地方病性牛白血病に対する補体依存性抗体細胞障害試験の診断的応用

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Application of Complement-Dependent Antibody Cytotoxicity Test for Diagnosis of Enzootic Bovine Lymphosarcoma

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ABSTRACT. Complement-dependent antibody cytotoxicity (CDAC) test of peripheral blood lymphocyte (PBL) using monoclonal antibodies against tumor-associated antigen (TAA) expressed on enzootic bovine lymphosarcoma (EBL) cells was evaluated for its usefulness in the diagnosis of EBL. Twenty-seven out of 28 EBL cattle showed cytotoxic index (CI) greater than 31.7 which was considered as minimum value for positive EBL. The CI of PBL and sarcoma cell from solid tumor of one remaining cow was 28.0 and 61.0, respectively. On the other hand, one cow infected with bovine leukemia virus (BLV) but not affected with lymphosarcoma and 9 cattle uninfected with BLV showed less than 31.7 of CI. On the basis of the examination of hemogram, the TAA-positive cells were not always morphologically atypical. Since the TAA-positive cells are circulating in the peripheral blood of EBL cattle, the CDAC test of PBL using monoclonal antibodies is useful for diagnosis of EBL, and will be capable of a diagnostic procedure for aleukemic or subclinical cases of EBL.—KEY WORDS: bovine leukemia virus, CDAC test, enzootic bovine lymphosarcoma, monoclonal antibody, tumor-associated antigen.

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It is accepted that enzootic bovine lymphosarcoma (EBL) is caused by bovine leukemia virus (BLV) [11]. BLV infected cattle are diagnosed by detection of serum antibodies to BLV antigens or of BLV particles demonstrated in the cultured lymphocyte [12]. For diagnosed of EBL, clinical and hematological signs, such as palpation of tumors, lymphocytosis and increased number of atypical mononuclear cell count in the peripheral blood are effective. However, they are not helpful for diagnosis of aleukemic or subclinical cases of EBL [13].

Immunologically, it was reported that tumor cells in various species [5, 9, 15] as well as those of EBL [8, 14, 17, 18] have tumor-associated antigens (TAAs). Since a new technique to produce monoclonal antibody has been developed, TAAs were detected in various neoplasms [3, 6, 7]. Monoclonal antibodies against TAAs could be useful for diagnosis and therapy of malignancies [3].

Recently, Aida *et al.* [1, 2] obtained 13 monoclonal antibodies against TAAs expressed on EBL neoplastic cells. Further, Onuma *et al.* [16] suggested that these monoclonal antibodies might be useful for diagnostic tool of EBL. The purpose of this study is to apply complement-dependent antibody cytotoxicity (CDAC) test for the peripheral blood lymphocytes using the monoclonal antibodies for diagnosis of EBL.

MATERIALS AND METHODS

Animals: Blood samples were obtained from 38 cattle autopsied in the Department

of Veterinary Pathology of Iwate University from August 1983 to September 1984. The serum antibodies to BLV gp- or P-antigens were positive in 29 cattle by immunodiffusion (ID) test [12] but were negative in 9.

After the clinical examinations, the animals were sacrificed by exsanguination and autopsied. They were divided into 3 groups due to the results of autopsy and ID test. Group 1 was composed of 28 EBL cattle; group 2 was a single BLV positive cow without EBL but peritonitis lesions; group 3 was 9 BLV negative cattle affected with the other diseases, such as 3 cases of calf form bovine lymphosarcoma (CBL), 2 cases of congenital anomaly, and each one case of infectious thrombo-embolic meningoencephalomyelitis, encephalopathy, catarrhal enteritis and piroplasmosis (Table 1).

Target cells: Peripheral blood mononuclear cells (MNCs) were used as target cells for the CDAC test. The blood was collected from the jugular vein using anticoagulant EDTA. The MNCs were separated by Ficoll-Conray gradient as described previously [4], and the MNCs were suspended in phosphate buffer saline (PBS) to give 5×10^6 cells/m*l*.

Monoclonal antibodies against TAAs: Monoclonal antibodies c453 and c164 produced by Aida *et al.* [1, 2], which were specific for the common TAA expressed on EBL neoplastic cells, and reactive with all EBL neoplastic cells tested but not with normal bovine lymphocytes or BLV antigens, were used for the present study.

