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Study on Relationships between Serum Gamma-Globulin Concentration and Pathological Characteristics in Bovine Lymphosarcoma

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Abstract

We investigated the relationships between the serum γ-globulin concentration and the pathological characteristics in 4 types of bovine lymphosarcoma including the adult, calf, thymic and skin forms. In 5 cases of the adult form, there were extensive neoplastic proliferations in the lymph nodes and the γ-globulin concentration was low. Four cases of the calf form showed systemic lymph node involvement, and the concentration was low in one case and much lower in 3 cases. IgM was absent in 3 cases. In the thymic form, one case showed a slightly high concentration with systemic neoplastic lesions in the lymph nodes, whereas in the other the concentration was normal and lesions were localized. In a cow with the skin form, the concentration was low in the tumor stage but markedly increased in the regressive stage.

Discipline: Animal health
Additional key words: bovine leucosis, immunoglobulin, serum protein

Introduction

Bovine leukosis has been classified into enzootic and sporadic forms[2]. The former is almost invariably seen in bovine leukaemia virus (BLV)-infected adult cattle, and is referred to as the adult form[5]. The latter consists of 3 clinicopathological types; the calf, thymic and skin forms[14]. These neoplasms are malignancies of the lymphoid system[2], which is closely related to immunological functions. It is likely that generalized immunological disorders occur in cattle with the lymphoid malignancies. Abnormal electrophoretic patterns of serum proteins are recognized in some of the human lymphomas[16]. Although serum protein concentrations have been determined in some cattle with lymphosarcoma[1,9,10,15], few studies have dealt with the relations between serum protein concentration and pathological characteristics. The purpose of the present study was to analyze the relationships between the serum γ-globulin concentration and pathological characteristics in 4 types of bovine lymphosarcoma.

Materials and methods

1) Animals

Leukosis in cattle was classified into 4 groups: (1) the adult form including 5 cases, (2) the calf form consisting of 4 cases in which 3 cases older than the age of calves were included, due to the presence of characteristic calf form lesions[21,24], (3) the thymic form including 2 cases and (4) the skin form in a cow[11]. A 2-year-old healthy heifer, which was negative for BLV antibodies, was used as a control animal.

2) Hematological, serological and serum protein analyses

Number of peripheral lymphocytes was calculated based on leukocyte counts and the percentage of differential leukocytes in blood smears stained with Giemsa solution. The agar gel immunodiffusion test was applied to serum samples for the demonstration of antibodies to BLV[13].

The total serum protein concentrations were determined by the biuret method according to the

method of Coles[3]. The serum protein patterns were examined by electrophoresis on a cellulose-acetate membrane[4,8] and the electrophoretic graphs were analyzed using a spectrophotometer DU-8 (Beckman Co., U.S.A.). Agar gel immunoelectrophoresis was carried out on microscope slides using anti-bovine γ-globulin rabbit serum (Miles Lab., U.S.A.) and anti-bovine IgM rabbit serum (μ-chain specific, Miles Lab., U.S.A.)[8].

3) Cell surface markers

Mononuclear cells were isolated from the peripheral blood and neoplastic tissues of cattle with lymphosarcoma, and also from the peripheral blood, lymph nodes and thymus of a healthy heifer by the sodium metrizoate/Ficoll procedure (Lymphoprep, Nyegaard Co., Norway). The erythrocyte rosette-forming (RF) test for the demonstration of T-cells was performed by Paul's method[17]. Aliquots of 1×10^6 mononuclear cells suspended in 100 μl of RPMI-1640 medium were mixed with 200 μl of 1% sheep erythrocytes (SRBC). The suspension was incubated at 37°C for 10 min and later at 4°C overnight. A drop of 0.5% trypan blue was added and 200 live cells were counted. When each cell adhered to more than 3 SRBC, the reaction was considered to be positive. For the surface membrane immunoglobulin (sIg), a marker of B-cells, 1×10^6 cells were resuspended in 50 μl of 1:20 dilution of fluoresceinated anti-bovine IgM rabbit serum (μ-chain specific. EY Lab., U.S.A.) and incubated at 4°C for 30 min. Then 200 live cells were counted under a Zeiss Axiophoto microscope equipped with an epifluorescent apparatus. The standard deviation (SD) of mean was calculated from the results of RF and sIg tests.

