Pathologic Studies on Juvenile Bovine Leukosis

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Abstract. A diagnosis of juvenile bovine leukosis was made in eleven animals, which were examined to clarify the pathologic situation in the bovine leukosis. These animals had been selected by routine laboratory examinations from among 178 animals with bovine leukosis during the past 11 years. Clinico-pathologically, three of the eleven animals were tentatively classified into the thymic type, five into the calf type and the other three into the intermediate type in which both thymic and calf type lesions were associated. The distribution of lesions was somewhat different from that of the adult type of leukosis. The calf and thymic types seemed to be located at the two extremes in a single entity of juvenile leukosis. As exceptional cases, one case of the calf type had more than 160 tumorous lesions in the subcutis, and another one of the intermediate type a neoplastic invasion into osseous tissues in the cranial region. The neoplastic cells were not different from those of adult type, and lymphocytic in nature in eight and reticulum-cellular in three animals. Neither bovine leukemia virus (BLV) nor serum BLV antibodies were detected from four animals examined.

Bovine leukosis is a lymphoreticular proliferative disease of cattle. It is accepted that BLV is associated with enzootic adult bovine leukosis. On the other hand, sporadic juvenile leukosis is considered to be non-contagious from an epidemiological point of view [2-4, 10, 15, 16, 31]. According to Eilmann [9], a single case of juvenile leukosis was recorded by Gerlach in 1868.

The juvenile leukosis has been subdivided into calf and thymic types due to the age of occurrence, as well as the distribution of lesions [7, 8, 16, 43, 44]. The former type may affect calves less than 6 months of age and develop a lymphadenopathy. The latter may affect young animals from 7 to 24 months old and cause thymic lesions predominantly. The causative agent is still obscure. No BLV association is known in the juvenile type at present.

Pathologic research works, as well as case

reports have been published on both calf [3, 5-7, 9, 11, 14-17, 19, 21-23, 27, 29, 31, 32, 34-40, 42-46, 48-50] and thymic [1, 3, 7, 8, 15, 16, 18, 20, 29, 31, 33, 50, 51] types. Most of them, however, had been done before BLV was isolated. Very few investigations have been made since that time to differentiate an animal infected with BLV from any other animal. Only available was a case report made by Chander et al. [6].

According to the knowledge of today on adult type leukosis, BLV infection occurs to some of the fetuses of leukotic animals infected with BLV [28]. It should be borne in mind neoplastic lesions may logically develop by the age of 24 months [41] which is also the age of occurrence of the juvenile type. Neoplastic lesions are multicentric in the calf type, as well as in the adult type. A thymic lesion is also observed in the adult type. Under these circumstances, it is im-

Table 1. Number of autopsy cases of bovine leukosis during 11-year period

Year	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	Total
Thymic type	0	0	0	0	1	0	0	1	0	1	0	3
Intermediate type	0	0	0	2	0	0	0	1	0	0	0	3
Calf type	1	0	0	0	2	0	1	0	0	1	0	5
Adult type	5	6	4	9	14	23	12	25	19	21	29	167
Total	6	6	4	11	17	23	13	27	19	23	29	178

Table 2. Outline of animals examined

Type	Animal No. ¹⁾	Autopsy No. ²⁾	Age (months)	Breed ³⁾	rbc ⁴⁾	wbc ⁵⁾	MNC ⁶⁾	Cell type ⁷⁾
Thymic	1	1100*	4	JB	1,170	18.5	15.9	L
"	2	1087	12	HF	—	—	—	"
"	3	1169*	36	JB	446	19.0	19.0	"
Intermediate	4	1785	11	HF	—	—	—	"
"	5	1056**	19	"	625	51.3	43.3	"
"	6	1779	22	"	—	—	—	"
Calf	7	1203*	11	"	630	9.5	6.4	R
"	8	1787	12	SH	—	—	—	L
"	9	1803	12	JB	—	—	—	"
"	10	1010	20	"	450	183.0	180.0	R
"	11	670	22	HF	270	27.0	24.5	"

¹⁾ All were female, except No. 1 which was steer.

²⁾ *Electron microscopy for BLV and immunodiffusion tests for p- and gp-antibodies were negative. ** Immunodiffusion tests were negative.