CDAC test: CDAC test was performed by trypan blue dye exclusion method and cytotoxic index (CI) was calculated as described previously [2, 16]. Briefly, 5 μl of rabbit complement, 5 μl of antibody, 5 μl of target cell suspension were mixed together in the well of a microplate and incubated at 37°C for 45 min. After incubation, 5 μl of 1.0% trypan blue solution was added to the well, and the viability of cells were counted. The antibody was replaced by PBS for each test to serve as the control. CI was calculated by the following formula:

$$CI=\{(\%\text{ of viable cell in control well}-\%\text{ of viable cell in test sample})/\%\text{ of viable cell in control well}\}\times100$$

The CI values of c453 and/or c164 greater than 31.7 was considered as positive [2, 16].

Hematological examination: Blood from the jugular vein were used for hematological examination. Differential count of white blood cells was performed with blood smears stained with May-Giemsa solution. Atypical mononuclear cells (AtMC) categorized as "neoplastic cells" [13], lymphocytes and monocytes were counted collectively as MNC in the present study.

RESULTS

Thirty-eight cattle were grouped according to histological diagnosis into 3 and their CIs are shown in the left half of Table 1. The group 1 cattle showed 28.0 to 95.5 of CI, and the mean and its standard deviation (SD) were 64.0±19.2. CI of the group 2 cow showed 20.4. The range of CI, and the mean and its SD were 8.2 to 31.0 and 18.5±7.8 in group 3, respectively. CIs of 3 calves with CBL were 31.0, 20.9 and 18.7.

Hematological findings of 38 cattle were shown in the right half of Table 1. Four (Case Nos. 6, 16, 20 and 27) out of 28 cattle of group 1 were diagnosed as aleukemic EBL. Because, their mononuclear cell counts were within a normal range based on the following 3 criteria, 1) hematological diagnostic key of European Communities [10], 2) less than 5% of AtMC in the hemogram [13] and 3) less than 1,000/cmm of AtMC in the peripheral blood [13].

Fig. 1 illustrates the relationships between CI and the absolute number of MNC. Further, Fig. 2 shows the relationships between CI and the percentage of AtMC/MNC. Although it was hard to find out any

Table 1. Histopathological diagnosis, cytotoxic index (CI) and hematological finding

Group No.	Case[a] No.	Histological diagnosis[b]	CI	Hematological finding[c]					
				WBC $\times 10^3$/cmm	% of MNC in hemogram	% of AtMC in hemogram	No. of MNC/cmm	No. of AtMC/cmm	AtMC/MNC (%)
1	1	EBL	95.5	36.8	82.0	64.5	30,176	23,736	78.7
	2	EBL	89.0	408.0	98.0	93.0	399,840	379,440	94.9
	3	EBL	85.1	15.4	56.0	41.5	8,624	6,391	74.1
	4	EBL	85.0	23.5	74.5	3.5	17,508	823	4.7
	5	EBL	85.0	422.0	56.5	22.5	238,430	94,950	39.8
	6	EBL	83.7	10.9	46.0	0.5	5,014	55	1.1
	7	EBL	82.0	12.4	36.0	13.0	4,464	1,612	36.1
	8	EBL	80.6	12.8	74.5	9.5	9,536	1,216	12.8
	9	EBL	80.0	19.7	55.5	14.0	10,934	2,758	25.2
	10	EBL	78.5	28.0	87.0	23.0	24,360	6,440	26.4
	11	EBL	73.7	292.0	98.5	88.5	287,620	258,420	89.8
	12	EBL	73.5	12.8	81.5	5.5	10,432	704	6.7
	13	EBL	70.9	63.8	85.0	70.0	54,230	44,660	82.4
	14	EBL	68.0	13.7	63.0	9.0	8,631	1,233	14.3
	15	EBL	65.0	19.9	57.0	8.5	11,343	1,692	14.9
	16	EBL	59.0	13.9	36.5	4.0	5,074	556	11.0
	17	EBL	52.0	16.6	45.5	4.5	7,553	747	9.9
	18	EBL	51.5	11.5	51.0	27.0	5,865	3,105	52.9
	19	EBL	50.5	58.2	89.0	77.5	51,798	45,105	87.1
	20	EBL	50.5	16.6	16.0	2.0	2,656	332	12.5
	21	EBL	48.0	11.3	53.0	11.5	5,989	1,300	21.7
	22	EBL	48.0	15.8	51.5	18.0	8,137	2,844	35.0
	23	EBL	47.9	23.6	58.5	32.0	13,806	7,552	54.7
	24	EBL	40.0	202.0	99.5	95.5	200,990	192,910	96.0
	25	EBL	38.5	9.9	63.5	21.0	6,287	2,079	33.1
	26	EBL	37.0	10.3	37.5	11.5	3,863	1,185	30.7
	27	EBL	36.7	9.5	35.5	4.0	3,373	380	11.3
	28	EBL	28.0	14.6	52.5	10.5	7,665	1,533	20.0
2	29	Peritonitis	20.4	19.4	14.5	1.0	2,813	194	6.9
3	30	CBL	31.0	7.0	30.0	4.5	2,100	315	15.0
	31	CBL	20.9	7.6	52.0	31.5	3,952	2,394	60.6
	32	CBL	18.7	12.8	82.0	3.5	10,496	448	4.3
	33	CA	12.4	7.1	43.0	3.5	3,053	249	8.2
	34	CA	8.2	5.2	36.5	3.0	1,898	156	8.2
	35	ITEME	25.0	5.8	28.0	1.5	1,624	87	5.4
	36	Enc	24.7	10.4	28.5	0.5	2,964	52	1.8
	37	Enteritis	17.8	10.4	27.0	1.0	2,808	104	3.7
	38	Piroplasmosis	8.2	10.2	47.0	3.0	4,794	306	6.4

