正常ウシ胎仔におけるウシ白血病腫瘍関連抗原と交差する抗原の存在

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Existence of Antigens Cross-Linking to Tumor Associated Antigens of Enzootic Bovine Leukosis in Normal Tissues of the Bovine Fetus

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There are 4 different forms of bovine leukosis. Enzootic bovine leukosis (EBL) is a lymphosarcoma of cattle caused by bovine leukemia virus (BLV) [8]. The etiology of sporadic bovine leukosis (SBL) including calf, thymic and skin forms is unknown. Tumor-associated antigens (TAAs) have been demonstrated on BLV-transformed cells using hyperimmune serum against neoplastic cells by many authors [3, 5, 11, 13]. Recently, Aida et al. [1, 2] succeeded in obtaining 13 monoclonal antibodies specific for the TAA in EBL neoplastic cells. These monoclonal antibodies can be divided into three groups. Group 1 reacts with all EBL neoplastic cells tested; group 2, with some but not all of the EBL neoplastic cells tested; and group 3, only with autologous neoplastic cells. The common TAA which is recognized by group 1 monoclonal antibodies is a polypeptide with a molecular weight of 74,000. The partially common TAA recognized by group 2 monoclonal antibodies and the individually distinct TAA recognized by group 3 monoclonal antibody are polypeptides with a molecular weight of 64,000. Antigenic determinants on the 3 different TAAs occur independently from each other. It is apparent that the 13 monoclonal antibodies are very specific for EBL neoplastic cells because none of the antibodies reacted with peripheral blood lymphocytes (PBL) from 139 normal cattle, renal tissues from 3 EBL cattle, or renal and hepatic tissues from 4 fetuses [2]. Since there are no reports of tumor-specific antigen in the strict sense, we thoroughly examined the specificity of the monoclonal antibodies. In order to determine the nature of the TAAs, we carried out immunohistologic staining on sections of tissues from normal fetuses and non-EBL calves using the monoclonal antibodies.

Twelve bovine fetuses (Nos. 1–12) from 3.5 to 9 months of gestation and 3 calves (Nos. 13–15) are summarized in Table 1. Six of 12 fetuses were derived from EBL dams and one was from a dam affected with the calf form of bovine leukosis. Five were derived from normal dams and obtained at an abattoir. Three calves were 2 days, 18 days and 12 months old, respectively. The former two were diagnosed as congenital anomalies and the latter calf had perirectal abscesess at autopsy. Immunodiffusion tests [10] for detection of serum antibodies to BLV were performed on 9 of 15 animals. None had antibodies to BLV antigens in their sera, except for No. 14 which had BLV maternal antibody. Therefore, it was suggested that placental infection with BLV had not occurred. The superficial cervical lymph nodes, spleen, thymus, liver, kidney and bone marrow were removed from dissected cases (Nos. 1–4, 6–8, and 13–15). On 5 cases (Nos. 5 and 9–12) obtained at the abattoir, only the thymus was examined.

Each block of tissue, about 1 cm^3 in size, was divided into two parts. One part was fixed by periodate lysine paraformaldehyde (PLP) [7] for 4–5 hrs, and successively treated with 0.05 M sodium phosphate buffers at pH 7.4 containing 7, 15 and 25% sucrose for 4 hrs, respectively. Then, the tissue was rinsed in phosphate buffered saline (PBS) at pH 7.4, frozen rapidly by soaking into liquid nitrogen, and preserved at −80°C. The remaining part of the block was fixed in 10% formalin solution and embedded in paraffin for histologic examination.

Frozen sections of 4 μm thickness were washed three times in PBS at pH 7.4. Immunohistologic staining was carried out by the avidin biotin peroxidase complex (ABC) method [4] as described previously [12]. Briefly, sections were incubated with undiluted culture fluid of each monoclonal antibody for 30 minutes, followed by incubation with anti-mouse IgG horse serum,