4) Pathological examination

Tissue samples were fixed in 10% neutral buffered formalin and processed by a routine paraffin wax sectioning method. Thin sections were stained with haematoxylin and eosin (HE) or by silver impregnation.

Small blocks of neoplastic tissues were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate, and then examined under an electron microscope (JEOL 100CX).

The immunoperoxidase method was applied to paraffin sections. After deparaffinization, sections were incubated with primary antibodies; rabbit anti-bovine IgM (μ-chain specific, Miles Lab., U.S.A.), rabbit anti-bovine IgG (Fc specific, EY Lab., U.S.A.) or rabbit anti-bovine IgA (α-chain specific, Miles Lab., U.S.A.). A biotin-streptavidin system

Table 1. Results of hematological and serum protein analyses

No.	Breed	Age (years)	Sex	BLV antibodies	Number of lymphocytes ($10^3/\mu l$)	Albumin (g/dl)	Globulin (g/dl)			Total protein (g/dl)	IgM line
							α	β	γ		
(Adult form)											
1	H[a]	4.01	F	+	17.1	3.4	1.4	0.7	1.1	6.6	+[c]
2	H	8.0	F	+	9.3	3.8	2.0	1.1	1.2	8.1	+
3	H	2.0	F	+	21.0	4.5	1.2	0.8	1.5	8.0	+
4	H	5.0	F	+	8.3	3.6	1.5	0.7	1.7	7.5	+
5	J[b]	4.10	F	+	7.2	3.0	1.4	0.8	1.7	6.9	+
(Calf form)											
6	H	6.0	F	−	107.6	3.8	1.5	0.7	0.3	6.3	−
7	H	1.06	F	−	4.4	2.9	1.5	0.6	0.5	5.5	±[d]
8	H	0.03	F	−	4.4	2.9	1.1	0.7	0.5	5.2	−
9	H	2.04	F	−	60.2	2.3	2.0	1.3	1.7	7.3	−
(Thymic form)											
10	H	2.08	F	−	6.7	3.0	1.0	0.9	2.9	7.8	+
11	H	1.03	F	−	3.4	3.9	1.3	1.3	2.4	9.0	+
(Skin form)											
12Tu[e]	H	2.03	F	−	5.0	4.4	1.8	1.2	0.8	8.2	+
Re[f]						2.7	1.3	0.8	3.4	8.2	+
(Control)											
13	H	2.0	F	−	6.1	3.5	1.0	0.6	2.2	7.3	+

a): H; Holstein. b): J; Japanese Black. c): +; Clear line. d): ±; Weak line. e): Tu; Tumor stage. f): Re; Regressive stage.

(BioGenex Lab., U.S.A.) was used in the subsequent processes.

Results

1) Hematological, serological and serum protein analyses

The lymphocyte counts, antibodies to BLV, serum protein concentrations and the results of the immunodiffusion test are presented in Table 1. A marked increase of the number of peripheral lymphocytes was noted in 2 cases of the adult form (Nos. 1 and 3) and 2 cases of the calf form (Nos. 6 and 9). Antibodies to BLV were detected in all the cases of the adult form.

The albumin concentration was low in some animals and there were slight variations in α- and β-globulin concentrations among the cases.

