T and B Lymphocytes in Canine Lymphosarcoma

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ABSTRACT. T and B lymphocyte subpopulation was studied on eight cases of canine lymphosarcoma. All except one were the Tosa breed, 3–10 years old, and had participated in dog-fight. Marked increase in B lymphocyte counts and decrease in that of T lymphocyte were observed in the lymphocytic subpopulation of the peripheral blood, spleen and lymph nodes. Serological and morphological studies revealed no C-type viral particles, and transplantation of the neoplastic cells was unsuccessful into dogs. — KEY WORDS: B cell, canine lymphosarcoma, T cell.

Lymphosarcoma is one of the commonest malignancies of the dog occurring 20–24 cases per 100,000 dogs [3, 6]. The main anatomical forms are the multicentric, thymic and alimentary [6, 7]. The tumors can be transplanted by inoculation with the neoplastic cells [2, 9, 12, 18], but transmission of the disease due to the cell-free fluid has never succeeded [9]. In electron microscopic observations of canine lymphosarcoma cells, retrovirus-like particles were found and reverse transcriptase activity was confirmed in culture fluid of the neoplastic cells [1, 17, 20, 21, 23]. B-cell line of a canine lymphoma has been established and retroviruses have been isolated [22]. However, no C-type particle was detected in the tissues of the disease [13, 19]. Cell surface immunoglobulin (SIg) of malignant cells was detected in 8 dogs out of 11 with malignant lymphoma [4]. Stains [15, 16] found that most of 39 canine lymphosarcoma of multicentric form were B-cell type and the rest were classified into the mixed cell, T-cell and null-cell types, respectively.

The authors examined on the lymphocyte subpopulation in the peripheral blood and lymphoid organs in eight cases of the multicentric form of canine lymphosarcoma occurred in Aomori prefecture, Japan, during the three years from 1981 through 1983. Neoplastic cells from case No. 1 were transplanted into 3 dogs. Virological studies were also performed on 6 animals (Nos. 1-3, 5-7). Table 1 showed the outline of the cases used in the experiments. In all of the cases, marked enlargement of the superficial lymph nodes was seen. With the exception of one pointer breed, all dogs have been reared as dog fighter and had participated at fights. Case No. 1 was admitted to our Animal Hospital and died after observation of 19 days. Cases Nos. 2-8 were brought to our school for pathological diagnosis from small animal clinics in the city. Histopathological diagnosis was lymphosarcoma in all of the dogs. Suspensions of the peripheral blood lymphocytes (PBL), lymphocytes of the spleen and lymph node were used for T and B lymphocyte counts. They were prepared by the Ficoll-Hypaque gradient centrifugation technique, and the living cells were counted by
Table 1. Outline of examined cases

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Breed</th>
<th>Age (yr)</th>
<th>White blood cell count ( \times 10^9/\text{cm}^3 )</th>
<th>Lymphocyte count ( \times 10^9/\text{cm}^3 )</th>
<th>Neoplastic cells detected in peripheral blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tosa</td>
<td>3</td>
<td>77.0</td>
<td>70.8</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>Tosa</td>
<td>5</td>
<td>39.5</td>
<td>9.5</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>Tosa</td>
<td>4</td>
<td>11.8</td>
<td>1.3</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>Pointer</td>
<td>10</td>
<td>ND(^{b1})</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>Tosa</td>
<td>6</td>
<td>11.0</td>
<td>1.8</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>Tosa</td>
<td>4</td>
<td>33.7</td>
<td>16.9</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>Tosa</td>
<td>3</td>
<td>37.8</td>
<td>18.1</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>Tosa</td>
<td>3</td>
<td>18.9</td>
<td>15.3</td>
<td>No</td>
</tr>
</tbody>
</table>

a) All were male dogs.
b) Not done.

trypan blue exclusion [10]. Indirect immunofluorescent antibody staining (FA) was used for the determination of T lymphocyte. They were firstly reacted with rabbit anti-canine thymocyte serum (ATS) diluted 1:8, and secondary with fluorescein isothiocyanate (FITC) goat anti-rabbit IgG serum (Medical and Biological Lab., Ltd. Japan) diluted 1:20. For the determination of B lymphocyte, SIg was examined by direct FA using FITC rabbit anti-canine IgG serum (Medical and Biological Lab., Japan) diluted 1:16. The specificity of the methods of T and B lymphocyte counts used here was confirmed and being reported in a separate paper [10]. Alpha-naphthyl acetate esterase (ANAE) staining was used for T lymphocyte count in the present study. This method have been used as a cytochemical marker of human, mouse and bovine T lymphocytes [5, 8, 11, 14, 25].