Table 1. Outline of examined cases

Case No.	Gestation in month (or age)[a]	Breed[b]	Sex[c]	Diagnosis[d]	ID[e] gp P
1	3.5	JSH	M	EBL	ND
2	4	JB	F	EBL	− −
3	5.5	HF	F	EBL	− −
4	6	JB	M	EBL	− −
5	6	HF	M	Normal	ND
6	6	JSH	M	EBL	− −
7	6.5	HF	F	CBL	− −
8	7	JB	F	EBL	− −
9	7	HF	M	Normal	ND
10	8	HF	M	Normal	ND
11	9	HF	M	Normal	ND
12	?	HF	?	Normal	ND
13	(2d)	HF	M	CA	− −
14	(18d)	HF	F	CA	(+ −)
15	(12m)	JSH	C	Abcess	− −

a) d, days; m, months.
b) JSH, Japanese Shorthorn; JB, Japanese Black; HF, Holstein-Friesian.
c) M, male; F, female; C, castrated.
d) EBL, enzootic bovine leukosis; CBL, calf form of bovine leukosis; CA, congenital anomaly. Nos. 1–12 are of their dams; Nos. 13–15 are of the calves.
e) ID, immunodiffusion test. gp, P, serum antibodies to glycoprotein and protein antigen of BLV. +, positive; −, negative; ND, not done. Parenthesized, possibly maternal antibodies.

Table 2. Characterization of monoclonal antibodies against TAAs [1, 2]

Group	Clone	Cytoxicity titer of culture fluid	Immunoglobulin class	Classification of TAAs	Molecular weight[b]
1	c453	32	IgG_{2b}	common	74 K
	c164	64	IgG_{2b}	common	74 K
	c143	128	IgG_{2b}	common	74 K
2	885	32	IgG_1	partially common	64 K
	903	32	IgG_1	partially common	64 K
	2064	ND[a]	IgG_1	partially common	NT[c]
	4134	ND	IgG_{2b}	partially common	64 K
	4366	ND	IgG_1	partially common	64 K
3	311	ND	IgG_1	individually distinct	64 K

a) Not detect.
b) Estimated by Western blotting.
c) Not tested.

and with an avidin-biotinylated peroxidase complex (Vector Lab. Inc., Burlingame, Calif.). Diaminobenzidine tetrahydrochloride solution was used for peroxidase substrate chromogen, and 1% methyl green solution was used for counter staining.

As the primary antibodies, c453, c143 or c164 of group 1 were used on all tissues of all cases. Monoclonal antibodies, 885, 903, 2064, 4134 and 4366 of group 2, and/or 311 of group 3 were also

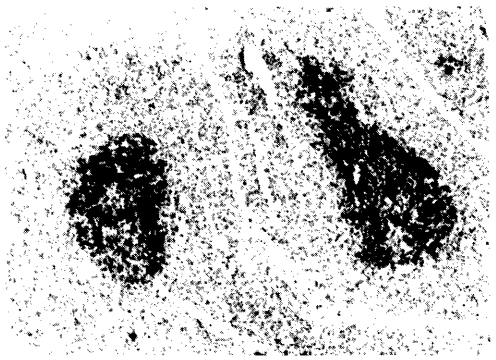

Fig. 1. Frozen section of fetal thymus (No.7) stained with the ABC method using c453 as the primary antibody. It shows the specific positive staining on the medulla. ×50.

Fig. 2. Magnification of Fig. 1. Cellular surface and cytoplasm reacted positively in the medulla (M), and negative in the cortex (C). ×500.

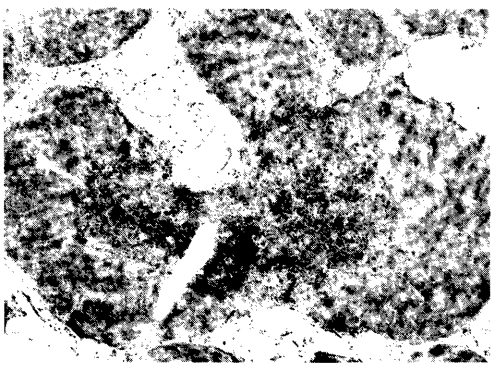

Fig. 3. Frozen section of thymus from a calf (No. 15) stained with ABC method using c143 as the primary antibody. The cortex is atrophic and adipose cells infiltrated along the interlobular conective tissue, but the specific positive reaction is still presented on medullary cells. ×50.

used on thymuses of Nos. 1, 5 and 9–12. The antibodies were characterized previously as shown in Table 2 [1, 2]. For negative controls, 1) PBS at pH 7.4, 2) 1: 1,000 diluted normal mouse serum, 3) c143 antibody absorbed by fetal thymic tissue and 4) c143 antibody absorbed by EBL neoplastic cells were used as the primary antibodies instead of the monoclonal antibodies. The specificity was confirmed by negative reactions in all control sections.