The γ-globulin concentration markedly decreased to 1.1–1.5 g/dl in 3 cases of the adult form (Nos. 1 to 3), and moderately to 1.7 g/dl in 2 cases of the same form (Nos. 4 and 5). In 3 cases of the calf form (Nos. 6 to 8), the γ-globulin concentration was 0.3–0.5 g/dl and much lower than that of the adult form. The other case (No. 9) showed a moderate hypogammaglobulinemia and the concentration of γ-globulin was 1.7 g/dl. In one case of the thymic form (No. 10), the γ-globulin concentration slightly increased to 2.9 g/dl. In the other case (No. 11), a normal value of 2.4 g/dl was recorded. In a cow

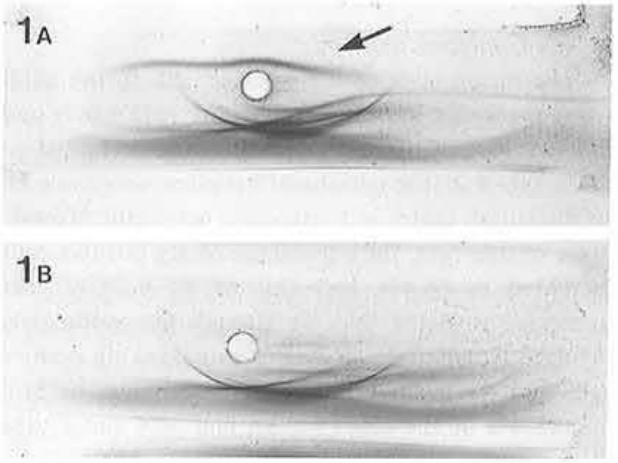

Fig. 1. Electrophoresis of sera from a healthy heifer (A) and leukotic cattle (No. 8) with calf form (B) IgM line (arrow) is absent in leukotic cattle.

Table 2. Results of surface marker and intracytoplasmic immunoglobulin examinations

No.	Surface markers of blood lymphocytes (%)		Surface markers in lymph nodes or thymus (%)		cIg[a] in lymph nodes		
	sIg[b]	RF[c]	sIg	RF	IgM	IgG	IgA
(Adult form)							
1	88.2	6.6	ND[d]	ND	–	–	–
2	90.2	8.8	66.4L[e]	6.4	–	–	–
3	80.8	1.7	ND	ND	–	–	–
4	78.3	7.3	51.0L	28.4L	–	–	–
5	72.8	4.3	54.5L	27.9L	–	–	–
Mean	82.8 ± 6.4[g]	5.7 ± 2.5	64.0 ± 2.2	20.9 ± 10.2			
(Calf form)							
6	9.1	2.0	ND	ND	–	–	–
7	7.3	14.3	8.7L	0L	–	–	–
8	10.0	11.8	7.1L	10.1L	–	–	–
9	4.2	0.5	2.8L	4.2L	–	–	–
Mean	7.6 ± 2.2	7.1 ± 5.9	6.2 ± 2.4	4.7 ± 4.1	–	–	–
(Thymic form)							
10	3.4	31.9	2.0T[f]	5.4T	–	–	–
11	27.6	53.2	5.1T	2.2T	–	–	–
Mean	15.5 ± 12.1	45.5 ± 10.6	3.5 ± 1.5	3.8 ± 1.6	–	–	–
(Skin form)							
12	ND	ND	ND	ND	–	–	–
(Control)							
13	36.9	35.8	(52.5L)[h] (4.6T)	(38.6L) (62.2T)	(+)[i]	(+)	(+)

a): cIg; Intracytoplasmic immunoglobulin-positive cells. b): sIg; Surface immunoglobulin-positive cells. c): RF; Cells positive for rosette-forming test. d): ND; Not done. e): L; Lymph nodes. f): T; Thymus. g): Standard deviation of the mean. h): Figures in parenthesis indicate values in normal lymph nodes(L) and thymus(T). i): Normal plasma cells.

with the skin form (No. 12), the concentration of γ-globulin decreased to 0.8 g/dl in the tumor stage, but increased to 3.4 g/dl in the regressive stage.

Immunoelectrophoresis revealed the absence of IgM lines in 3 out of 4 cases of the calf form (Nos. 6, 7 and 9) (Figs. 1A and 1B). The remaining one (No. 8) had a weak IgM line.