³⁾ JB, Japanese black; HF, Holstein-Friesian; SH, Shorthorn.

⁴⁾ Red blood cell count, $\times 10^4/\text{cmm}$.

⁵⁾ White blood cell count, $\times 10^3/\text{cmm}$.

⁶⁾ Mononuclear cell count, $\times 10^3/\text{cmm}$.

—, Not examined.

⁷⁾ L, Lymphocytic; R, Reticulum-cellular.

portant to perform pathologic reexaminations on juvenile leukosis with the etiological definition of the diseased animals with or without BLV infection borne in mind.

Materials and Methods

During an 11-year period since 1968, 178 cases of bovine leukosis were examined pathologically. Of them, eleven were regarded as cases of juvenile leukosis on the basis of the criteria of diagnosis of this disease established on age and clinicopathologic findings. They were presented in these studies.

They were autopsied after brief clinical examination. Sections were made from the organs and tissues of the whole body, and stained with hematoxylin and eosin or subjected to silver impregnation by Watanabe's method for histological observation.

Ultrastructural observation was performed on

neoplastic cells and tissues by the method described elsewhere [24].

Detection of BLV particles was performed in buffy-coat-cell culture by electron microscopy with 4 experimental animals [24].

Immunodiffusion tests against viral internal protein (p)- and viral membranous glycoprotein (gp)-antigen were carried out on the sera of 3 animals [25, 26].

Results

The number of autopsy cases of bovine leukosis in each year is shown in Table 1. Although an increase in incidence of adult type leukosis was obvious year after year during the observation period of 11 years, zero to 3 cases of juvenile leukosis occurred annually during the same period with a

Table 3. Distribution of neoplastic lesions

Type	Thymic			Intermediate				Calf				Positive rate
Animal No.	1	2	3	4	5	6	7	8	9	10	11	
Lymph nodes												11/11
cranio-cervical and axillary	+	++	+	++	+++	+++	+++	NE	NE	+++	+++	
subiliac, popliteal and supra-mammary	+	—	+	NE	+++	NE	+++	NE	+++	++	+++	
thoracic	+++	+	+	+++	+++	+++	+++	+	NE	+++	+++	
reno-iliac	—	NE	+	+	+++	+++	+++	NE	NE	+++	+++	
abdominal	+	—	+	+	+++	+++	+++	+++	+++	+++	+++	
Thymus	+++	+++	+++	+++	+++	+++	+	+	NE	—	NE	8/ 9
Bone marrow	+++	NE	+	NE	+++	NE	+	NE	NE	NE	—	4/ 5
Tonsils	—	—	++	+	+++	NE	+	—	—	—	—	4/10
Liver	—	—	++	+++	+++	+++	+++	+	++	+++	+	9/11
Spleen	—	—	+++	++	+++	+	—	—	+++	+++	+++	7/11
Kidneys	—	+	+++	++	+++	—	+	—	+++	+	—	7/11
Heart	—	—	++	+	+++	NE	—	++	+	+++	++	7/10
Lungs	+++	+++	+	+++	++	—	+	—	++	++	+	9/11
Thoracic cavity	+++	+++	+	+++	+++	++	+++	+++	—	+++	+++	10/11
Pancreas	—	—	+	NE	+++	NE	++	—	+	NE	NE	4/ 7
Forestomach	—	—	—	NE	—	NE	—	—	—	+++	+++	2/ 9
Abomasum	—	—	+	NE	+	NE	+++	+	++	+++	+++	7/ 9
Intestine	F	++	F	—	+	++	F	++	+	+++	+++	7/11
Uterus		—	++	NE	+	NE	+++	NE	+++	+	—	5/ 7
Ovaries		—	++	NE	+	NE	—	—	+++	+++	+	5/ 8
Mammary glands		NE	++	NE	+	NE	—	NE	+++	++	+	5/ 6
Abdomen	—	—	+	+	+	+	+++	+	+	+++	+++	9/11
Skeletal muscles	+++	+	+	NE	+++	NE	+++	NE	NE	++	+++	7/ 7
Central nervous system	+	—	+	NE	+++	NE	+	+++	+++	+	—	7/ 9
Other neo-plastic lesions	SM+		SM+		PE+++		SC+++	TH+	UB+	EY+	GB+++	
	GB+		PA+		PH+++			GB+		PA+	UB+++	
			TH+		SM+					TH+	AD+	
			GB+		PA+					GB+		
			UB+		TH+					UB+		
			AD+		GB+					AD+		
					AD+							

—, Microscopically negative; +, Microscopically positive; ++, Microscopically moderately positive; +++, Macroscopically positive; +++, Macroscopically marked.

