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## TUMOR-ASSOCIATED ANTIGEN AND CELL SURFACE MARKER IN CELLS OF BOVINE LYMPHOSARCOMA

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### Résumé

ANTIGENE ASSOCIE AUX TUMEURS ET MARQUEUR DE LA SURFACE CELLULAIRE DANS LES CELLULES DE LYMPHOSARCOMA BOVIN. — La détection d'antigène associé aux tumeurs et de marqueurs de surface des cellules B a été tentée dans des cellules lymphoïdes du sang périphérique (PBL) et des ganglions lymphatiques provenant de bovins infectés par le virus de la leucose bovine (BLV), et chez des bovins cliniquement normaux.

Un antigène spécifique associé à la tumeur a été trouvé dans les cellules PBL et les cellules de tumeurs lymphoïdes des bovins ayant la forme adulte du lymphosarcome (ALS), au moyen d'un sérum anti ALS. L'antigène a aussi été détecté dans les cellules PBL de bovins infectés par le BLV. Une réaction faible à l'antigène a été observée dans deux cas sur cinq de tumeur du veau. Aucune réaction significative pour l'antigène associé à la tumeur n'a été observée de la part des cellules lymphoïdes néoplasiques d'ALS dans les formes thymiques et cutanées. Le pourcentage de cellules B dans les cellules PBL et les ganglions lymphatiques d'ALS était plus élevé que chez les bovins normaux. Cependant, il semble qu'il y ait dans certains cas une perte des marqueurs de surface des cellules B dans certains cas d'ALS. On a observé une bonne relation entre l'augmentation du pourcentage de cellules B et l'apparition de l'antigène associé aux tumeurs dans les cellules PBL des bovins infectés par le BLV. Dans les cas sporadiques, le pourcentage de cellules B était plus faible parmi les cellules lymphoïdes de PBL et des ganglions que chez des bovins normaux.

### Introduction.

Bovine lymphosarcoma is a neoplasm of lymphoid tissue associated with BLV. Experimental inoculation with BLV can induce lymphocytosis or persistent lymphocytosis in young calves (Miller *et al.*, 1972) and lymphosarcoma in sheep (Olson and Baumgartner, 1976). The specific tumor-associated antigen

was found on neoplastic lymphoid cells from cattle and sheep with lymphosarcoma (Onuma and Olson, 1977 b). The antigen was also found in peripheral blood lymphocytes (PBL) of BLV infected cattle showing no evidence of lymphosarcoma. An increased B cell number was recognized in PBL and lymph node cells of cattle with persistent lymphocytosis and cattle with the adult form of lymphosarcoma

(ALS) cases (Muscoplat *et al.*, 1974 ; Takashima *et al.*, 1977 ; Weiland and Straub, 1975). Loss of normal B cell surface marker was also suggested in lymphoid tumors from some ALS cases (Takashima *et al.*, 1977). The purpose of the present report is to describe the relation between the appearance of tumor-associated antigen and the alteration in B cell number in neoplastic lymphoid cells.

## Materials and Methods.

### Animals.

Tissues from cattle with ALS (3163, 3167, 3169, 3170, 3183, 3191 and 3192) were obtained from an abattoir (Oscar Mayer and Co. Madison Wis., U.S.A.) ; and those with the calf form of lymphosarcoma (CLS : 3178 and 3182) were from farmers in Wisconsin, U.S.A. Tissue from cattle with the thymic form of lymphosarcoma (TLS ; 3185) were provided

by Dr. W.J. Jarrett, University of Glasgow, Glasgow, U.K. Tissues from cattle with 3 ALS (117, 128 and 129), one TLS (126) and two cases of the skin form of lymphosarcoma (SLS ; 114 and 127) were obtained from farmers in Hokkaido, Japan. Lymphosarcoma was diagnosed by both gross and histological examinations. PBL of BLV-infected cattle, either experimentally infected (289, 294, 340 and 500) or naturally infected (2197) were collected from an experimental herd at the University of Wisconsin, Madison, Wis., U.S.A. PBL from a naturally BLV-infected cow (131) came from an experimental herd at the Kitasato University, Towada, Japan. PBL from naturally BLV-infected cattle (I-7, I-11, I-23 and ch-4) were collected from farmers in Hokkaido, Japan. Tissues from 4 clinically normal cattle with histologically normal lymph nodes and PBL were also collected from abattoirs. Cattle with lymphosarcoma 3169, 3178, 3182 and 3185 were described in the previous paper (Onuma and Olson, 1977a).

Table 1. — Tests for tumor-associated antigen and slg bearing cells in neoplastic lymphoid cells of ALS.