2) Cell surface markers

The mean value of sIg-positive cells in the adult form markedly increased to 82.8% (SD ± 6.4) and that of RF-positive cells significantly decreased to 5.7% (SD ± 2.5) in peripheral lymphocytes (Table 2). In the lymph nodes with extensive neoplastic involvement of one case, the percentage of sIg-positive cells increased to 66.4% and that of RF-positive cells decreased to 6.4% (No. 2), though the moderately involved lymph nodes in 2 cases contained sIg-positive cells and RF-positive cells with percentages of 51.0 and 28.4% in one case (No. 4), and 54.4 and 27.9% in the other case (No. 5), respectively. Most of the neoplastic cells in the adult form were considered to harbor B-cell markers. In the calf form, the levels of sIg-positive cells and RF-positive cells were, respectively, only 7.6% (SD ± 2.2) and 7.1% (SD ± 5.9) in the peripheral lymphocytes, and 6.2% (SD ± 2.4) and 4.7% (SD ± 4.1) in the neoplastic lymph nodes. The neoplastic cells were considered to have neither a B- nor T-cell marker. In the thymic form, the percentages of sIg-positive cells and RF-positive cells in peripheral lymphocytes were different in each case. Although one case (No. 10) showed 31.9% of RF-positive cells, a similar value to that of a normal control, the percentage of sIg-positive cells decreased to 3.4%. In the other case (No. 11), the level of RF-positive cells increased moderately to 52.3%, while that of sIg-positive ones decreased slightly to 27.6%. In the neoplastic thymus, the mean values of sIg- positive cells and RF-positive cells were 3.5% (SD ± 1.5) and 3.8% (SD ± 1.6), respectively. The neoplastic cells in the thymic form were considered to have neither a B- nor T-cell maker. Surface markers of neoplastic cells in the skin form were not examined.

In a healthy heifer, sIg-positive cells and RF-positive cells amounted to 36.9 and 35.8% in the peripheral blood, 52.5 and 38.6% in the lymph nodes, and 4.6 and 62.2% in the thymus, respectively.

3) Pathological findings

Adult form: In 3 cases (Nos. 1 to 3), there was an almost generalized neoplastic involvement of the lymph nodes, which consisted of extensive proliferation of neoplastic cells. The original structure of many lymph nodes was almost completely absent except for one case (No. 3). In the moderately involved lymph nodes, the neoplastic cells tended to proliferate in the sinuses. The other 2 cases (Nos. 4

Table 3. Results of pathological examination of neoplastic lesions

No.	Lymph nodes	Spleen	Liver	Kidneys	Heart	Lungs	Abomasum	Uterus	Bone marrow	Thymus	Skin	Other organs
(Adult form)												
1	+++ G[a)]	+	−	+	+++	+	+++	+++	−	−	−	++
2	+++ G	−	−	−	+	−	+	+	−	−	−	++
3	++ G	+++	−	+++	+++	++	+	+++	−	−	−	+++
4	+ L[b)]	−	−	−	+++	−	+++	+++	−	−	−	++
5	+ L	−	−	−	+++	−	+++	+++	−	−	−	++
(Calf form)												
6	+++ S[c)]	+++	+++	+++	+	+	−	++	++	−	−	+++
7	+++ S	+++	+++	+++	−	+++	−	+	+	+	−	+++
8	+++ S	−	+++	++	−	+	−	+	+++	+	−	++
9	+++ S	++	+	++	+	++	+	+	+	−	−	+
(Thymic form)												
10	++ S	+++	++	++	−	−	−	++	+	+++	−	+
11	+ L	−	−	−	−	−	−	−	−	+++	−	−
(Skin form)												
12	+ L	−	−	+	−	−	−	−	−	−	+++ B[d)]	−
(Control)												
13	−	−	−	−	−	−	−	−	−	−	−	−

+++ : Severe neoplastic lesions without normal structure of the organs. ++ : Moderate neoplastic lesions with small area of normal structure of organs. + : Mild lesions with slight neoplastic cell proliferation. − : No lesions. a): G; General distribution. b): S; Systemic distribution. c): L; Local distribution. d): B; Biopsied material.