Lymphocytes of the dogs with lymphosarcoma were cultured in Dulbeccco’s minimum essential medium supplemented with 15% fetal calf serum, sodium pyruvate (1 mM) and antibiotics. Lipopolysaccharide (LPS), 5-iodo-2'-deoxyuridine (IUdR) or 12-O-tetradecanoyl-phorhol-13-acetate (TPA) were added as required. The PBL and lymph node-cells of cases Nos. 5, 6 and 7 were cultured for 48–120 hours in the medium with or without IUdR or TPA. The cultured cells were smeared on slide glasses and fixed in acetone. The reactivity between the cultured cells and the sera of cases Nos. 1-3, 5-7 was investigated by indirect FA. All or some of the PBL, spleen and lymph node cells from the six cases (Nos. 1-3, 5-7) were cultured for 24, 48, 72 and 120 hours in the medium with or without LPS (50 \( \mu \text{g/ml} \)), IUdR (25–100 \( \mu \text{g/ml} \)) or TPA (20 \( \mu \text{g/ml} \)). The cells were observed by electron microscopy for detection of virus or virus-like particles. MDCK, a canine cell line; MviLu, a mink cell line; and primary cultured cells of the canine kidney, testicle, bone marrow and spleen were cocultivated with PBL from case No. 1. After 9 days of culture, all of them were passaged in a Leighton tube and 60 mm Petri dish. After 5 days of culture, the cover slip of the Leighton tube was fixed in acetone and indirect FA test was performed with the serum of case No. 1. The cultured dishes were observed for 46 days.

In case No. 1, T and B lymphocytes were counted four times during the 19 days of observation. As shown in Table 2, the results indicated that there was always a marked increase in the rate of B lymphocytes as against decrease of T lymphocytes. As shown in Fig. 1, the percentage of subpopulations of T and B lymphocytes were 21.9 \( \pm 18.9 \) and 68.4 \( \pm 22.6 \) in PBL, 20.0 \( \pm 13.1 \) and 78.1 \( \pm 12.6 \) in the spleen and 4.7 \( \pm 4.6 \)
and 86.2 ± 7.6 in average of five lymph nodes of the lymphosarcoma dogs. In the 21 control dogs, the T and B lymphocyte subpopulations were 52.7 ± 3.5 and 36.3 ± 3.2 in the PBL, 39.6 ± 2.8 and 44.8 ± 3.8 in the spleen and 49.4 ± 3.6 and 39.0 ± 2.7 in the lymph nodes. Cell count was unable to accomplish for the PBL of case No. 4 and the spleen of case No. 2. Since the lymph nodes of the eight dogs were tested at several sites in each animal, the sites examined were not constant. The data for 21 control dogs clinically normal were shown in mean ± standard deviation and not individually. These results showed that there was a marked increase in B lymphocyte counts and decrease in T lymphocyte counts through all of the lymphoid tissues of the lymphosarcoma dogs.

Five mongrel dogs, 3 weeks old in age, were inoculated with 16 × 10^7 PBL cells of case No. 1 in the heart ventricle and 1 × 10^7 spleen cells of the same case (No. 1) as a booster shot on the 13th day. The subpopulations of T and B lymphocytes were counted after 51, 84 and 126 days of the second inoculation in the five inoculated dogs. The rates of T and B lymphocytes were within a normal range. These dogs were observed for

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### Table 2. Population of T and B lymphocytes in the peripheral blood lymphocytes of case No. 1

<table>
<thead>
<tr>
<th>Days of observation</th>
<th>Lymphocyte(%)</th>
<th>ANAE(%)</th>
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<tbody>
<tr>
<td></td>
<td>T cell</td>
<td>B cell</td>
</tr>
<tr>
<td>1</td>
<td>8.5</td>
<td>80.0</td>
</tr>
<tr>
<td>8</td>
<td>8.0</td>
<td>83.0</td>
</tr>
<tr>
<td>13</td>
<td>8.5</td>
<td>79.0</td>
</tr>
<tr>
<td>19</td>
<td>7.0</td>
<td>86.0</td>
</tr>
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</table>

### Eight cases of lymphosarcoma

<table>
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<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Percentage of positive cells (%)</td>
<td>T</td>
<td>B</td>
<td>T</td>
<td>B</td>
<td>T</td>
<td>B</td>
<td>T</td>
</tr>
</tbody>
</table>

**Fig. 1.** Percentage of T and B lymphocytes in tissues as determined by ATS and SIg.

Lnn-4: Superficial inguinal lymph node. Lnn-5: Medial iliac lymph node.
** Number in parenthesis is number of samples tested.
Vertical bar = Standard deviation from mean value.
5 months after the second inoculation, but no obvious change in the differential count of the white blood cell nor clinical symptoms was observed. All of the samples for virological studies resulted in negative with indirect FA and electron microscopy. The cocultured PBL from case No. 1 with indicator cells showed no changes through 46 days of observation. No single cell cultures of the PBL, spleen and three lymph nodes of case No. 7 showed any growth in medium with TPA, but maintained for only 40 days.

There have been a few reports on canine lymphosarcoma in Japan [24] and the pathogenesis of the disease is still obscure. In the present report, a marked increase in B lymphocyte counts and a decrease in T lymphocyte were demonstrated in all of the affected dogs examined. The presence of C-type viral particles was unable to demonstrate by serological and morphological studies. Seven out of the 8 dogs used in the examination had been reared for dog fighting. At present it is not clear if there are some connections between the dog fighting and the onset of lymphosarcoma. Further study for the detection of transmissible agent will be necessary.

REFERENCES