Case No.	Form	BLV antibodies	Tissues	BLV	B cell <sup>a)</sup> (%)	Tumor-associated antigen <sup>b)</sup> fixed-cell	living cell	Viability (%)
3163	ALS	+	lymph node		97	++	+	93
3167	"	+	PBL	+	27	++	++	99
			lymph node		71	+	++	79
3169	"	+	PBL	+	10	+	++	90
			lymph node	+	65	+	+	85
3170	"	+	PBL	-	16	+	+	88
			lymph node	-	87	±	++	92
3183	"	+	PBL	+	2	++		93
3191	"	+	PBL		75	+		89
			lymph node		52	+		92
3192	"	+	PBL		84	++		93
			lymph node		84			96
103	"	+	PBL		75			95
			lymph node	+	91			98
			PBL	+	58			98
107	"	+	lymph node	+	15	+		95
			abomasum		18			95
			PBL	+	72			96
119	"	+	lymph node	+	20	++		89
			heart	+	56			92

a) Percentage of surface immunoglobulin-positive cells.

b) Antigen was detected by fluorescent antibody test using anti-ALS serum. Results were expressed as ± to ++ : ±, questionable fluorescence ; ++ bright green fluorescence ; -, none.

Table 2. — Tests for tumor-associated antigen and slg bearing cells in neoplastic lymphoid cells of CLS, TLS and SLS.

Case No.	Form	BLV antibodies	Tissues	BLV	B cell (%)	Tumor-associated Fixed cell	tumor-associated Living cell	Viability (%)
3178	CLS	—	PBL	+	2.0	+	+	90
3182	"	—	PBL	—	3.0	+		96
117	"	—	lymph node	—	0.1			70
128	"	—	PBL	—	2.4	—	—	75
			lymph node	—	1.0	—	±	90
129	"	—	PBL	—	2.0	—	—	80
			lymph node	—	0.7	—	±	93
3185	TLS	—	PBL	—	0.6	±	+	90
126	"	—	lymph node	—	5.5	—		85
114	SLS	—	PBL	—	3.2	—		98
			lymph node	—	1.0	—		90
127	"	—	PBL	—	7.5	—	—	86
			skin tumor	—	0.2	—	—	100

Results are expressed as in Table 1.

*Serological tests.*

To detect antibodies to BLV, immunodiffusion test was done using glycoprotein antigen as previously described (Onuma *et al.*, 1977). BLV was detected in short-term lymphocyte culture or long-term monolayer culture by fluorescent antibody (FA) or complement fixation (CF) tests (Miller and Van der Maaten, 1974; Onuma *et al.*, 1977). Recently, the syncytium assay has been used to detect BLV (Diglio and Ferrer, 1976).

*Cell preparations.*

Fresh bovine tumor was collected in Eagle's minimal essential medium with 10 % fetal calf serum and antibiotics. Tumor cells were teased out and filtered through gauze to obtain suspensions of single cells as described previously (Onuma and Olson, 1977b). Lymphocytes were separated from blood by a Ficoll-Hypaque gradient or Ficoll-Conray gradient as described by Boyüm (1968). Viability of these cell preparations was determined by trypan blue exclusion test. Cell preparations with viability of more than 70 % from lymph nodes and more than 86 % from PBL were studied for detection of surface immunoglobulin (slg) bearing cells.

*Surface immunoglobulin (slg) immunofluorescence.*

Lymphocytes ( $2 \times 10^6$ /ml) in PBS were stained with fluorescein-conjugated anti-bovine IgG serum at 37°C for 30 minutes. The number of positive cells was determined by counting 200 cells with the fluorescence microscope under oil immersion.

*Detection of tumor-associated antigen.*

The FA test was performed to detect the tumor-associated antigen in both living cells and fixed cells using anti-ALS serum (Onuma and Olson, 1977 b). Acetone-fixed cells were incubated with anti-ALS serum at 37°C for 30 minutes, washed and then stained with FITC-conjugated anti-bovine  $\gamma$ -globulin rabbit serum. A single cell suspension from fresh tumor and PBL was prepared as described above. Living cells were stained with FITC conjugated anti-ALS  $\gamma$ -globulin.

**Results**

Ten ALS cases were examined by FA test

Table 3. — Tests for tumor-associated antigen and slg bearing cells in lymphoid cells of BLV-infected and normal cattle.

Case No.	Form	BLV antibodies	Tissues	BLV	B cell (%)	Tumor-associated antigen Fixed cell	Tumor-associated antigen Living cell	Viability (%)
289	Exp. Infect.	+	PBL	+	55	+		92
294	"	+	"	+	28	-		97
340	"	+	"	+	30	-		99
500	"	+	"	+	58	+		94
2197 <sup>(a)</sup>	Natural Infect.	+	"	+	59	++	+	92
131 <sup>(a)</sup>	"	+	"	+	70	+	+	97
1-7	"	+	"		45			95
1-11	"	+	"		26			87
1-23	"	+	"	+	75		+	88
ch-4	"	+	"	+	93		+	98
3187	Normal	-	PBL	-	21	-		89
3189	"	-	Lymph node	-	24	-		84
3190	"	-	Lymph node	-	22	-		75
H-1	"	-	Lymph node	-	17	-		84
		-	PBL	-	30	-		82