and 5) showed a localized neoplastic involvement of the lymph nodes. The various extra-nodal tissues were also invaded (Table 3). Ultrastructurally, neoplastic cells varied in size from small cells with scanty cytoplasm to large cells with abundant cytoplasm. The nuclei showed irregular contours with indentations, and contained coarse chromatin and large nucleoli (Fig. 2). The rough-surfaced endoplasmic reticulum (RER) was poorly developed. There were a few mitochondria and small areas of Golgi apparatus in the cytoplasm, and free ribosomes were abundant, especially in the large cells.

Calf form: In all the cases, systemic neoplastic involvement was observed in the lymph nodes, and also characteristic involvement was observed in the Glisson capsule of the liver and in the bone marrow[24]. The neoplastic tissues were also present in other sites (Table 3). In 3 cases (Nos. 6 to 8), the lymphatic parenchyma was crowded with neoplastic cells, and normal lymphatic structures were absent. The other case (No. 9) showed very small areas of normal lymphatic tissue surviving among severe neoplastic proliferation. Ultrastructurally, the size of the neoplastic cells differed in each case ranging from small to medium. Small cells had scanty cytoplasm with poorly developed RER and a few mitochondria, and their nuclei were oval and chromatin was coarse. Medium-sized cells displayed oval nuclei and abundant cytoplasm, in which organelles similar to those of small cells were observed (Fig. 3).

Thymic form: One case (No. 10) showed a large mass of neoplastic thymus and systemic neoplastic lesions, which resembled those of the calf form. In the other case (No. 11), neoplastic lesions were localized in the thymus and a few lymph nodes surrounding it. Ultrastructurally, the neoplastic cells were small with round or slightly indented nuclei with condensed chromatin and inconspicuous nucleoli. The organelles were poorly developed like those in the calf form (Fig. 4).

Skin form: In addition to the neoplastic lymphoid-cell proliferation in the skin, there were palpable swellings of the superficial and pelvic lymph nodes in the tumor stage. In the regressive stage, the neoplastic cells disappeared from the dermis, and most of the neoplastic cells in the lymph nodes and kidneys degenerated with infiltration of normal lymphocytes and macrophages[11]. Numerous plasma cells infiltrated many lymph nodes without neoplastic involvement. Ultrastructurally, neoplastic cells were polygonal in shape, and the organelles consisted of poorly developed RER, a few mitochondria and numerous ribosomes. The nuclei were round to oval in shape, and contained a small amount of chromatin and distinct nucleoli. Nuclear convolution was

