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Spontaneous Follicular Center Cell Lymphomas of B Cell Origin in Cataract Mice

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Spontaneous lymphomas from a strain of hereditary cataract (CAC-nct/+) mice were examined by light and electron microscopy and by immunohistochemical reaction for the mouse heavy and light immunoglobulin chains. Lymphomas occurred in 28 out of 45 male cataract mice and in 34 out of 52 females at 25 to 65 weeks of age. All of the lymphoma-bearing mice showed an enlargement of the spleen and mesenteric lymph nodes, and some mice also had hepatomegaly. Morphologically, all tumors were composed of a mixed population of small and large cells. Neoplastic cells had features of follicular center cell lymphomas, such as scant to moderate amounts of cytoplasm and cleaved and/or round nuclei with a large nuclear-to-cytoplasmic ratio. Large cells were often admixed with small cells, and had vesicular nuclei with prominent nucleoli juxtaposed to the nuclear membrane. Intracytoplasmic eosinophilic inclusions were observed in occasional cells, but Golgi apparatus was poorly developed and rough-surfaced endoplasmic reticulum was scant, unlike those in plasma cells. C-particles were seen in all lymphoma-bearing mice by electron microscopy. Intracisternal A-particles were detected in some mice. Immunohistochemically, neoplastic lymphoid cells were positive for the kappa light chain and the surface/cytoplasmic immunoglobulin M. These results indicate that lymphoid cell neoplasms found in hereditary cataract mice originate from follicular center B cells.

Key words: Lymphoma — B cell — Follicular center cell — Cataract mice — Immunohistochemistry

Mouse hematopoietic cell neoplasms are generally classified according to the system proposed by Dunn in 1954.[1] In addition to the morphological classification, more recent immunomorphologic classification[2–4] have been described. Most of the murine thymic lymphoma/leukemia are composed of lymphoblasts or medium-sized lymphocytes with frequent involvement of adjacent and distant tissues and the incidence may have a peak at three to six months of age.[3,4] Some strains of mice also develop B cell lymphomas more than one year after birth.[3,5–7] The murine B cell lymphomas increase in number with age and may be a suitable model system for the study of human non-Hodgkin's lymphoma/leukemia.[8]

Here, we report morphologic and immunohistochemical characteristics of lymphomas arising in a strain of hereditary cataract (CAC-nct/+) mice with a high rate and an early occurrence.

MATERIALS AND METHODS

Mice The hereditary cataractous strain of mice (CAC-nct/+) used in the present study was raised from a conventional closed mouse colony, "the General Purpose Colony," at the National Institute of Health, Tokyo in 1957.[9] A substrain of the cataract mice has been maintained and continuously inbred by strict sister-brother matings in an environment-controlled animal facility at Senju Pharmaceutical Laboratory, Osaka, since 1961.

Since 1985, the number of pups in a litter has been markedly decreasing (ca. 4.2/litter), and the adult animals showed severe abdominal distention and lethargy, and died within several weeks. Most cases of this peculiar spontaneous disease appeared in the cataract mice younger than one year. The cumulative incidence was 62.2% (28/45) in male and 65.4% (34/52) in female cataract mice. The average age of death caused by these abnormalities was nine months. The mice were monitored serologically and were free from the following pathogens: Sendai virus, mouse hepatitis virus, *Mycoplasma pulmonis, Bordetella bronchiseptica,* and *Corynebacterium kutscheri.* No lymphoma/leukemia or other neoplastic lesion had been recorded in breeding or in retired cataract mice up to one year of age by late 1984.

Three males and nine females of these lymphoma-bearing cataract mice in moribund condition were kindly provided by the above Laboratory. Animals were killed under ether anesthesia and subjected to further observations (Table I).

Pathological procedures Necropsies were done on each animal and all organs were fixed in 10% neutral buffered formalin. For more accurate immunohistochemical analyses, it was best to drain the fixative within 24–48 h and replace it with 10% neutral buffered formalin . The organs and tissues were embedded in paraffin, sectioned at 4 μm, and then stained with hematoxylin and eosin (HE). Additional stains such as methylgreen-pyronine,

Giemsa, periodic acid-Schiff, and silver impregnation were also employed. Blood smear preparations were stained with May-Giemsa. At necropsy, imprints of the cut surface of the spleen and mesenteric lymph nodes were prepared and immediately fixed in Gendre's fluid (80 volumes of 90% ethyl alcohol saturated with picric acid, 15 volumes of 40% formaldehyde solution and 5 volumes of glacial acetic acid)[10] at room temperature for 10 min. The slides were rinsed with 80% alcohol, then stained with HE. For transmission electron microscopic examination, small parts of the spleen and mesenteric lymph nodes of all mice were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in Epon. Ultrathin sections were cut and stained with uranyl acetate and lead citrate.

