Experimental Transmission of Canine Malignant Lymphoma to the Beagle Neonate

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SUMMARY

Two serial passages of canine malignant lymphoma (CML) were accomplished in 2 litters of Beagle neonates with suspensions of viable CML whole cells. In these experiments 3 of 15 dogs inoculated with a single dose of either a cell suspension or a cell-free extract developed malignant lymphoma at 50, 53, and 80 days postinoculation. Two dogs with overwhelming CML were preirradiated, while one dog was not X-irradiated. Thus, a significant feature of this work was the successful transmission of CML without pretreatment of total-body irradiation. Malignant lymphoma did not occur in the third serial passage, although animals had enlargement of the superficial lymph nodes. Biopsies of these enlarged lymph nodes revealed lymphoid hyperplasia but not malignant lymphoma. The clinical signs and pathologic lesions were similar to those reported for naturally occurring malignant lymphoma of dogs and cats. Electron microscopic examination revealed intracytoplasmic structures which were made up of individual particles that measured 18 to 22 m\(^\mu\) in diameter.

INTRODUCTION

Malignant lymphoma in the dog (CML) has attracted considerable attention because of its histopathologic similarity to Burkitt's lymphoma of man (1, 9). Malignant lymphoma of the dog has been used by several investigators in transmission studies and in morphologic investigations at the ultrastructural level in an attempt to determine the etiologic agent, and to compare or contrast the findings with those in leukemia of man. Lymphoma accounts for approximately 2 to 3% of all tumors observed in dogs (13).

Lombard et al. (8) reported that four neoplasms of the dog were known to be transplantable: venereal sarcoma, anaplastic thyroid carcinoma, papilloma, and mastocytoma. Of the transplantable tumors, the papilloma and mastocytoma have been transmitted with a cell-free filtrate (8).

Cellular transmission of lymphoma in domestic chickens and rodents has been accomplished with relative ease by inoculating cells into the same species. Cellular transfer of lymphoma in domestic animals has, however, proved difficult. Recently, Moldovanu et al. (1966) reported cellular transfer of malignant lymphoma to X-irradiated mongrel puppies.

In the present study, a spontaneous CML was found to be transmissible to Beagle neonates. Macroscopic, microscopic, and ultrastructural characteristics of experimental CML are described in detail. Clinical and pathologic characteristics of spontaneous CML have been described in previous reports (2, 6, 13, 16, 18).

MATERIALS AND METHODS

Spontaneous Malignant Lymphoma. The initial donor dog was a 20-month-old, female, purebred Doberman Pinscher with acute malignant lymphoma. Clinical history and signs included a sudden onset, dyspnea, anorexia, listlessness, and hydrothorax. Radiographs of the thoracic cavity revealed a tumorous mass. A hemogram indicated anemia and lowered platelet count, both of which have been common findings in over 65 spontaneous CML cases examined in our laboratory. In the thoracic cavity, there was approximately 200 ml of fluid which contained numerous neoplastic lymphocytes. The tumor mass observed at necropsy was located bilaterally in the anterior half of the thoracic cavity. The tumor appeared to involve the thymus and anterior mediastinal lymph node and extended to the trachea and esophagus dorsally and the lungs posteriorly. Many of the superficial and deep body lymph nodes were enlarged and edematous. The liver and spleen were markedly enlarged.

Microscopically, the affected lymph nodes and anterior thoracic tumor mass consisted of a uniform distribution of neoplastic lymphocytes (Fig. 1). The lymph node architecture was completely obliterated with proliferating lymphocytes. Neoplastic lymphoid cells were characterized by prominent, round to ovoid nuclei rich in chromatin, conspicuous cytoplasm, prominent nucleoli, and mitotic figures. Other organs affected with lymphocytic infiltration (Table 3) had a similar cell type as mentioned above with variable replacement of tissue parenchyma.

