Early Lesions in Cats Inoculated with Feline Leukemia Virus

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SUMMARY

Morphological alterations in the thymus, lymph nodes, and bone marrow were determined at 2-week intervals in 21 cats inoculated at birth with feline leukemia virus and in 19 age-matched control cats. Severe thymic atrophy was detected at 5 weeks of age. Thymic atrophy was due primarily to extensive depletion of cortical thymocytes while the medullary components of many thymic lobules persisted. Apparent stages in the development of lymphosarcoma from atrophic thymuses were detected in cats examined between 11 and 19 weeks of age. Lymphosarcoma was detected as early as 9 weeks in some cats, by examination of bone marrow biopsies.

INTRODUCTION

Feline LSA2 (feline leukemia) occurs spontaneously and can be produced experimentally in outbred animals that commonly share the same environment with man (14, 18, 20, 23, 36, 37, 43). The viral etiology and major anatomic patterns of the disease have been well documented (2, 5, 12—15, 17—20, 23, 32, 33, 36, 37, 43). However, relatively little is known of the alterations that precede the onset of LSA in the cat (17). Jarrett (16), Anderson et al. (3), and Hoover et al. (14) have observed thymic atrophy, lymphoid depletion, wasting disease, and apparent increased susceptibility to infection in cats inoculated with FeLV, suggesting that the thymic-lymphatic system and immune response of pre-leukemic cats may be abnormal. The objectives of this study were to determine the morphological alterations in the thymus, lymph nodes, and bone marrow during the preneoplastic and early neoplastic phases of experimental feline LSA and to correlate lesions in these tissues with skin allograft survival time (34).

MATERIALS AND METHODS

Animals and FeLV Inoculation. A total of 40 pathogen-free cats were used in this study. The cats were obtained from a cesarean-derived, disease-free breeding colony which has been maintained in strict isolation for more than 6 years (38). No spontaneous case of LSA has occurred in the colony since its origin. Twenty-one cats were inoculated i.p. on the day of birth with 1.0 ml of an inoculum containing FeLV. Nineteen cats were used as age-matched, uninoculated controls. The inoculum consisted of a 0.45-μm filtrate of a 20% (w/v) homogenate of bone marrow and lymph node prepared from a gnotobiotic cat inoculated with the Rickard isolate of FeLV3 (36). The viral stock has been examined for contamination with mycoplasma, feline reovirus, feline panleukopenia virus, and feline syncytial virus.4 No extraneous microbial agents have been detected. The disease induced in gnotobiotic and pathogen-free cats by the i.p. administration of this inoculum has been described (14). LSA occurred in all of the inoculated cats that were allowed to survive to 20 weeks of age (14).

Inoculated litters were housed in isolation rooms separate from control litters. The kittens were left with their dams until 12 weeks of age to minimize any stress associated with weaning which could influence thymic size. All of the kittens in a given litter were either inoculated or used as controls. Inoculating one-half of the kittens in each litter was not considered to be desirable, since horizontal transmission of FeLV from inoculated to control kittens in the same litter would be anticipated (8, 14). The queens had not suckled any previous litters inoculated with feline oncornaviruses.

 Necropsy and Sample Collection. Two inoculated and 2 control cats were necropsied at 1, 3, 5, 7, 9, 11, 13, 15, 17, and 19 weeks of age (weeks postinoculation). Three inoculated cats were available at 17- and 19-week intervals. On the day of necropsy, the following procedures were performed on each cat. Blood was collected for a complete hemogram and body weight was recorded in g. Bone marrow aspirates were obtained from the proximal femur with an 18-gauge, 1.5-inch Osgood biopsy needle. Smears of aspirated marrow were prepared on coverslips and stained with Wright and Giemsa stains, and differential counts of 500 cells were performed independently on each sample by 2 of us (G. J. K., E. A. H.). The mean of the 2 counts was used to compare inoculated and control cats.