For immunohistochemical examination, representative formalin-fixed paraffin sections of the spleen, mesenteric lymph nodes, and bone marrow of the 12 cataract mice were examined using the streptavidin-biotin-peroxidase (SA-B) method (Stravigen kit, BioGenex Laboratories, San Ramon, CA).[11] The rabbit anti-mouse heavy (A, M, G_1, G_{2a}, G_{2b}, and G_3) and light (kappa and lambda) immunoglobulin chain antibodies were purchased from Litton Bionetics (Kensington, MD). In addition, rat anti-mouse thy-1 (Sera-Labs., Sussex, England) was employed to treat the serial sections described above for the avidin-biotin-peroxidase complex method.

For comparison, one male B6C3F$_1$ mouse (102-week-old) with spontaneous follicular center cell (FCC) lymphoma and one female B6C3F$_1$ mouse (104-week-old) with immunoblastic lymphoma were also examined morphologically and immunohistochemically in the same manner.

RESULTS

Cataracts occurred in both eyes from 16 to 22 days of age as a pin-head nuclear opacity and were matured by the age of four to five weeks. All lymphoma-bearing cataract mice clinically showed marked abdominal distention with rough coat and staining hair. The enlarged spleen and superficial lymph nodes could be palpated. At autopsy, spleen weight was increased more than tenfold in comparison with that of normal mice. Some mice showed an enlargement of the lymph nodes of the whole body, including mesenteric, inguinal, renal and mandibular nodes. Also, hepatomegaly with many grayish nodules was found.

Table I summarizes the microscopic distribution of tumor cells in affected mice. All tumors were morphologically composed of a mixed population of small (6–10 μm in diameter) and large cells (10–16 μm). Some tumor cells were cleaved and others were noncleaved.

Microscopic examinations of the imprints of the spleen and mesenteric lymph nodes revealed that the neoplastic large cells had scant to moderate amounts of cytoplasm and round or cleaved nuclei. The neoplastic small cells had a scant cytoplasm and cleaved nuclei with peripherally marginated chromatin. Some of these cells had apparent eosinophilic intracytoplasmic inclusion (Fig. 1).

Table I. Distribution of Tumor Cells in Various Organs/Tissues

Mouse No.	Age (wk)	Sex	Organs/tissues						
			Spleen	Lymph nodes	Liver	Bone marrow	GALT	Thymus	Peripheral blood
1	40	F	+	+	+	0	+	0	0
2	31	M	+	+	+	+	+	0	0
3	31	F	+	+	+	0	+	0	0
4	52	F	+	+	+	0	+	0	0
5	49	F	+	+	+	0	+	0	+
6	40	F	+	+	0	0	+	0	0
7	34	F	+	+	+	+	+	0	+
8	63	F	+	+	+	0	+	0	0
9	65	M	+	+	0	0	+	0	0
10	61	F	+	+	+	+	+	0	+
11	25	F	+	+	0	0	+	0	0
12	42	M	+	+	+	0	+	0	0
13	102	M	+	+	+	+	0	0	0
14	104	F	+	+	0	0	+	0	0

Mouse No. 1–12: cataract mice, No. 13 and 14: B6C3F$_1$ mice. GALT: gut-associated lymphoid tissues. + = present; 0 = none.

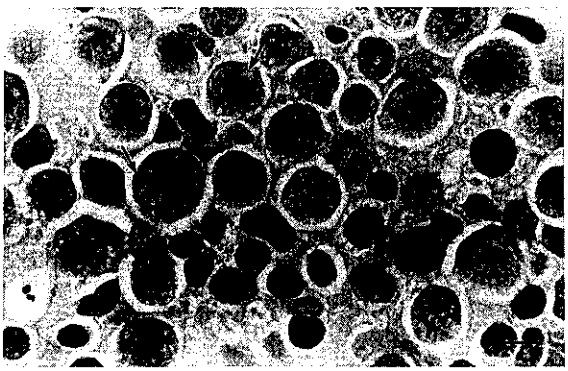

Fig. 1. Imprints of the spleen. FCC lymphoma cells have apparent eosinophilic intracytoplasmic inclusion bodies (arrows). Cataract mouse (Case 1). Gendre's fluid fixative and HE stain. Bar=10 μm.