Experimental Animals. Purebred Beagle neonates of both sexes, varying in age from birth to 3 days, were used (Table 1). There were three serial passages of CML attempted, utilizing 3 litters of Beagle neonates (17 dogs). Using single doses of inoculum, 11 dogs were given viable whole cells and 4 cell-free
Canine Malignant Lymphoma

Table 1

<table>
<thead>
<tr>
<th>Donor of malignant lymphoma</th>
<th>Passage generation</th>
<th>Recipients</th>
<th>Age inoculated (days)</th>
<th>Sex</th>
<th>Irradiated</th>
<th>Tissues inoculated</th>
<th>Route &amp; amount</th>
<th>Cell counts (cells/ml)</th>
<th>Termination*</th>
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<tr>
<td>10019</td>
<td>1</td>
<td>30030</td>
<td>Birth</td>
<td>F</td>
<td>Yes</td>
<td>Thymus</td>
<td>i.p. 1.5 ml</td>
<td>8.5 x 10^6</td>
<td>50 days</td>
</tr>
<tr>
<td>30031</td>
<td></td>
<td>30032</td>
<td>Birth</td>
<td>M</td>
<td>Yes</td>
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<td>i.p. 1.5 ml</td>
<td>8.5 x 10^6</td>
<td></td>
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<tr>
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<td></td>
<td>30034</td>
<td>Birth</td>
<td>F</td>
<td>No</td>
<td>Thymus</td>
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<td>8.5 x 10^6</td>
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<tr>
<td>30035</td>
<td></td>
<td>30036</td>
<td>Birth</td>
<td>M</td>
<td>No</td>
<td>Thymus</td>
<td>i.p. 1.5 ml</td>
<td>8.5 x 10^6</td>
<td></td>
</tr>
</tbody>
</table>

Table 2

<table>
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<tr>
<th>Donor of ML</th>
<th>Passage* generations</th>
<th>Total No. of dogs</th>
<th>No. of animals with ML</th>
<th>Lymph nodes enlargement</th>
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</thead>
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<td>6</td>
<td>2/6</td>
<td>4/6</td>
</tr>
<tr>
<td>30032</td>
<td>2</td>
<td>5</td>
<td>1/4</td>
<td>2/4</td>
</tr>
<tr>
<td>30036</td>
<td>3</td>
<td>6</td>
<td>0/5</td>
<td>5/5</td>
</tr>
</tbody>
</table>

Serial passage of canine malignant lymphoma (CML) to three litters of Beagle neonates. Total body irradiation: 140 Peak Kilovolts (PKV), 3 mm Al (aluminum) HVL (half-value layer), 20 milliamperes (MA), 32 seconds (60.8 R).

"Dogs 30030, 30032, and 30036 were killed; the other dogs are still alive and apparently normal.

Results

Three of 11 Beagle neonates inoculated with the cell suspensions developed frank malignant lymphoma at 50 and 80 days.
in the first passage and 53 days in the second passage (Tables 1, 2). Two of the three dogs with malignant lymphoma were preirradiated, while one dog was not X-irradiated. In no case was CML transmitted using cell-free extracts. Even though CML did not occur in the third serial passage, dogs inoculated with either a cell suspension or a cell-free extract had enlargement of the superficial lymph nodes. Biopsies of these enlarged lymph nodes with subsequent histologic examination revealed lymphoid hyperplasia but not malignant lymphoma.

Clinical signs included sudden onset with listlessness, extreme weakness, loss of weight, inappetence, diarrhea, dyspnea, and distention of the abdomen. Radiographs revealed an opaque mass in the anterior mediastinal region, and a fluid line in the abdominal cavity. Paracentesis disclosed fluid in the peritoneal cavity which contained anaplastic lymphoid cells. Masses were palpable in the abdominal cavity. In Dogs 30030 and 30032, there were nodular masses in the abdominal and thoracic musculature and subcutaneous tissues. In Dog 30032, the superficial lymph nodes were somewhat enlarged, whereas in Dogs 30030 and 30089, the superficial lymph nodes were normal in size. Enlarged lymph nodes were also observed in dogs inoculated with either cellular suspensions or cell-free extracts; however, malignant lymphoma did not develop in these dogs (Table 2).