a) In Case No. 28, CI of lymphosarcoma cells is 61.0.

b) EBL: Enzootic bovine lymphosarcoma, CBL: Calf form bovine lymphosarcoma, CA: Congenital anomaly, ITEME: Infectious thrombo-embolic meningoencephalomyelitis, Enc: Encephalopathy, Enteritis: Catarrhal enteritis.

c) WBC: White blood cell count, MNC: Mononuclear cells, AtMC: Atypical mononuclear cells.

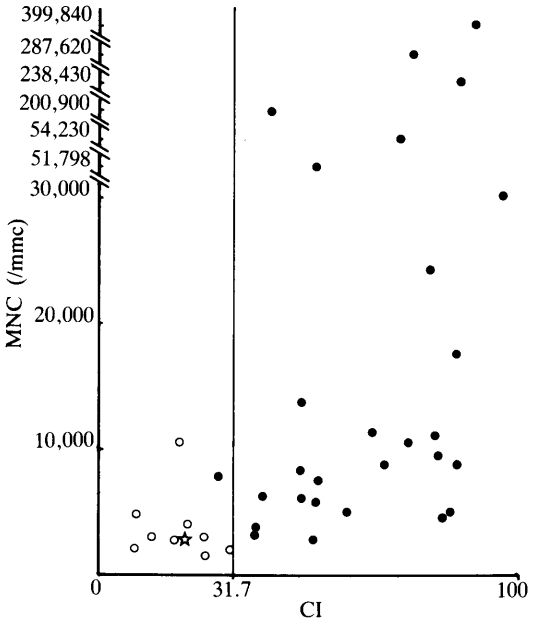

Fig. 1. Relationship between cytotoxic index (CI) and mononuclear cell (MNC) count ●: EBL, ☆: Infected with BLV but without neoplastic lesion, ○: Free from BLV infection,

Table 2. Comparison of cytotoxic index (CI) and hematological diagnosis (HD) of enzootic bovine lymphosarcoma

CI	HD	Number of cases
+	+	23
+	−	4
−	+	1
−	−	0

correlation between CI and the hematological figures, there was a tendency that CI was higher than the percentage of AtMC/MNC.

Twenty-three out of 28 cattle of the group 1 diagnosed as EBL by both CDAC and hematological examination. Remaining 4 cattle showed CI positive but negative for hematological examination, and the other one showed positive for hematology but negative for CI, and there was no cattle negative for both CI and hematology (Table 2).