NE, Not examined; F, Follicular hyperplasia; SM, Submaxillary glands; GB, Gallbladder; PA, Parotid glands; TH, Thyroid glands; UB, Urinary bladder; AD, Adrenal glands; PE, Peritoneum; PH, Pharynx; SC, Subcutis; EY, Eyes.

mean incidence of a single case per year.

Hematologic findings of 6 animals examined are shown in Table 2.

Pathologic findings

From the lesions observed, 3 animals were

proved to be affected with the thymic type, 5 with the calf type, and the other 3 with the intermediate type. The distribution of neoplastic lesions is shown in Table 3.

Thymic type: The thymus was remarkably enlarged. The largest one (No. 1) was

45×15×10 cm, weighing approximately 7 kg. The enlarged thoracic thymus occupied more than one third of the thoracic cavity and was located in the anterior part of the cavity (Fig. 1). The cervical thymus of an animal (No. 3) surrounded the trachea, esophagus, and blood vessels, and infiltrated into the neighboring muscular tissue. Its cut surface swelled up slightly and showed an obvious lobular pattern grayish yellow or pink in color. Histologically, proliferative neoplastic cells extended from the medulla toward the cortex. They were not uniform in the mode of proliferation in all the lobules, but in general the lobular pattern was fairly well preserved. They were lymphocytic in nature and contained a round or oval nucleus.

Two of 3 animals presented macroscopic tumorous lesions only in the thoracic cavity, and the remaining one (No. 3) in various organs located in both thoracic and abdominal cavities (Figs. 5, 6). In this group of three, no or a few microscopic lesions were observed in the digestive and urogenital organs, except the kidney.

Intermediate juvenile type: The lesion of this type was produced by overlapping of those of both thymic and calf types. It was characterized by tumorous enlargement of the thymus and lymph nodes of the whole body with marked neoplastic cell infiltration (Fig. 7) and accompanied with additional lesions in the bone marrow, liver, spleen (Fig. 8), kidneys, tonsils (Fig. 9) and other organs, as shown in Table 3.

The neoplastic cells observed were lymphocytic in nature in this series. They proliferated from medulla to cortex in the thymus. As a peculiar change, neoplastic invasion was observed in the periosteum and osseous tissue of the mandible, vomer and sphenoid bone in animal No. 5 (Fig. 2). Its lesions were soft and elastic to the touch.

Their surface was irregularly swollen and brownish yellow in color. The neoplastic cells proliferated in the periosteum and infiltrated into osteum and bone marrow.

Calf type: Neoplastic lesions were detected in all the lymph nodes examined in 5 animals of this type. The largest of them was as large as the child's head and located in the hepatic lymph node of animal No. 7. The neoplastic cells were infiltrated in medullary sinuses and cords, and further in the cortex of lymph nodes. Occasionally, marked neoplastic proliferation extended to the capsule and pericapsular adipose tissues. The original architecture was hardly preserved in such nodes. The proliferated cells were lymphocytic in nature in 2 animals and reticulum-cellular in the other 3 animals. Neoplastic lesions were commonly observed in various organs and rather mild except those in the lymph nodes.

It is peculiar finding in animal No. 7 that more than 160 tumorous nodes were present in the subcutis. These nodes were apparently projected from the body surface, and ranged from the pigeon's egg to the human fist in size. They were elastic and soft to the touch. Most of them were well defined and brownish yellow in color (Figs. 3, 4). Histologically, they consisted of large polymorphic cells with a moderate amount of reticular fibers (Fig. 10).

Electron microscopy

Lesions and buffy coat cells were examined in 4 animals. Lymphocytic neoplastic cells were observed in 3 animals. Most of them were round and oval in shape and contained a nucleus frequently concaved or indented. Chromatin tended to be restricted to the marginal area of the nucleus. Organelles were sparsely distributed almost all over the cytoplasm (Fig. 11). Nuclear pockets were recognized in several neoplastic cells

of a single animal (No. 5). Nuclear bodies were occasionally observed in all the 3 animals.