Results are expressed as in Table 1. a) Persistent lymphocytosis was found.

using ALS antiserum (Table 1). Tumor-associated antigen was found on the membrane and in the cytoplasm of lymph node cells and PBL in all ALS cases tested. Antibody to BLV glycoprotein antigens was detected in sera from all of the ALS cases, and BLV antigen was detected in lymph nodes or PBL from 6 of 7 cases. Tumor cells with viability of more than 79 % of cells were studied for detection of slg bearing cells. As shown in Table 1, increased B cell percentage was found in 9 of 10 cases. However, different B cell percentages were observed in different preparations from the same animals. In case No. 3183, the percentage of B cells was lower than that in normal cattle.

Nine sporadic bovine leukosis cases including 5 CLS, 2 TLS and 2 SLS were examined by FA test using ALS antiserum for detection of the tumor-associated antigen (Table 2). Weak or questionable positive reaction was observed in 4 CLS and one TLS cases but not SLS cases. None of these sporadic cases had antibody to BLV antigen. The slg bearing cells of these cases were also examined. The percentages of slg bearing cells from these cases were lower than those from the normal bovine cells. PBL from 10 BLV infected cattle showing no evidence of tumor were examined for the tumor-associated antigen using ALS antiserum (Table 3). Six cases were positive for the antigen. The B cell percentage of these positive 6 cases was higher than that of control BLV negative animals. For the other 4 cases, B cell percentage was in normal range except No. 1-7. Cases 2197 and 131 showing persistent lymphocytosis and a high percentage of B cells gave positive reaction for the tumor-associated antigen. PBL from 10 more BLV infected cattle showing no evidence of tumor were also examined. B cell percentage was in normal range and no positive reactions were found (data was not shown).

## Discussion

Specific tumor-associated antigen in lymphoid cells of BLV-infected cattle were described in a previous paper (Onuma and Olson, 1977 b). In ALS cases, the tumor-associated antigen was found on the membrane and in the cytoplasm of neoplastic lymphoid cells. An increased B cell number was recognized in ALS cases, however, different B cell percentages were observed in diffe-

rent preparations from the same animal in the present observation. When B cells were detected by both erythrocyte antibody-complement (EAC) rosette, and slg immunofluorescence assays, EAC rosettes positive cells were fewer than slg positive cells in the same preparation of ALS cases (Takashima *et al.*, 1977). These neoplastic cells with the tumor-associated antigen are B cell origin and may have lost their normal B cell surface marker during neoplastic transformation.

With regard to sporadic bovine leukosis, the etiology of the disease is still unknown. A weak reaction for the tumor-associated antigen was found in 2 of 5 cases of CLS. No significant reaction for the antigen of lymphosarcoma cells from ALS was found in the thymic and skin forms. The evidence that the neoplastic lymphoid cells from the sporadic leukosis could not be identified as B cells suggests that the neoplastic cell from these cases may not be of B cell origin. The tumor-associated antigen was also found in PBL from BLV-infected cattle showing no evidence of lymphosarcoma. B cell percentage in PBL from these cases in which the antigen was found was higher than that of BLV negative animals. However, the antigen was not detected in PBL from cattle showing no significant increase in B cell percentage. Therefore, a good correlation can be pointed out between

an increase in B cell percentage and the appearance of the antigen. Case 289 which was experimentally BLV-infected, had the antigen but no lymphosarcoma when tested. However, three years later and nearly 11 years after it was inoculated it died with lymphosarcoma. The demonstration of this kind of antigen on PBL from BLV-infected cattle with no evidence of tumor may help the development of a new diagnostic test for bovine leukosis.

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### Summary

The detection of tumor-associated antigen and B cell surface marker was attempted in lymphoid cells of peripheral blood (PBL) and lymph nodes obtained from cattle with lymphosarcoma, cattle infected with bovine leukosis virus (BLV), and clinically normal cattle.

As a result, specific tumor-associated antigen was found in PBL and lymphoid tumor cells from cattle with the adult form of lymphosarcoma (ALS) using anti-ALS serum. The antigen was also detected in PBL of BLV-infected cattle. A weak reaction for the antigen was found in 2 of 5 cases of calf form. No significant reaction for the tumor-associated antigen of neoplastic lymphoid cells from ALS was found in the thymic and skin forms. The percentage of B cells in PBL and lymph nodes from ALS was higher than that in normal cattle. However, a loss of B cell surface marker was suggested in some ALS cases. A good relation was obtained between an increased B cell percentage and an appearance of the tumor-associated antigen in PBL of BLV-infected cattle. In lymphoid cells of PBL and lymph nodes from sporadic cases, the percentage of B cells was lower than that observed in normal cattle.

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