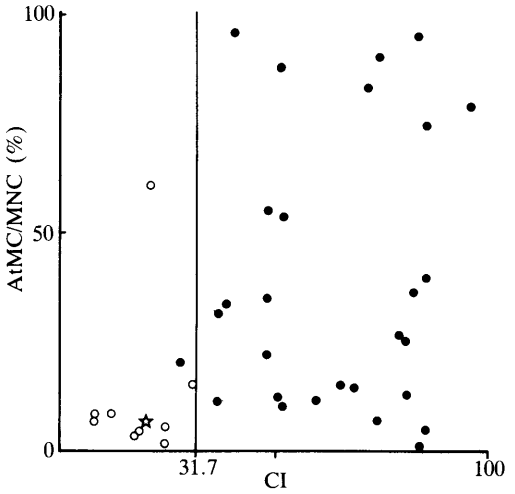

Fig. 2. Relationship between cytotoxic index (CI) and atypical mononuclear cell (AtMC)/mononuclear cell (MNC) ●: EBL, ☆:Infected with BLV but without neoplastic lesion, ○: Free from BLV infection.

DISCUSSION

We previously examined peripheral blood lymphocytes from 133 BLV negative cattle by CDAC test to determine the maximum nonspecific cytotoxicity of the monoclonal antibodies, and had reported that the mean CI and SD were 13.3±7.8 [2]. The CI greater than 31.7 (1.5 times more than that of the mean plus SD of the negative controls) was regarded as CI-positive. Although 27 among 28 EBL cattle of group 1 were positive for CI, one EBL cow (Case No. 28) showed negative for CI, whose neoplastic cells from lymphosarcoma tissue showed 61.0 of CI value (Table 1).

If TAA expresses only on AtMC observed by hematological examination, the CI value should be correlated with the rate of AtMC/MNC. However, there was no direct correlation between the two, and CI value tended to be somewhat higher than the rate of AtMC/MNC (Fig. 2). The above result indicates that apparently normal cells may be positive for TAA.

On the other hand, CI value was lower

than the rate of AtMC/MNC in some cases having extremely increased AtMC (Case Nos. 2, 11, 13, 19, 23, 24). Although the exact reason is still obscure, some kind of release of TAA in AtMC might occur in excessively proliferated cells.

Four cattle diagnosed as aleukemic EBL by hematological diagnosis showed positive for CI, though one was negative for CI and positive hematologically (Table 2). In contrast, all cattle of the group 2 and 3 were negative for CI. Therefore we confirmed that TAAs are good tumor marker of EBL, and monoclonal antibodies against TAAs are useful tool for diagnosis of EBL, as suggested previously [16], and will be capable of a diagnostic procedure for aleukemic or subclinical cases of EBL.

The CI values was less than 31.7, and negative in all of the 3 CBL calves (Table 1). However, c143, one of the common monoclonal antibodies, recognizing different epitope from c453 or c164 [1] used in the present study, is reactive with neoplastic cells of sporadic leukosis including CBL (unpublished data), and further studies will be needed to analyze the relationship between TAAs on EBL and CBL.

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要　約

地方病性牛白血病に対する補体依存性抗体細胞障害試験の診断的応用：鈴木雅実・岡田幸助・大島寛一・沼宮内茂・間　陽子[1)]・小沼　操[2)]（岩手大学農学部家畜病理学教室，[1)]北海道大学獣医学部家畜伝染病学教室，[2)]酪農学園大学家畜微生物学教室）――地方病性牛白血病（EBL）腫瘍細胞に存在する腫瘍関連抗原（TAA）に対する単クローン性抗体を用いた末梢血リンパ球の補体依存性抗体細胞障害試験（CDAC）によるEBLの生前診断の可能性について検討した。EBL牛28例中27例は細胞障害指数（CI）31.7以上を示した。残り1例はCI 28.0を示したが，肉腫細胞を標的細胞とした場合のCIは61.0であった。一方，牛白血病ウイルス（BLV）感染未発症牛1例ならびにBLV非感染牛9例はすべてCI 31.7未満を示した。また，末梢血血液像との関連から，形態的に異型性を示さない細胞でもTAAを有する細胞が存在することが示された。以上の成績から，EBL発症牛ではTAA保有細胞が末梢血中を循環しており，末梢血を用いてのCDACはEBLの生前診断に応用可能であり，従来の血液検査でEBL陰性と判定される非白血性EBLおよび亜臨床的EBLの生前診断の可能性が示唆された。