Results of hematologic examinations disclosed that dogs with malignant lymphoma characteristically had anemia and a lowered platelet count. However, the relative and absolute lymphocytic counts were not substantially elevated.

**Morphology**

**Gross.** Experimentally transmitted CML involved primarily the organs and lymph nodes of the thoracic and abdominal cavities. Typically, the thymus and mediastinal lymph nodes were so greatly enlarged that the heart was displaced posteriorly. In the abdominal cavity: the liver was markedly enlarged, friable, and had discrete whitish nodules or diffuse mottling; the lymph nodes were extensively enlarged and had a homogeneous whitish color throughout; the pancreas had a soft texture and resembled lymphoid tissue; and the omentum was mottled; the lymph nodes were extensively enlarged and had a homogeneous whitish color throughout; the pancreas had a soft texture and resembled lymphoid tissue; and the omentum was mottled. Infiltrating lymphocytic cells in the thymus were morphologically similar to those described for the lymph nodes. There was, however, such proliferation of lymphocytic cells that Hassall's corpuscles (Fig. 6) and interstitial connective tissue were invaded, resulting in architectural obliteration. In the liver the lymphocytic proliferation was usually limited to the perportal areas (Fig. 7) and, in some instances, there was destruction of hepatic tissue (Fig. 8). Infiltration of lymphocytic cells in the kidney was confined to the cortex, particularly near the corticomedullary junction. The spleen was not markedly enlarged, yet the capsule and sinusoids were infiltrated with lymphoid cells that obstructed its architecture. Many intercostal muscle fibers were destroyed and replaced by infiltrating lymphoid cells (Fig. 10). Other organs and tissues were also infiltrated with lymphocytic cells (Table 3).

**Ultrastructure.** Electron microscopic examination revealed an abundance of intraerytoplasmic crystalline structures in tissues of all three dogs affected with experimental malignant lymphoma. The crystalline structures were made up of individual particles which measured 18 to 22 m\(\mu\) in diameter (Fig. 11).

**DISCUSSION**

A previous report by Moldovanu et al. (11) indicated that cell-induced CML can be accomplished by inoculating radiated mongrel newborn puppies repeatedly. Our investigation demonstrated unequivocally that cellularly induced malignant lymphoma is possible in the dog without pretreatment of total body irradiation. In our experiments CML developed in 3 of 15 attempts in 50 to 80 days after inoculation using a single dose of inoculum.

In agreement with our results, Moldovanu et al. found that dogs inoculated with either cell-free extracts or CML cell suspensions developed lymphadenopathy. It is well known that, rather than being a pathognomonic indication of early CML, lymphadenopathy may be initiated by several infectious agents. Since Moldovanu et al. reported difficulty with "intercurrent" infection, it is possible that much of the lymph node enlargement they observed was related to an infectious agent. Our results indicate that lymphadenopathy was most frequent in dogs of the third serial passage (Table 2), yet based on clinical examination and biopsied lymph node specimens, CML was not present. With the aid of a barrier system for housing dogs, we have been able to control distemper and infectious hepatitis.

There are two possible explanations for the development of cellularly induced CML: (a) the cells inoculated could have multiplied and metastasized, and hence acted similarly to an in vivo tissue culture system, or (b) an agent could have been...
adenopathy was most frequent in the superficial and mesenteric nodes of the lymph nodes revealed lymphoid hyperplasia but not associated with the inoculated cells which, while multiplying, did not infect, and transform more cells, which then may result in a latency (50-80 days) at which malignant lymphoma occurred in these transmissions, along with the inability to obtain localized growth at the inoculation site, may rule out simple transplantation as previously described (10, 11, 14).