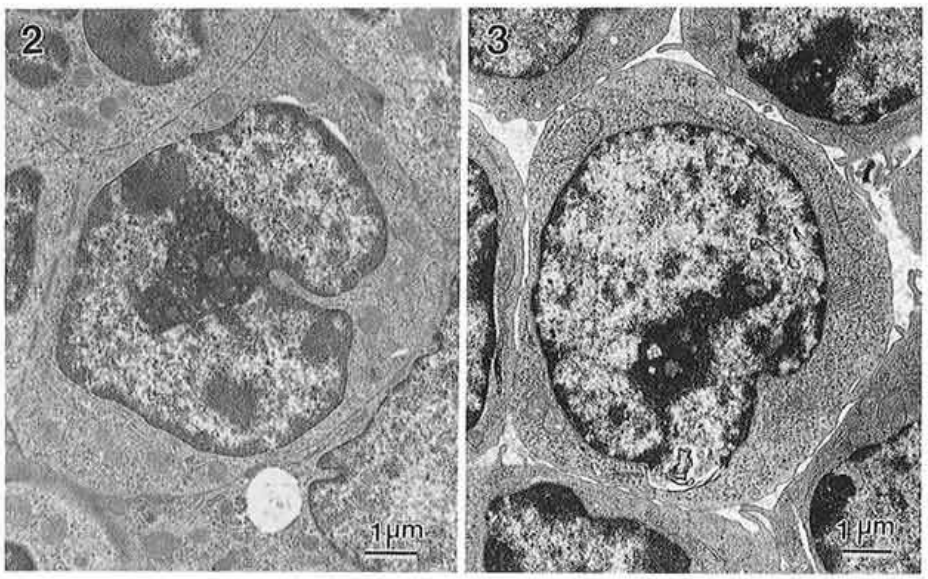

Fig. 2. (left) Electron micrograph of neoplastic cell in adult form (No. 2)
The cell shows on irregularly indented nucleus and poorly developed organelles. (× 3,300)

Fig. 3. (right) Electron micrograph of neoplastic cell in calf form (No. 9)
The cell shows on oval-shaped nucleus and poorly developed organelles. (× 3,300)

not observed (Fig. 5).

Intracytoplasmic IgM, IgG and IgA were not detected in the neoplastic cells of the 4 types of bovine lymphosarcomas (Table 3). In contrast, the lymph nodes of a healthy heifer contained plasma cells and immunoblasts, which gave a positive reaction for IgM, IgG or IgA.

Discussion

Human lymphomas are frequently accompanied by abnormalities of serum γ-globulin, such as hypergammaglobulinemia with M-component and hypogammaglobulinemia[7,16,18,19,23]. Electrophoretic patterns for serum γ-globulin have revealed normal, increased or decreased concentrations in bovine lymphosarcomas[1]. In the present study, animals in the adult form had antibodies to BLV and typical lymphosarcoma as described previously[24]. The values of serum protein suggest that an increase of involved lymph nodes causes a decrease of the level of serum γ-globulin. Since the neoplastic cells gave a positive reaction for sIg, they were presumably derived from the B-cell series[20]. However, these neoplastic cells showed a poorly developed RER and lacked intracytoplasmic immunoglobulins. It is considered that the neoplastic cells could not differentiate into immunoglobulin-producing cells, and extensive proliferation of these cells in the lymph nodes may cause a decrease of the concentration of serum γ-globulin.

The characteristic lesions of the calf form consisted of systemic involvement of the lymph nodes and neoplastic proliferation in the bone marrow and in the Glisson capsule of the liver[21,24]. Although the calf form is most common in calves younger than 6 months of age, similar lesions may be observed in adolescent or adult cattle without antibodies to BLV[24], as in the 3 cases in the present study. Neither a B- nor T-cell marker was present in our cases, and the inability to produce immunoglobulins was demonstrated by the absence of intracytoplasmic immunoglobulins and poorly developed RER. Severe hypogammaglobulinemia in 3 cases (Nos. 6 to 8) was probably due to the absence of normal tissue in the lymph nodes, which was replaced by neoplastic proliferation. The moderate decrease of the concentration of serum γ-globulin in one case (No. 9) was presumably associated with the small area of residual lymphatic tissues in the lymph nodes.

The thymic form showed typical involvement in the thymus and the neoplastic cells had neither a B- nor T-cell marker. These cells may originate