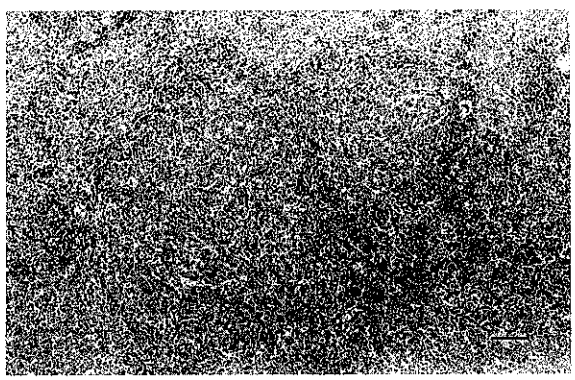

Fig. 2. Diffuse infiltration and proliferation of FCC lymphoma cells in the spleen. Cataract mouse (Case 1). HE stain. Bar=50 μm.

Fig. 3. Neoplastic proliferation of IgM-positive cells in the femoral bone marrow. Cataract mouse (Case 2). SA-B method. Bar=50 μm.

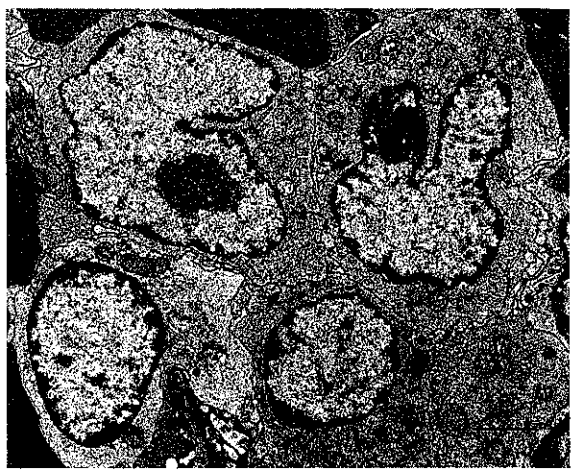

Fig. 4. Large FCC lymphoma cells in the spleen. Cataract mouse (Case 1). Uranyl acetate and lead citrate stain. Bar=2 μm.

In the spleen, diffuse proliferation of the tumor cells resulted in loss of the white pulp (Fig. 2). Non-neoplastic plasmacytes were found within lymphomas in some mice. An increase of reticulum fibers was not obvious in the sections with silver impregnation. Tumor cells also proliferated in the mesenteric lymph nodes and gut-associated lymphoid tissues, particularly in the Peyer's patches. Mild to moderate infiltration of tumor cells was observed in the liver, bone marrow, kidney, lung, and other lymph nodes. Some liver had dense nodules of tumor cells that were associated with the sublobular veins or portal areas and occasionally compressed hepatic cords. In the bone marrow, four of the 12 cataract mice with neoplastic proliferative lesions showed granulomatous appearance in marginal areas of the femoral bone marrow (Fig. 3). In three mice, lymphomas had progressed to leukemia. Anisocytosis of erythrocytes was also seen on the May-Giemsa-stained blood films.

The thymus showed different degrees of involution in all mice and was replaced with fatty tissues.

In ultrastructural examinations, neoplastic large cells with cleaved or non-cleaved nuclei often had obvious nucleoli juxtaposed to the nuclear membrane. The cytoplasm of neoplastic large cells (Fig. 4) was particularly rich in free ribosomes and rough-surfaced endoplasmic reticulum (RER). Neoplastic small cells showed a large nuclear-to-cytoplasmic ratio and had a round nucleus with inconspicuous nucleoli, and RER developed with different grades in each cell. C-particles budded from the surface of tumor cells in all cases (Fig. 5). Intracisternal A-particles were seen in well-developed RER of occasional cells.

The results of immunohistochemical examination are summarized in Table II. Neoplastic lymphoid cells found

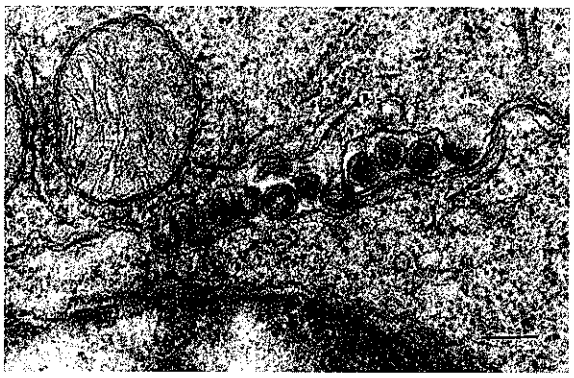

Fig. 5. Budding of C-particles from tumor cells in the spleen. Cataract mouse (Case 1). Uranyl acetate and lead citrate stain. Bar=200 nm.