Reticulum-cellular neoplastic cells were round or irregular in shape with cytoplasmic projections and blebs in animal No. 7. The nucleus was polymorphic and concave in most of them. An onion skin structure was detected in some of the nuclei. Ribosomes were abundant and many polysomes formed. The Golgi apparatus, rough-surfaced endoplasmic reticulum, and lysosomes were also detected (Fig. 12).

Virological findings

No C-type virus particles were detected in the buffy-coat cell culture with PHA or concanavalin A stimulation in any of the 4 animals examined. No serum antibodies against p- or gp-antigen were detected in the serum of any of the 3 animals examined (Table 2).

Discussion

The occurrence of juvenile type leukemia is less common than that of adult type leukemia. In this series, a diagnosis of juvenile type leukemia was made in only 11 animals among a total of 178 animals with bovine leukemia encountered during the past 11 years.

In the 3 animals with thymic type leukemia studied in the present investigation, marked tumorous swelling of the thymus was accompanied with lesions in the lymph nodes, lungs, thoracic cavity, bone marrow, kidneys, submaxillary glands, gallbladder, central nervous system, skeletal muscle, spleen, liver, intestine, ovaries, pancreas, etc. The distribution of neoplastic lesions was not so markedly different from that in the animals reported by previous authors [1, 8]. The neoplastic cells were lymphocytic and proliferated predominantly in the

medullary portion of the thymus. The lesions seemed to be initiated from the organ. Furthermore, the neoplastic proliferation was not the same in intensity throughout the thymus, but varied from one portion to another. These findings are similar to those obtained by Theilen and Dungworth [43] from the thymus affected with calf type leukemia, and by Okada et al. [27].

All the present cases of calf type leukemia exhibited marked swelling of most of the lymph nodes in the whole body. Comparatively mild lesions were observed in the thymus, tonsils, bone marrow, and other organs and tissues. Neoplastic proliferation was mild in the bone marrow and thymus, but severe in the abomasum, central nervous system, ovaries, intestine, mammary glands, urinary bladder, gallbladder, thyroid glands, and adrenal glands, as compared with the respective organs of the cases reported by Theilen et al. [43, 44]. Difficulty in making a differential diagnosis on the basis of morphologic findings would remain, as animal No. 10 showed the distribution of lesions which overlapped that of lesions of adult type leukemia.

In general, neoplastic lesions are observed commonly in the hematopoietic organs, and less frequently in the digestive and urogenital organs in the case of calf type leukemia. A similar tendency was shown macroscopically by the present animals with calf type leukemia. Histologically, such lesions were occasionally found in all the organs and tissues.

Animal No. 7 somewhat differed in the distribution of lesions from any other animal of the same group. It presented more than 160 neoplastic lumps in the subcutaneous tissue of the whole body. These findings seemed to be identical with those of the case reported by Smert [38]. In his brief descrip-

tion Smert explained that a heifer was 'covered with lumps', which were associated histologically with muscular fibers. Accordingly, it is necessary to distinguish such subcutaneous lesions clearly from those of skin leukosis [2-4, 16].

In the present investigation, the authors proposed a category of the intermediate type of juvenile leukosis. This type was noticed in 3 animals in which marked neoplastic lesions were presented in both thymus and lymph nodes. Such cases as presented to be of the intermediate type were reported by Dungworth et al. [8] and Theilen and Dungworth [43]. The animals examined by Okada et al. [27] and Sato et al. [34] may have been affected with the intermediate type.

The existence of the intermediate type indicates that there are no essential differences among the 3 juvenile types, except the severity of neoplastic proliferation, the two extremes of which are presented by the thymus and lymph nodes. It is reasonable to consider that the thymic and calf types may be grossly classified into one category, or the juvenile type, as several previous authors [2, 4, 10, 15] already asserted. On the other hand, there seemed to be a significant difference in the distribution of lesions between the juvenile and adult types. Lesions were predominant in the hematopoietic organs and commonly seen in the mammary, submaxillary, parotid, thyroid, and adrenal glands, gallbladder and pancreas in the case of juvenile leukosis, as compared with those in the case of adult type leukosis.