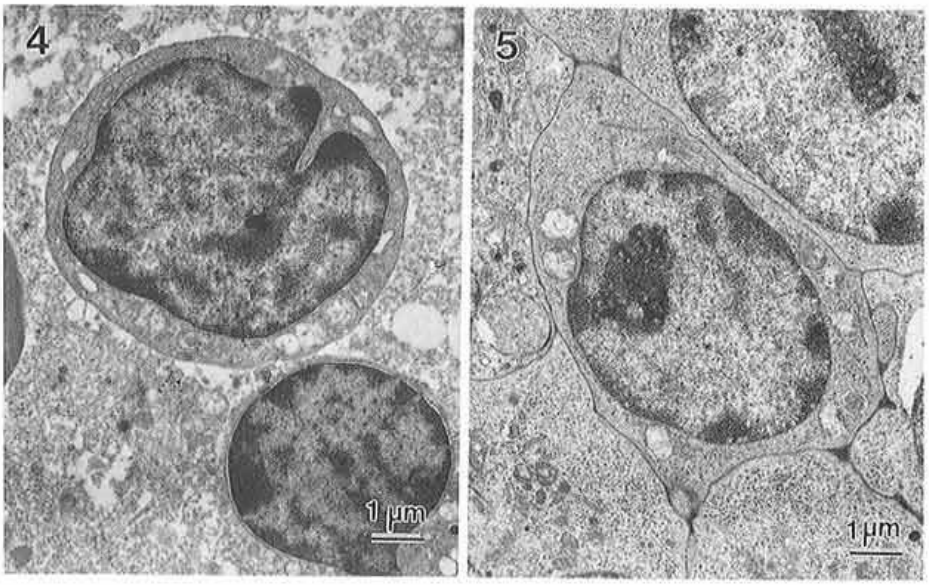

Fig. 4. (left) Electron micrograph of neoplastic cell in thymic form (No. 10)
The cell shows on oval-shaped nucleus with a small indentation and poorly developed organelles. (× 3,300)

Fig. 5. (right) Electron micrograph of neoplastic cell in skin form (No. 12)
The cell shows a round nucleus and poorly developed organelles. (× 3,300)

from thymocytes lacking a T-cell marker due to malignant transformation. In one case, the γ-globulin concentration slightly increased despite the generalized involvement of the lymph nodes, which may be associated with the activation of remaining B-cells by the stimulation of neoplasic cells. It is suggested that the normal value in the other case was due to the fact that in the various lymph nodes without neoplastic lesions normal immunological functions were maintained.

In the skin form, the neoplastic lesions were formed in the lymph nodes and various organs as well as in the skin. These lesions may regress within a few months after the initial recognition of the tumor[11]. In a study of cutaneous lymphosarcoma, the neoplastic cells gave a negative reaction for the RF test[11], while another study showed that the neoplastic cells had a marker for helper/inducer T-cells[25]. In our study, hypogammaglobulinemia in the tumor stage may be associated with the proliferation of neoplastic cells in the lymph nodes, and hypergammaglobulinemia in the regressive stage may be due to the presence of numerous plasma cells in the lymph nodes.

A lower level of IgM fraction in the sera was observed in BLV-positive cattle with persistent lymphocytosis[6]. In cattle with lymphosarcoma, the absence of IgM was pointed out[22], although the level of IgM did not decrease significantly in other studies[15]. Ichijo described a decrease of IgM level in the adult form and IgG in the calf form[9]. No IgM line was detected in our 3 cases with the calf form. It was reported that the immunoblasts, which correspond to one stage of B-cell differentiation, produced initially IgM during differentiation to plasma cells[12]. When the lymphoid tissues are damaged rapidly by malignant growth, the IgM-producing cells may be affected first. In the remaining case of the calf form, with a weak IgM line, it is possible that IgM-producing cells may remain elsewhere including the extra-nodal lymphoid tissues or that IgM which had already been produced may keep a detectable concentration in the serum.