Cell-free transmitted malignant lymphoma in the cat has been demonstrated (7). Jarrett et al. (5) has demonstrated, by electron microscopy, virus-like particles in the tissues of cats with lymphoma. Although cell-free extracts have not transmitted lymphoma in the dog, the presence of C-type virus-like particles in naturally occurring CML (3) suggests a viral etiology. It is possible that inoculated cells could temporarily grow in the recipient, thus giving a viral agent a chance to replicate, infect, and transform more cells, which then may result in a widespread malignancy. Temporary growth could explain the transient lymphadenopathy noticed 25 to 45 days postinoculation. Biopsy specimens from dogs having transient enlargement of the lymph nodes revealed lymphoid hyperplasia but not lymphoma.

It has been reported that, in spontaneous CML, lymphadenopathy was most frequent in the superficial and mesenteric lymph nodes (2, 6, 13, 16). Our results indicate, however, that lymph nodes that appear normal in size may contain neoplastic cells (Table 3). The donor dog had neoplastic involvement of the thymus, which has been a rare finding in spontaneous CML (2, 6, 13, 16). This could be related to age since, in most dogs, lymphoma occurs between 5 and 8 years of age (2, 13). Our results indicated that in transmitted CML the thymus, visceral organs, and abdominal body lymph nodes were involved in all three dogs. Thus the experimentally induced CML simulates spontaneous lymphoma of the cat, which is predominantly a visceral form with little or no peripheral lymphadenopathy (4, 15). The thymic involvement in experimental lymphoma of the dog also resembles the thymic form described for the cat (4, 15).

Several investigators have reported the close histopathologic similarity of spontaneous CML to that of Burkitt's lymphoma (1, 9). This has been based on the histologic "starry-sky" pattern, a nonspecific phenomenon which has been observed in spontaneous malignant lymphoma of dogs (1, 9), cattle (16), and cats (19).

ACKNOWLEDGMENTS

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Fig. 1. Iliac lymph node from initial donor dog (10019), a 20-month-old female Doberman Pinscher. Notice the monotonous uniformity of neoplastic lymphocytes. H & E, x 680.

Fig. 2. First passage of cellularly induced malignant lymphoma in Dog 30032. At birth the dog was pretreated with 60.8 R total body radiation. It was then inoculated with a cell suspension prepared from Donor 10019 (Fig. 1). (A), Liver, notice the nodular areas which protrude above the surface and the enlarged hepatic lymph nodes. (B) Kidney and enlarged renal lymph node. (C) Markedly enlarged mesenteric lymph node. (D) Pancreas.

Fig. 3. Dog 30089, representing the second serial passage of malignant lymphoma induced by inoculating a cell suspension prepared from 30032 (Fig. 3). Subject was inoculated at 2 days of age without radiation pretreatment and was killed when 53 days old. Notice the enlarged liver (A) and the thickening of the central veins and portal triads (arrow). (B) Kidney and renal lymph node. (C) Mesenteric lymph node. (D) Portion of thymus. (E) Portion of omentum.

Fig. 4. Mesenteric lymph node obtained from Dog 30089. Notice the invasion and proliferation of lymphocytic cells in the perinodal tissue. H & E, x 130.

Fig. 5. Higher magnification of Fig. 4. Notice monotonous distribution of lymphocytic cells. H & E, x 680.

Fig. 6. Thymic malignant lymphoma. Hassel’s corpuscle (arrow) is being invaded by neoplastic lymphocytes. H & E, x 680.

Fig. 7. Liver from Dog 30089. Proliferation of invading lymphocytic cells at portal triad. H & E, x 130.

Fig. 8. Liver from Dog 30032. Notice massive lymphocytic cell infiltration; only a few hepatic cells remain. H & E, x 680.

Fig. 9. Pancreatic malignant lymphoma. Overwhelming replacement of pancreatic parenchyma by neoplastic lymphoid cells. H & E, x 375.

Fig. 10. Section taken from intercostal muscle of Dog 30089. Invading lymphocytic cells have proliferated and replaced many muscle fibers. H & E, x 130.

Fig. 11. Intracytoplasmic crystalline structures. Notice the uniform geometric configuration. The crystalline structures were made up of individual particles which measured 18 to 22 mm in diameter. Uranyl acetate-lead stain.
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