Spontaneous Lymphoma in a Japanese White Rabbit

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ABSTRACT. Lymphoma was observed in a 4-month-old female Japanese White rabbit. Grossly, the markedly enlarged mesenteric lymph nodes, prominent Peyer's patches of jejunum, splenomegaly, and enlargement of tracheobronchial lymph nodes, adrenal glands and ovaries were observed. Histologically, neoplastic lymphoid cells proliferated diffusely showing frequent mitotic figures and a characteristic 'starry sky' appearance. Their basophilic cytoplasm contained a few lipid droplets. The mesenteric lymph nodes, Peyer's patches of jejunum, and tracheobronchial lymph nodes were largely replaced by the tumor tissues. The stomach, small intestines, especially the jejunum, liver, spleen, ovaries, and adrenal glands were heavily infiltrated with neoplastic cells. These results suggest that the present lymphoma may have originated from the gastrointestinal lymphoid tissue.— KEY WORDS: gastrointestinal lymphoma, rabbit.

Information on spontaneously occurring neoplasms in the rabbit is insufficient, although rabbits have widely been used in various toxicity tests and pharmaceutical studies. Several case reports have been published on lymphomasarcoma in rabbits of the multicolored English type [4], New Zealand White strain [3, 7, 9, 13, 14, 16] and Netherlands Dwarf strain [10]. Lymphosarcoma has also been reported in the WH and WH/J strains of rabbits, with an autosomal recessive inheritance [5, 6]. Myeloid leukemia has been described in rabbits of the IIIep strain, in which susceptibility was shown to be conferred by a single autosomal recessive gene [12]. We describe the results of pathological and ultrastructural observations of the first case of spontaneous lymphoma in a Japanese White rabbit.

Thirty female Japanese White rabbits (JW-NIBS strain) were purchased from Laboratory Animal Research Station (Yamanashi) at the age of 4 months. They were housed individually in aluminum wiremesh floor cages (44 × 22 × 35 cm) in a barrier-sustained room controlled at 23 ± 2°C, 50 ± 20% relative humidity, and a 12-hr light-dark cycle. The animals had free access to a standard laboratory diet and tap water.

The affected rabbit showed depression and anorexia in the acclimatization period, and the animal was found dead one week later. The rabbit weighed 1.6 kg at death and was subjected to a complete necropsy. Grossly, there were greatly enlarged mesenteric lymph nodes (50 ± 15 × 10 mm), prominent Peyer's patches in the jejunum, splenomegaly (90 ± 20 × 15 mm) with indistinct white pulp, enlargement of the tracheobronchial lymph nodes, adrenal glands, and ovaries. The thymus was atrophied. The stomach adhered to the jejunum at the fundal portion and small brown foci, 2–3 mm in diameter, were scattered on the gastric mucosal surface. In the jejunum, there were mural thickening and a perforation, approximately 3 mm in diameter, close by the adherent site to the stomach. A small amount of intestinal contents was observed in the abdominal cavity. There were no abnormal findings in the other organs except discoloration of the liver.

Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin (HE). Selected sections were stained by the methods of periodic acid-Schiff (PAS) and Watanabe's silver impregnation for reticulin. Frozen sections from the enlarged lymph nodes were stained with oil red O. Paraffin wax embedded sections were also stained immunohistochemically by the avidin-biotin-peroxidase complex technique [11] using a commercial kit for goat antibodies (HISTOFINE SAB-PO(G) Kit, Nichirei Corp., Tokyo). Goat polyclonal antibodies to rabbit IgG (H+L) (Southern Biotechnology Associates, Inc., Bringham, AL) diluted 1:5,000, rabbit IgA (Nordic Immunological Laboratories, Tilburg, The Netherlands) diluted 1:1,000, and rabbit IgM-µ chain specific (Bethyl Laboratories Inc., TX, U.S.A.) diluted 1:100 were used as primary antibodies. The positively-labeled areas in sections were visualized by 3,3'-diaminobenzidine tetrachloride and the sections counter-stained with hematoxylin. Small pieces of formalin-fixed tumor tissue were examined by electron microscopy after being postfixed in osmium tetroxide and processed conventionally.