 A complete necropsy was performed, and tissues were fixed in phosphate-buffered formalin. The thymus and the mesenteric, pharyngeal, mandibular, and popliteal lymph nodes were carefully dissected from surrounding fascia and adipose tissue and were weighed after fixation. The proportion

1 Supported by NIH Contract PH-43-65-1001 from the Special Virus Cancer Program, and by NIH Grants RR-05463 and GM-1052.
2 The abbreviations used are: LSA, lymphosarcoma; FeLV, feline leukemia virus.
3 Kindly provided by Dr. C. G. Rickard, Department of Pathology, New York State Veterinary College, Ithaca, N. Y. 14850.
4 In collaboration with Dr. D. Holmes and Dr. J. H. Gillespie, Department of Microbiology, New York State Veterinary College, Ithaca, N. Y. 14850.

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of the body weight comprised by each of these tissues was expressed as mg of tissue per g of body weight.

RESULTS

Incidence of Lesions of LSA. All 3 cats that were allowed to survive to 19 weeks of age developed LSA. An additional 4 cats killed between 9 and 15 weeks of age also had lesions of LSA. Of the 7 cats with LSA, lesions were present in the thymus of 4, the bone marrow of 4, the eyes of 3, the lymph nodes of 2, and the intestine of 1. No significant lesions were detected in other tissues.

Thymus. Atrophy of the thymus was the earliest lesion detected in the cats inoculated with FeLV. Severe thymic atrophy was evident at 5 weeks of age but was not present at 3 weeks of age (Table 1; Fig. 1). Of the inoculated cats necropsied between 5 and 19 weeks of age, 11 had severely atrophic thymuses, 4 had ISA replacing the thymuses and 3 had thymuses within normal limits (Table 1).

Atrophy of the thymus was principally due to extreme depletion of cortical thymocytes. In most severely atrophied thymuses, only the medullary portions of thymic lobules remained. The atrophic thymic lobules were separated by loose areolar connective tissue (Figs. 2 and 3). The size of the medullary portions of lobules in atrophic thymuses was initially similar to that of controls (at 5 weeks) but, thereafter (at 9 to 17 weeks), partial-to-complete dissolution, collapse, and fusion of the medullary remnants of many thymic lobules occurred. At no time was a significant degree of pyknosis or karyorrhexis of thymic cells detected.

Alterations in the epithelial component within and between atrophic thymuses were variable. Some Hassall's corpuscles were inconspicuous and atrophic, some were within normal limits, and some were prominent due to extreme keratinization and mineralization. The latter change was also observed to a milder degree in the thymuses of 2 of the 19 control cats.

The microscopic appearance of the grossly atrophic thymuses of 2 inoculated cats varied from the above pattern. The surviving medullary components of the thymic lobules were expanded due to insinuation of lymphoblasts and lymphocytes throughout the lobules (Fig. 4). The lymphoid cells did not tend toward cortical alignment, focal aggregation, or follicle formation. On the basis of similar observations in mice inoculated with murine leukemia virus (4, 40), this histological alteration in atrophic thymuses was interpreted as a preneoplastic lesion. The bone marrow smears from 1 of the 2 cats contained 10% lymphoblasts and undifferentiated blasts and was considered diagnostic of LSA (see below).

LSA arising from the thymus was detected in 4 cats, including each of the 3 inoculated cats that were killed at 19 weeks of age (Fig. 5; Table 1). Masses of large lymphoblasts, with interspersed histiocytic cells with abundant clear cytoplasm, obliterated thymic lobules and extended into the mediastinal fascial planes (Fig. 6). Additional cytological features of the populations of neoplastic cells were abundant mitotic figures, hyperchromatism, multiple nucleoli, and clear cytoplasmic vacuoles. Hassall's corpuscles were the most persistent histological remnant of the thymic lobules involved with LSA (Fig. 7). Thymic lobules that were adjacent to an expanding LSA were usually atrophic. However, some lobules in thymuses involved with LSA did contain distinct cortical