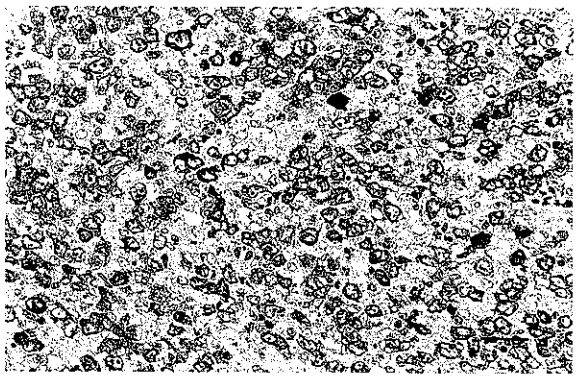

Fig. 6. Photomicrograph of the spleen stained for IgM. FCC lymphoma cells are positive for IgM. Cataract mouse (Case 1). SA-B method. Bar=20 μm.

Table II. Immunohistochemical Features of Tumor Cells

Mouse No.	Light chain		Heavy chain					
	Kappa	Lambda	IgM	IgA	IgG_1	IgG_{2a}	IgG_{2b}	IgG_3
1	+	0	+	+	0	0	0	0
2	+	0	+	0	0	0	0	0
3	+	0	+	0	0	0	0	0
4	+	0	+	+	0	0	0	0
5	+	0	+	0	+	+	0	+
6	+	0	+	0	0	0	0	0
7	+	0	+	0	0	0	0	0
8	+	0	+	0	0	0	0	0
9	+	0	+	0	0	0	0	0
10	+	0	+	0	0	0	0	0
11	+	0	+	0	0	0	0	0
12	+	0	+	0	0	0	0	0
13	+	0	+	0	0	0	0	0
14	+	0	0	0	0	0	0	+

Mouse No. 1–12: cataract mice, No. 13 and 14: $B6C3F_1$ mice. +=A large number of tumor cells are positive. 0=A large number of tumor cells are negative.

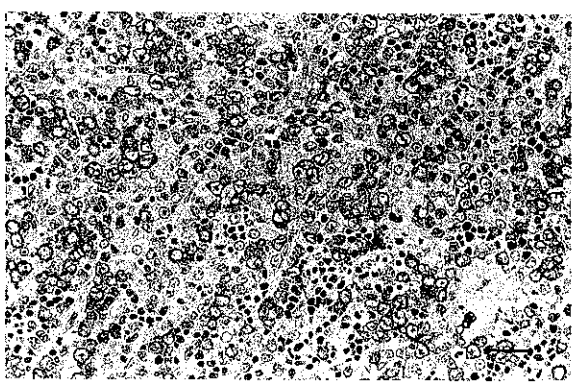

Fig. 7. Photomicrograph of the spleen stained for IgM. Spontaneous FCC lymphoma in an aged $B6C3F_1$ mouse. SA-B method. Bar=20 μm.

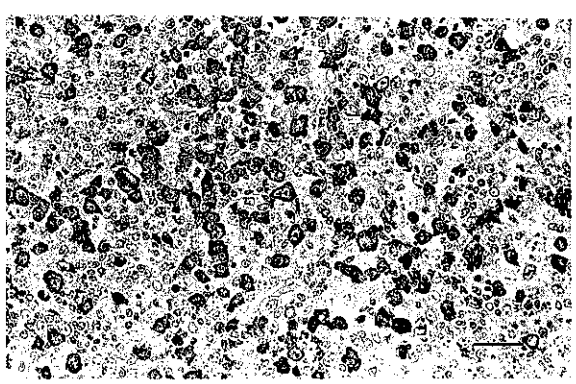

Fig. 8. Photomicrograph of the spleen stained for IgG_3. Spontaneous immunoblastic lymphoma in an aged $B6C3F_1$ mouse. SA-B method. Bar=20 μm.

in all cataract mice were positive for the kappa light chain and immunoglobulin (Ig) M on the surface of the cell membrane, in the perinuclear space and/or in the cytoplasm of tumor cells in all tissues examined (Fig. 6). These kappa light chain- and IgM-positive cells were diffusely distributed in every section. Tumor cells found in three out of the 12 cataract mice were positive for two heavy chains (M and A or M and G). However, there was no striking correlation of immunological isotype with morphologic features of neoplastic small and large cells. On the other hand, tumor cells of FCC lymphoma found in a $B6C3F_1$ mouse were strongly positive for

kappa light chain and surface/cytoplasmic IgM (Fig. 7), and those of immunoblastic lymphoma found in another $B6C3F_1$ mouse were positive for kappa light chain and cytoplasmic IgG_3 (Fig. 8).