The neoplastic cells were lymphocytic in 8 and reticulum-cellular in 3 animals. The findings of these 11 animals were similar to those reported by some previous authors [12, 26, 47, 48] in the case of adult type leukosis.

Thymoma must be differentiated from

the thymic type of leukosis. It is a neoplasm of epithelial cells accompanied with proliferation of thymic lymphocytes [13, 30]. In the present investigation, no evidence of epithelial origin was detected in the neoplastic cells by electron microscopy or any other method of examination.

All the 4 animals examined were negative for detection of BLV by electron microscopy and for determination of serum antibodies against p- and gp-antigens by the immunodiffusion test. Accordingly, the viral etiology of juvenile leukosis is unknown. At present, it seems reasonable to consider that etiological factors associated with juvenile leukosis may be different from those associated with adult type of leukosis caused by BLV.

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要 約

若齢牛白血病に関する病理学的研究：大島寛一・小見邦雄・岡田幸助・沼宮内 茂（岩手大学農学部獣医学科家畜病理学教室）——1968年から1978年に至る11年間にウシ白血病と診断された178例中11例が若齢牛白血病と診断された。これらは臨床病理学的に、胸腺型が3例、子ウシ型が5例、および両型の病巣を併せ持つ中間型が3例であり、いずれも散発的な発生を示した。腫瘍病巣は胸腺、リンパ節、肝臓、脾臓、腎臓、肺、骨髓などのほか、乳腺、顎下腺、耳下腺、甲状腺、胆のう、副腎、膵臓などにもしばしば発現し、成牛型の病巣分布と異なる様相を示した。また、若齢型3型の病巣分布の間には移行が認められた。珍らしい症例として160余コの皮下腫瘍結節を示す子ウシ型の1例ならびに咽喉頭および頭部領域の骨組織に肉腫病巣を見る中間型の1例を記載した。腫瘍細胞は成牛型のそれと異なるものではなく、8例がリンパ球性、3例が細網細胞性であった。ウシ白血病ウイルス（BLV）および血清中におけるBLV抗体は検索を行なった4例すべてから検出出来なかった。

Explanation of Figures

- Fig. 1. Markedly enlarged thymus (T) located in the anterior portion of the lungs (L). Thymic type, case No. 1.
- Fig. 2. Neoplastic invasion in periosteum of the sphenoid bone (dark area with arrows) in the dorsal view of cranial cavity. Intermediate type, case No. 5.
- Fig. 3. Yearling heifer with several dozens of subcutaneous lumps. Calf type, case No. 7.
- Fig. 4. Subcutaneous view of the same side as in Fig. 3. Multiple lumps are formed independently in the subcutaneous tissue.
- Fig. 5. Marked infiltrative proliferation of neoplastic cells in the periportal area of liver. Thymic type, case No. 3. H-E staining, $\times 130$.
- Fig. 6. Perialveolar accumulation of neoplastic cells. Thymic type, case No. 3, $\times 130$.
- Fig. 7. Neoplastic proliferation in thymic medulla and remaining cortical lymphocytes (left bottom). Intermediate type, case No. 4. H-E staining, $\times 260$.
- Fig. 8. Neoplastic proliferation in the red pulp of spleen. W: White pulp. Intermediate type, case No. 5. H-E staining, $\times 260$.
- Fig. 9. Diffuse proliferation of neoplastic cells in the tonsil. The cells in the lymphoid tissue are similar to those in the follicle (F). Intermediate type, case No. 5. H-E staining, $\times 510$.
- Fig. 10. Large reticulum-cellular neoplastic cells associated with reticular fibers in a subcutaneous lump shown in Fig. 4. Watanabe's silver impregnation method, $\times 510$.
- Fig. 11. Lymphocytic neoplastic cells in the thymus. They are round or ovoid in shape with comparatively smooth surface. They contain mitochondria, rough-surfaced endoplasmic reticulum, lysosomes, and ribosomes in the cytoplasm, and nucleoli in the nuclei. Intermediate type, case No. 5, $\times 10,100$.
- Fig. 12. Reticulum-cellular neoplastic cells in subcutaneous tumor shown in Fig. 4. They are irregular in shape and size with extending cytoplasmic projections. Collagen fibers are observed in intercellular spaces, $\times 10,000$.