| Table 1
| Weight of the thymus and mesenteric node of control and FeLV-inoculated cats |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Control cats    |                 | FeLV-inoculated cats |
|                 | Thymus wt.      | Mesenteric node wt. | Thymus wt.      | Mesenteric node wt. |
| Age (wk)        | mg/g body wt.  | g               | mg/g body wt.  | g               |
| 1               | 1.30           | 6.70            | 0.23           | 0.18            |
| 2               | 1.61           | 5.05            | 0.54           | 1.70            |
| 3               | 1.50           | 4.57            | 0.50           | 1.80            |
| 4               | 1.46           | 4.26            | 0.36           | 1.04            |
| 5               | 1.59           | 4.61            | 0.87           |                 |
| 6               | 0.94           | 1.90            | 0.55           | 1.11            |
| 7               | 1.95           | 3.52            | 0.98           | 1.76            |
| 8               | 2.10           | 2.18            | 2.06           | 2.16            |
| 9               | 1.55           | 1.54            | 3.27           | 3.27            |
| 10              | 1.81           | 1.68            | 2.83           | 2.63            |
| 11              | 4.31           | 3.35            | 3.06           | 2.36            |
| 12              | 4.39           | 3.95            | 3.01           | 2.71            |
| 13              | 1.73           | 1.78            | 2.30           | 2.38            |
| 14              | 2.51           | 1.73            | 2.56           | 1.76            |
| 15              | 2.11           | 1.61            | 1.88           | 1.44            |
| 16              | 1.72           | 1.01            | 2.74           | 1.67            |
| 17              | 1.63           | 0.93            | 3.55           | 2.03            |
| 18              | 3.52           | 2.39            | 3.20           | 2.18            |
| 19              | 3.78           | 2.72            | 2.78           | 1.94            |
| a Lymphosarcoma present. |

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and medullary zones, implying either that uniform thymic atrophy did not precede the onset of thymic LSA in all cases or that partial regeneration occurred during LSA induction.

**Lymph Nodes.** Lesions of LSA were present in the mesenteric node of one cat and the sternal node of a 2nd cat. Distinct populations of neoplastic lymphoblasts were present in the peripheral and medullary sinuses and in the paracortical zones (Fig. 8). Although the nodes were enlarged, only mild histological architectural distortion had occurred. The neoplastic cells were 20 to 25 μm in diameter and had large round or indented nuclei with finely dispersed but darkly staining chromatin and prominent nucleoli. No neoplastic cells were detected in the germinal centers. The lymph nodes of the remaining FeLV-inoculated cats could not be distinguished from those of controls by histological examination. Although thymic atrophy was present in many of the inoculated cats, no significant paracortical depletion was detected in the lymph nodes.

The mesenteric, pharyngeal, and mandibular nodes of 3 inoculated cats were moderately enlarged but did not contain microscopic evidence of LSA. The enlargement was due principally to expansion of the medullary sinus tissue of the nodes. Each of the cats, however, did have lesions of LSA in either the thymus or bone marrow.

**Bone Marrow and Blood.** In 4 cats, LSA could be diagnosed on the basis of bone marrow biopsy. Three of the 4 cats with positive marrow biopsies had concomitant visceral lesions of LSA detected at necropsy. In 3 other cats with early thymic LSA detected at necropsy, marrow biopsies revealed myelogenous hyperplasia in 2, and values within the normal limits in the 3rd.

The criteria used for diagnosis of LSA from marrow cells were (a) a total lymphocyte count of 46% of all marrow cells (control mean ± 2 S.D.), or (b) a total of at least 10% lymphoblasts plus undifferentiated blast cells in the marrow (control mean = <1%). The morphology of lymphoblasts and more mature lymphocytes in a marrow smear from a representative cat is illustrated in Fig. 9.

The most notable feature of the bone marrow counts of the control SPF cats between 5 and 19 weeks of age was the large number of lymphocytes present (mean, 29.6%; Table 2). The mean total myeloid cell count of the FeLV-inoculated cats was significantly higher, and the mean total lymphoid cell count was significantly lower than corresponding control values (Table 2).