Histologically, neoplastic cells proliferated diffusely and were arranged in sheets in affected organs (Fig. 1A). Characteristic 'starry sky' patterns consisting of macrophages with abundant clear cytoplasm containing tumor cells or cell debris were frequently observed (Fig. 1A). Neoplastic lymphoid cells were moderate to large in size, round to polygonal in shape, and had round or oval, occasionally indented nuclei with one to several nucleoli (Fig. 1B). The cytoplasm was scant to moderate in amount, basophilic and slightly vesicular. A few lipid droplets were demonstrated in cytoplasm of neoplastic cells by the oil red O stain but no PAS-positive reactions were found. Mitotic figures were frequently seen. Ultrastructurally, the neoplastic cells were in close contact with neighboring cells and the cell surface was smooth (Fig. 1C). Their nuclei contained prominent nucleoli and moderate amounts of heterochromatin, that clumped at the nuclear envelope. The cytoplasm contained abundant free ribosomes and polyribosomes, a few lipid droplets, several small round mitochondria, and poorly developed rough-surfaced membranes. The neoplastic cells were subject to a complete necropsy. Grossly, there were greatly enlarged mesenteric lymph nodes (50 ± 15 × 10 mm), prominent Peyer’s patches in the jejunum, splenomegaly (90 ± 20 × 15 mm) with indistinct white pulp, enlargement of the tracheobronchial lymph nodes, adrenal glands, and ovaries. The thymus was atrophied. The stomach adhered to the jejunum at the fundal portion and small brown foci, 2–3 mm in diameter, were scattered on the gastric mucosal surface. In the jejunum, there were mural thickening and a perforation, approximately 3 mm in diameter, close by the adherent site to the stomach. A small amount of intestinal contents was observed in the abdominal cavity. There were no abnormal findings in the other organs except discoloration of the liver.

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immunohistochemically negative for rabbit IgG, IgA, and IgM-µ chain. The neoplastic cells were histologically negative for rabbit IgG, IgA, and IgM-µ chain. The neoplastic cells in the present case had characteristics of immature or blastic lymphoid cells, showing a high mitotic activity, poorly developed cytoplasmic organelles, abundant free ribosomes and polyribosomes, and no immunostaining for IgG, IgA or IgM-µ chain. These observations suggest a rapid growing property of the tumor, coinciding with acute clinical course of the present case. In addition to these findings, the present neoplastic cells possessed a few lipid droplets and no PAS reactive granules in their cytoplasm. From clinical, gross and histological features, the present lymphoma appeared to bear some resemblance to Burkitt's tumor [2, 8], since a frequent 'starry sky' appearance and cytoplasmic lipid droplets in neoplastic cell are regarded as prominent histological features of Burkitt's tumor in man [2, 8]. However, polymorphic and indented nuclei of tumor cells, which were occasionally observed in the present case, differed from histological features of Burkitt's tumor [2]. Lymphoma of rabbits has not yet been classified into subtypes, probably because of the rarity of the tumors, while lymphoid neoplasms in human beings [8] and such species of animals as cattle, dogs, cats, horses, and pigs, have been classified in detail [15]. A recent study has indicated that neoplastic lymphocytes of lymphosarcoma in a New Zealand White rabbit were of T-cell origin [13]. There was no evidence suggestive of tumor cell origin in the present lymphoma. Cytological and immunohistochemical analyses of cell surface antigens and search for other markers of neoplastic cells remain to be performed in further studies.

Ultrastructurally, virus-like particles have been observed in neoplastic tissue from the kidney in a New Zealand White rabbit with lymphosarcoma [7]. A viral agent has also been implicated in rabbit hereditary lymphosarcoma by demonstrating 70 S RNA and RNA-directed DNA polymerase associated with particles that banded in the density region of type C RNA viruses [1]. Electron microscopy on neoplastic cells of the present case failed in detecting any structures representative of viral particles.

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REFERENCES

LYMPHOMA IN A RABBIT

Fig. 1. Lymphoma of the mesenteric lymph node. A: Diffuse, sheet-like proliferation of neoplastic cells showing a characteristic ‘starry sky’ appearance. HE stain. × 80. B: Neoplastic lymphoid cells show pleomorphism with round or oval, occasional indented nuclei and scant to moderate cytoplasm. HE stain. × 800. C: Electron micrograph showing compactly packed neoplastic cells. The cytoplasmic organelles are poorly developed. × 4,000.

Fig. 2. Lymphoma. A: The jejunum, diffuse infiltration of neoplastic cells in mucosa, submucosa and muscular layers. B: The liver with centrilobular fatty change. Neoplastic cells infiltrate around the portal triad. C: Ovarian tissue is largely replaced by tumor tissue. D: Perivascular infiltration of neoplastic cells in white pulp of the spleen. HE stain. × 80.