All tissues examined were apparently histologically normal. Results of immunohistologic staining are shown in Table 3. Specific positive staining was obtained on the cells in the thymic medulla (Figs. 1, 2) from all fetuses by using the group 1 antibodies. Staining with antibody groups 2 and 3 as the primary antibody showed no reaction. Although the positive cells were thought to be both lymphoid and reticular cells, reticular cells were more numerous than lymphoid cells. In addition, there were a few negative cells among the positive cells in the thymic medulla. Lymphocytes in the cortex were negative. The specific positive reaction was also obtained in the thymic medulla of calves (Nos. 13–15) (Fig. 3), although the cellular reactivity and frequency were less than in those of the fetal thymus. Furthermore, a few positive cells were demonstrated with group 1 antibodies in the superficial cervical lymph nodes of 8 out of 10 cases examined and in the spleens of 5 of 10 cases tested. No positive cells were detected by immunohistologic staining using group 1 antibodies as the primary antibody in the kidney, liver and bone marrow of all cases examined.

In a previous paper [2], we examined the reactivity of the same antibodies to liver and kidney from 4 fetuses by cytotoxicity and fluorescent antibody tests, and obtained negative results. In the present study, positive cells were detected in the thymus, spleen and lymph node of fetuses and calves by immunohistologic staining. The TAAs were undetectable on lymphocytes derived from BLV-free cows; however, a low percentage of the cells converted to positive when stimulated with a mitogen in our preliminary experiments. Since the TAA is expressed on some SBL neoplastic cells (our unpublished data) as well as on EBL neoplastic cells, the antigen might not be induced by BLV. Perhaps the TAA is reexpressed on bovine neoplastic cells if

Table 3. Results of immunohistologic staining on fetal or calf tissues[a)]

Case No.	Tissue: / Antibody:	Thymus			Lymph node[b)]	Spleen	Liver	Kidney	Bone marrow
		Group 1	Group 2	Group 3	Group 1				
1		+	−	ND	−	−	−	−	ND
2		+	ND[a)]	ND	−	+	−	−	ND
3		+	ND	ND	+	+	−	−	ND
4		+	ND	ND	+	+	−	−	ND
5		+	−	−	ND	ND	ND	ND	ND
6		+	ND	ND	+	−	−	−	−
7		+	ND	ND	+	+	−	−	ND
8		+	ND	ND	+	+	−	−	−
9		+	−	−	ND	ND	ND	ND	ND
10		+	−	ND	ND	ND	ND	ND	ND
11		+	−	ND	ND	ND	ND	ND	ND
12		+	−	ND	ND	ND	ND	ND	ND
13		+	ND	ND	+	−	−	−	ND
14		+	ND	ND	+	−	−	−	−
15		+	ND	ND	+	−	ND	ND	ND

a) Not done.
b) Superficial cervical.

lymphoid cells are transformed. But we cannot state that TAA is oncofetal antigen, because the antigen was detected in calf tissues. Although the nature of the TAAs is not clear at this writing, the TAAs detected by group 1 monoclonal antibodies might be lymphocyte differentiation antigens.

The neoplastic cells in EBL are thought to be B cell origin [6, 9, 14, 15]. However, the TAA is expressed mainly in the T cell region of the thymic medulla. It is important to define its biological significance that a differentiation antigen of T cells might be expressed in B cell lymphosarcomas.

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要　　約

正常ウシ胎仔におけるウシ白血病腫瘍関連抗原と交差する抗原の存在（短報）：岡田幸助・坂口一平・沼宮内茂・間　陽子[1)]・小沼　操[2)]・大島寛一（岩手大学農学部家畜病理学教室，[1)]理化学研究所ライフサイエンス筑波研究センター，[2)]酪農学園大学家畜微生物学教室）――ウシ白血病腫瘍関連抗原の本態を明らかにする目的で，正常ウシ胎仔および非地方病性ウシ白血病の仔牛から得られた各組織について，単クローン性抗体を用いた酵素抗体法により検索した。全例の胸腺髄質のリンパ様細胞と細網細胞に特異反応がみられたが，皮質のリンパ球は陰性であった。検索した10例中８例のリンパ節および５例の脾臓に少数の陽性細胞が認められた。