Packed erythrocyte volume was significantly lower in cats inoculated with FeLV, compared with controls (Table 3). No instances of true leukemia were observed in any of the cats inoculated with FeLV. LSA could not be diagnosed by analysis of hemograms from any of the 7 cats with lesions of LSA.

**DISCUSSION**

The results of this study indicate that atrophy of the thymus occurs in the preneoplastic period following experimental FeLV infection. The apparent sequence of early depletion of cortical thymocytes, diffuse infiltration or proliferation of lymphoblasts in the surviving portions of atrophic thymic lobules, and subsequent origin of LSA from the thymus indicates that the morphogenesis of the viral-induced thymic LSA in cats is similar to that in mice (4, 7, 11, 27, 40). In mice, similar thymic lesions also precede LSA induced by irradiation (21, 22) or carcinogen treatment (9, 35). Yet to be resolved is the possibility that leukemogenesis in the cat is thymic dependent, as it is in mice (29, 30).

We have found that thymic atrophy in the cats reported here correlated with depression of the cell-mediated immune response, as assessed by cutaneous allograft rejection (34). Paracortical lymphoid depletion in lymph nodes was not present. The absence of paracortical lymphoid depletion is not surprising, since peripheralization of presumable postthymic lymphoid cells occurs by 33 days of gestation in cats (1). Consequently, the lymph nodes of newborn kittens are morphologically well developed when FeLV inoculation occurs. On the basis of the period of over 5 months required for decay of the postthymic cell population of adult mice following thymectomy (28, 31, 41, 42), the interval required for lymphoid depletion following complete thymic ablation in cats would be expected to exceed the duration of this experiment. However, the relationship of the thymus to the ontogeny of the immune response of cats has not been

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**Table 2**

*Mean bone marrow counts of control and FeLV-inoculated cats*

| Age range, 5 to 19 weeks. | 
|---|---|---|
| Bone marrow count (%) in | Total myeloid cells | Total erythroid cells | Total lymphoid cells |
| Controls | 12 | 32.9 ± 8.6 | 38.3 ± 10.3 | 29.6 ± 7.8 |
| FeLV-inoculated | 18 | 43.4 ± 17.9 | 35.2 ± 17.5 | 21.0 ± 14.1 |
| Probability | <0.025 | >0.10 | =0.025 |

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*a Mean ± S.D.
*b Seven of 18 cats had values > (control X ± 2 S.D.).
*c Two of 18 cats had values > (control X ± 2 S.D.); 6 of 18 cats had values < (control X ± 2 S.D.).
*d By t test.
Table 3

Mean hemogram values of control and FeLV-inoculated cats

<table>
<thead>
<tr>
<th>Cats</th>
<th>No. tested</th>
<th>Total hemoglobin (g/100 ml)</th>
<th>Packed cell volume (%)</th>
<th>Total leukocytes per cu mm (X 10^3)</th>
<th>Total neutrophils per cu mm (X 10^3)</th>
<th>Total lymphocytes per cu mm (X 10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>15</td>
<td>11.00 ± 1.30^a</td>
<td>32.5 ± 3.89</td>
<td>14.21 ± 7.00</td>
<td>5.63 ± 2.50</td>
<td>6.88 ± 4.53</td>
</tr>
<tr>
<td>FeLV-inoculated</td>
<td>18</td>
<td>9.63 ± 1.86^b</td>
<td>28.78 ± 5.78</td>
<td>18.00 ± 9.45</td>
<td>10.80 ± 8.06</td>
<td>5.21 ± 3.33</td>
</tr>
<tr>
<td>Probability^c</td>
<td>&gt;0.10</td>
<td>&lt;0.025</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
</tr>
</tbody>
</table>

^a Mean ± S.D.
^b Six of 18 cats has values < (control X – 2. S D.).
^c By t test.

investigated, and analogies to the thymic function of adult rodents may be invalid. Furthermore, a wasting disease characterized by generalized lymphoid atrophy, in addition to thymic atrophy, has been observed previously in some cats inoculated with FeLV (3, 14, 16).