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References

1) Abramova, E. N., Kondratef, V. S. & Sytinskii, I. A. (1974): The biochemistry of leucosis in cattle. *Vet. Bull.*, **44**, 689–711.
2) Bendixen, H. J. (1965): Bovine enzootic leukosis. *Adv. Vet. Sci.*, **10**, 129–204.
3) Coles, E. H. (1974): Plasma proteins. *In* Veterinary clinical pathology (2nd ed.). W. B. Saunders Co., London, 558–560.
4) Ek, N. (1969): Studies on electrophoresis on cellulose acetate membrane of bovine serum proteins in healthy animals. *Acta Vet. Scand.*, **10**, 118–126.
5) Ferrer, J. F. (1980): Bovine lymphosarcoma. *Adv. Vet. Sci. Comp. Med.*, **24**, 1–68.
6) Gate, I. M. H., Lavin, M. F.& Daniel, R. C. W. (1990): Serum immunoglobulin concentrations in cattle naturally infected with bovine leukemia virus. *Zbl. Vet. Med.*, **B37**, 575–580.
7) Harris, J. & Bagai, R. C. (1972): Immuno deficiency states associated with malignant disease in man. *Med. Clin. North Am.*, **56**, 501–514.
8) Hirai, S. et al. (1989): Experimental methods of electrophoresis. Bunkodo Co., Tokyo, 21–212 [In Japanese].
9) Ichijo, S., Kim, D. H. & Konishi, T. (1981): Clinical and clinicopathological findings on bovine leukosis III. Blood clinico-chemical findings. *J. Vet. Med. (Jpn.)*, **724**, 640–645 [In Japanese with English summary].
10) Ishihara, K. et al. (1980): Clinical studies on bovine leukemia in Japanese black cattle IV. Serum immunoglobulin concentrations in leukemic cattle and those with negative and positive serum antibodies to bovine leukemia virus. *Jpn. J. Vet. Sci.*, **42**, 427–434.
11) Ishino, S. et al. (1988): Histopathological observations on regression of skin lymphosarcoma in five cows. *Zbl. Vet. Med.*, **A35**, 578–585.
12) Jelinek, D. F. & Lipsky, P. E. (1987): Regulation of human B-lymphocyte activation, proliferation and differentiation. *Adv. Immunol.*, **40**, 1–59.
13) Kono, Y. et al. (1982): Changes in antibody titers in cattle infected clinically and subclinically with bovine leukemia virus. *Int. J. Cancer*, **30**, 655–657.
14) Marshak, R. R. (1968): Criteria for the determination of the normal and leukotic state in cattle. *J. Natl. Cancer Inst.*, **41**, 243–263.
15) Matthaeus, W. & Straub, O. C. (1975): Das serum γ-Globulinbild bei leukosekranken Rindern und Schafen. *Zbl. Vet. Med.*, **B22**, 758–763.
16) Michaux, J-L. & Heremans, J. F. (1969): Thirty cases of monoclonal immunoglobulin disorders other than myeloma or macroglobulinemia. A classification of disease associated with the production of monoclonal-type immunoglobulins. *Am. J. Med.*, **46**, 562–579.
17) Paul, P. S. et al. (1979): Enumeration of T-cells, B-cells and monocytes in the peripheral blood of normal and lymphocytic cattle. *Clin. Exp. Immunol.*, **35**, 306–316.
18) Prasad, A. (1958): The association of hypogammaglobulinemia and chronic lymphatic leukemia. *Am. J. Med. Sci.*, **230**, 610–613.
19) Rosset, G., Farquet, J-J. & Cruchaud, A. (1978): The serum immunoglobulins in lymphoproliferative malignancies. *Europ. J. Cancer*, **14**, 369–376.

20) Takashima, I. et al. (1977): B-lymphocytes and T-lymphocytes in three types of bovine lymphosarcoma, *J. Natl. Cancer Inst.*, **59**, 1205–1209.
21) Theilen, G. H. & Dungworth, D. L. (1965): Bovine lymphosarcoma in California. III. The calf form. *Am. J. Vet. Res.*, **26**, 696–709.
22) Trainin, Z. et al. (1973): Levels of IgM and IgG in the sera of normal and leukaemic calves. *J. Comp. Pathol.*, **83**, 87–90.
23) Ultmann, J. E. (1959): The clinical implications of hypogammaglobulinemia in patients with chronic lymphocytic leukemia and lymphocytic lymphosarcoma. *Ann. Intern. Med.*, **51**, 501–516.
24) Yamamoto, H. et al. (1982): Histopathological definition of the adult and calf types of bovine leukosis. *Natl. Inst. Anim. Health Q. (Jpn.)*, **22**, 115–125.
25) Zwahlen, R. D., Tontis, A. & Scheider, A. (1987): Cutaneous lymphosarcoma of helper/inducer T-cell origin in a calf. *Vet. Pathol.*, **24**, 504–508.

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