortex (corresponding to our "cortical atrophy"); a concurrent or subsequent proliferation of the medullary reticular cells (corresponding to our stage of "regeneration"); malignant transformation of the reticular cells and the eventual development of thymic lymphosarcoma (corresponding to our stages of lymphosarcoma in situ and lymphosarcoma).

We propose that Gross leukemia arises in the inbred C3H(f)/Bi mouse after a similar sequence of histologic changes in the thymus. This particular sequence appears probable for the reasons that follow.

Cortical atrophy, if at all related to the subsequent development of lymphosarcoma, must precede rather than follow lymphosarcoma. The data in Table 2 support, although admittedly do not prove, this relationship. It is seen from the table that cortical atrophy was the earliest abnormality to appear, and it generally tended to occur earlier than lymphosarcoma.

Other personal observations on more than twenty mice sacrificed with far-advanced Gross leukemia have shown complete replacement of the thymus with typical lymphosarcoma cells and not cortical atrophy.1 Furthermore, other investigators who have studied the histology of far advanced Gross leukemia have not reported the presence of cortical atrophy (5, 12).

It is possible that one lobe of the thymus atrophies while the other lobe goes on to develop lymphosarcoma which subsequently obliterates all evidence of the atrophic lobe. In many, if not all, instances of atrophy this does not happen, as shown by the regenerative changes that we frequently observed in otherwise atrophic lobes.

Authors in addition to Arnesen support the observation that a stage of cortical atrophy precedes lymphosarcoma arising in the thymus. Dunn and Moloney (9) reported a preleukemic atrophy of the thymus in BALB/c mice given injections of Moloney virus. They apparently did not observe

1 Unpublished observations.
the stage of development we have called regeneration. Irradiation-induced mouse leukemia arising in the thymus is preceded by a period of atrophy (15, 25). Rappaport and Baroni (21) observed thymic atrophy in Swiss mice prior to the development of hydrocarbon-induced lymphosarcoma.

Levinthal et al. (17) studied the development of leukemia in hybrid mice (offspring of AKR females and C57/Bi males) given injections of passage A virus. These authors observed a thymic atrophy in a few mice which had been given injections, but they did not consider it significant, because they observed a similar atrophy in their control mice. However, since 75 per cent of their hybrid control animals developed a spontaneous leukemia, it is possible that the atrophy observed in control and virus-injected animals was a preleukemic change.

It would appear that the change we have called "regeneration" must follow rather than precede cortical atrophy. Early regenerative changes always occurred in an otherwise atrophic lobe (Fig. 16) and were never observed in a normal lobe. On the other hand, the clusters of large and medium lymphocytes, commonly observed in the cortex of control and injected control animals of all ages, were also seen in the experimental animals prior to the development of cortical atrophy. These clusters, interspersed with small lymphocytes (Figs. 4, 5), were easily distinguishable from the monomorphic closely packed large lymphocytes seen in intermediate stages of regeneration (Fig. 9).

The changes that we have labeled "regeneration" are clearly related to the subsequent development of lymphosarcoma. In fact, advanced regeneration, such as illustrated in Figs. 14 and 15, merges with LSA in situ and can be distinguished from it only by arbitrary criteria. We accept as a basic premise that lymphosarcoma is a progressive condition, and therefore regeneration must precede rather than follow lymphosarcoma. We never observed focal lymphosarcoma in an otherwise normal lobe, nor did we see it in an otherwise atrophic lobe.

Our observations do not rule out the possibility that repeated episodes of atrophy and regeneration occur, and they do not insure that a lobe showing atrophy or regeneration at the time of sacrifice would always have developed lymphosarcoma.

RELATIONSHIP TO OTHER TYPES OF MOUSE LEUKEMIA

As already noted, the pathogenesis of Gross leukemia is similar to other forms of mouse leukemia. The striking similarities in histogenesis between Gross leukemia and spontaneous leukemia of the AK mouse are of particular interest, because the Gross virus was originally derived from AK mouse tissue.

In other forms of mouse leukemia the thymus does not play a major role in the histogenesis of the disease. Friend leukemia of the Swiss mouse begins in the spleen (19). The initial changes are similar to those observed by us in one animal with early splenic involvement (Fig. 25). The thymus in Schwartz's mouse leukemia is not involved until late in the course of the disease (24), and the thymus apparently plays no part in the pathogenesis of spontaneously occurring leukemia in the C58 mouse (20). The pathogenesis of leukemia in the DBA mouse treated with methylcholanthrene has not been well studied. It has been claimed by some investigators that leukemia does not begin in the thymus (18), but other authors have expressed the opposite view (1, 2).

THEORETICAL CONSIDERATIONS

It can be seen from our results that the classical definitions of "malignancy" are inadequate when describing the morphologic transitions from "normal" to "malignant." By arbitrary criteria we have divided the "pre-malignant" changes into three sequential categories (cortical atrophy, regeneration, lymphosarcoma in situ), which in reality form a continuum. It is probable that under the conditions of our experiment (0.1 cc. of passage A virus injected in young C57(f)/Bi mice) the scattered regenerative cells, seen in an otherwise atrophic thymus (Fig. 16), are obligated to grow in a "malignant" manner. These cells are indistinguishable with the light microscope from normal medium and large lymphocytes (Figs. 4, 5). It is only by their eventual abnormal growth pattern that regenerative cells subsequently fulfill classical criteria for "malignancy."