Although the mechanism of thymic atrophy was not revealed by histological study, there was no evidence that massive destruction of thymic cells occurred in situ, which might be expected if direct viral lysis of thymocytes occurred. An alternative explanation for atrophy of the thymus could involve abnormalities in the traffic of thymic precursor cells from the bone marrow, since continual replacement of thymocytes appears to depend on continual supply of hemolymphatic stem cells from the marrow (10, 26), and FeLV has an affinity for bone marrow cells (24, 25, 37). Atrophy of the thymus could be an indirect effect of FeLV infection which is mediated through increased secretion of endogenous adrenocorticosteroids. The observations of Santisteban et al. (39), which indicate that thymic atrophy in mice infected with LDH virus is mediated through the action of the adrenal gland, are particularly relevant in this regard.

We consider protein-calorie malnutrition to be an unlikely primary cause of thymic atrophy in cats inoculated with FeLV, since the cats killed at the earliest interval at which marked thymic atrophy was detected (5 weeks) were in good physical condition, with body weights comparable to controls. It is also unlikely that preleukemic thymic atrophy is caused by the action of an undetected virus other than FeLV, since the FeLV inoculum and the cats from our disease-free cat colony have been examined for the presence of other known feline viruses and none have been detected.

Examination of bone marrow biopsies did permit early detection of LSA in some cats. However, 3 of 7 cats with concurrent visceral lesions of LSA had negative marrow biopsies. Thus, although a useful diagnostic adjunct, the reliability of marrow examination for diagnosis and sequential evaluation of LSA in cats appears limited. In none of the cats inoculated with FeLV, including 7 with concurrent LSA, could the disease be diagnosed by examination of peripheral blood. The only significant differences between hemograms of control versus inoculated cats were in erythrocytic parameters. These data are compatible with those regarding spontaneous feline LSA, which indicate that leukemia only occurs in approximately 10% of all cases (2, 5, 12, 13).

REFERENCES

Fig. 1. Bottom, atrophic thymuses from 2 cats inoculated with FeLV, compared with thymuses (top) from 2 control cats at 5 weeks of age.

Fig. 2. Thymus from a 7-week-old control cat. There is a distinct cortical mantle of thymocytes resulting in definable cortical and medullary zones in each thymic lobule. X 125.

Fig. 3. Thymus from a 7-week-old control cat. There is a distinct cortical mantle of thymocytes resulting in definable cortical and medullary zones in each thymic lobule. X 125.

Fig. 4. Expansion of a lobule in an atrophic thymus into the perilobular adipose tissue due to diffuse proliferation of lymphoblastic cells. This change, which occurred in the atrophic thymuses of 2 cats inoculated with FeLV, was considered preneoplastic. X 125.

Fig. 5. Thymic lymphosarcoma in 3 cats killed at 19 weeks of age. Top, thymus of a control cat of the same age. X 125.

Fig. 6. Invasion of neoplastic lymphoblasts into the perilobular adipose tissue in an early thymic LSA in a cat 19 weeks of age. X 125.

Fig. 7. Hassall's corpuscles in a thymic lobule nearly obliterated by LSA. Interspersed histiocytic cells with abundant pale-staining cytoplasm appear as irregular clear spaces diffusely distributed among the neoplastic lymphoblasts. X 125.

Fig. 8. Early lesion of LSA in the mesenteric lymph node of a cat inoculated with FeLV. Neoplastic lymphoblasts (between arrows) are present in the peripheral sinus. Below the neoplastic cells is a portion of a normal cortical follicle containing a peripheral layer of mature lymphocytes surrounding the germinal center composed of nonneoplastic lymphoblasts and reticulum cells. X 315.

Feline Lymphosarcoma


Fig. 9. Lymphoblasts and more mature lymphocytes in a bone marrow smear from a cat with LSA detected by bone marrow biopsy. Giemsa stain, X 1250.
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