DISCUSSION

During the past three decades, this strain of cataract mice has been the most commonly used mouse strain for ophthalmological research. Although the cataract mice have been maintained and inbred at 15 institutes in the world, there are relatively few comprehensive reports of background data.[9, 12, 13] We know of no publication describing a substrain of the cataract mice with a high rate and an early occurrence of spontaneous lymphoma/leukemia as described in this report.

In rodents, the terminology used to describe hematopoietic neoplasms is quite varied. Many investigators have simply referred to hematopoietic neoplasms as lymphomas, leukemias, or lymphoreticular diseases.[14–16] According to the standardized nomenclature and diagnostic criteria of the Society of Toxicologic Pathology in the USA,[17] the present lymphoid neoplasms arising from a strain of hereditary cataract mice were diagnosed as FCC lymphomas derived from B cells for the following reasons. In our cases, neoplastic lymphoid cells were cohesive in tissue section, and had scant to moderate amounts of cytoplasm and cleaved and/or round nuclei with a large nuclear-to-cytoplasmic ratio. Larger cells were often admixed with small cells, and had vesicular nuclei with prominent nucleoli often juxtaposed to the nuclear membrane. Mitotic index was generally low. Intracytoplasmic inclusions in tumor cells were scattered on some HE-stained imprints and were positive for cytoplasmic Ig. Ig inclusions, however, can not be equivalent to plasmacytic differentiation; immunohistochemically, FCC has massive cytoplasmic Ig, but ultrastructurally, the Golgi region is poorly developed and RER is scant in inclusion-containing cells, unlike those in plasma cells. Irregular signet ring nuclei found in our lymphoma cells are also one of the characteristics of cleaved cells that is FCC, not plasma cells.[18–20] Generally, immunoblastic lymphomas in mice are characterized histologically by large noncohesive cells with abundant cytoplasm. Round to oval nuclei are large and vesicular with multiple, conspicuous nucleoli. Nuclear-to-cytoplasmic ratios are small to intermediate. Immunoblastic lymphoma cells have conspicuous amphophilic immunoglobulin staining cytoplasm. Lymphomas of B immunoblasts and plasma cells are characterized as postfollicular B cells, which are closer to terminal differentiation and are actively secreting Ig protein products.[2, 4, 8] In plasma cell lymphomas,[2, 4] neoplastic cells are small to large, and noncohesive with abundant amphophilic cytoplasm. Nuclei are round to oval and have irregularly marginated condensed chromatin. Mitotic activity is variable. Hence, lymphomas found in hereditary cataract mice were diagnosed as FCC lymphomas, although the classification of hematopoietic tumors in rodents is not fully developed, unlike that in human beings.[21, 22]

The etiology of FCC lymphomas in mice remains unsolved.[2, 3, 8] An earlier study has evaluated the influence of common viral infections on the incidence of spontaneous tumors in the NCI-NTP studies and revealed that Sendai virus infection caused a consistent increase in lymphoma prevalence in male $B6C3F_1$ mice.[23] However, the cataract mice used in this study were free from Sendai virus. Moreover, murine T cell lymphomas and related leukemias have been described for many years in laboratory mice as results of infection with murine leukemia virus (MuLV),[24–26] expression of endogenous virus,[26] exposure to chemical carcinogens[27, 28] and irradiation.[25, 29, 30] Mice infected with irradiation-induced MuLV developed lympho-splenomegaly, hypergammaglobulinemia, profound immunosuppression, and terminal B cell lymphomas.[31, 32] Oncornaviruses have also been established as etiologic agents in mice lymphoma/leukemia. Ultrastructurally, C-particles were observed as features of budding from the cell surface of tumor cells with well-developed RER and abundant free ribosomes. A-particles were also seen in the cisterna of RER. Such viruses may be transferred to mice vertically or immediately after birth by unknown routes.

Recently, we amplified and analyzed protooncogene activation in our lymphoma cells by means of the thin-tissue section technique. No neoplastic lymphoid cells in cataract mice contained a mutation in codon 61 of the c-Ha-*ras* gene (unpublished data), although activations of K- and/or N-*ras* oncogenes as well as non-*ras* genes have been detected in spontaneous and chemically induced lymphomas in $B6C3F_1$ mice.[33, 34]

The relationship between the lymphomas and these possible lymphomagen factors is still uncertain. In addition to those viruses, probably associated with development of the lymphomas, the immunological status of this strain of cataract mice needs to be elucidated: both cataracts and B cell lymphomas are morphological and functional abnormalities in aging animals, and oncogenic viruses may be activated under certain immunological conditions such as immunodeficiency.[2, 35]

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