As previously noted, lobes showing late atrophy and/or early regeneration contain many cells with morphologic features intermediate between the reticular cell and large lymphocyte. We agree with Arnesen (3) that the regenerative cell arises from a transformation of the reticular cell.

In one of our experimental animals (mouse #19, Table 2) an entire thymic lobe was replaced by closely packed cells with morphologic features intermediate between reticular cells and large lymphocytes (Fig. 17). It is possible that this proliferation of cells actually represented a reticulum-cell sarcoma in situ. On the other hand, it is possible that in this animal "regeneration" was in a late phase, and differentiation of reticular cells to medium and large lymphocytes had not yet occurred.

Although the Gross virus is in some way associated with the proliferation of cells, it is also
associated with cell destruction. In all areas of the tumor dying cells are numerous. It is possible that the increased cell death is not due to the virus per se but is in some nonspecific way related to the rapid proliferation of cells.

Large and medium lymphocytes, contrary to Axelrad’s and van Der Gaag’s claim (5), are commonly found in the subcapsular zone of adult mice. It has been suggested that animals older than 3 weeks are resistant to the induction of leukemia by injection of virus because they lack the subcapsular zone of large lymphocytes seen in younger mice (5). This theory is unlikely (a) because large and medium lymphocytes are found in the subcapsular zone of older mice and (b) because, with repeated passage, the Gross virus will induce leukemia in older mice (14).

ACKNOWLEDGMENTS

We are grateful to Dr. Ludwik Gross for supplying us with mice for the preparation of virus and for breeding purposes.

REFERENCES


FIG. 6.—Medulla of thymus pictured in Figure 1 showing reticular cells (R) and small lymphocytes (SL). X1200.

FIG. 7.—Thymus showing cortical atrophy (CA) involving one lobule of one thymic lobe. Medulla (M), cortex (C). X60.

FIG. 8.—Thymus showing marked cortical atrophy of one lobe (AL) and regeneration of other lobe (RL). Regenerating lobe has “cortex” (C) and medulla (M). X60.

FIG. 9.—Same thymus pictured in Figure 8. Note prominence of reticular cells and decreased number of small lymphocytes in atrophic lobe (AL). Compare atrophic lobe to Figure 4. The other lobe (RL) shows a moderately advanced stage of regeneration. The entire cortex is packed with medium and large lymphocytes but individual cell borders remain distinct. Note the monomorphous appearance of this cortex in contrast to the pleomorphism, illustrated in Figures 4, 5. X600.

FIG. 10.—Regenerative lobe of Figures 8 and 9 showing islands (Is) of medium and large lymphocytes encroaching upon the medulla (M) and presumably proliferating there. X600.

FIG. 11.—High-power view of atrophic lobe shown in Figures 8, 9. Note the paucity of small lymphocytes (SL) and the prominence of the reticular cells (R). There are many cells (T) that have morphologic features of both the reticular cell and the large lymphocyte (LL). X1200.
FIG. 12.—Extreme cortical atrophy of entire thymic lobe. X60.

FIG. 13.—High-power view of Figure 12 showing only rare small lymphocyte. Many of the cells (T) demonstrate features intermediate between reticular cells (R) and large lymphocytes. X1200.

FIG. 14.—Advanced regenerative changes with almost complete replacement by medium and large lymphocytes (AR). The other lobe shows early regenerative changes in an otherwise atrophic lobe (ER). X60.

FIG. 15.—Higher power of thymus pictured in Figure 14, to show interface between advanced regeneration (AR) and early regeneration (ER). At this magnification, the lobe showing early regeneration (ER) closely resembles the pure cortical atrophy illustrated in Figure 9, but there are more reticular cells and large lymphocytes and fewer small lymphocytes than in Figure 9. X400.

FIG. 16.—Small clusters of packed medium and large lymphocytes (CL) in the otherwise atrophic lobe shown in Figures 14 and 15. Reticular cells (R), large lymphocytes (LL), and cells with morphologic features of both the large lymphocyte and the reticular cell (T) are seen. X1200.

FIG. 17.—Cells are intermediate in morphology between reticular cells and large or medium lymphocytes (mouse #19, Table 2). X1200.
FIG. 18.—Thymus showing LSA in situ. X60.

FIG. 19.—High-power view of Figure 18 showing syncytial packing (S) of cells and large numbers of degenerating cells (d). Where individual cell borders are distinct, the cells resemble large lymphocytes (LL). X600.

FIG. 20.—One lobe showing focal lymphosarcoma in an otherwise regenerative lobe. Note the syncytial packing of cells. X400.

FIG. 21.—Another area of same lobe seen in Figure 20. In this area the cells are less densely packed, and there is less cellular debris and phagocytosis. However, invasion and/or lifting of the capsule has occurred (Ire). X600.

FIG. 22.—High-power view of area shown in Figure 20. Note phagocytosis (P) of cellular debris by reticular cells. X1200.

FIG. 23.—Spleen showing LSA cells beneath the splenic capsule. X600.
The Histogenesis of Gross's Viral Induced Mouse Leukemia

Stephen B. Goodman and Matthew H. Block