Tumor-associated Antigens on Bovine Leukemia Virus-induced Bovine Lymphosarcoma Identified by Monoclonal Antibodies

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INTRODUCTION

EBL or the adult form of bovine lymphosarcoma is a lymphoproliferative disease caused by BLV (23). The neoplastic cells of tumor lymph nodes have the B-cell surface marker (28, 29, 31) and TAA (12, 17, 25, 26, 28). Immunological studies with highly adsorbed antisera have shown that tumor cells from cattle with EBL have TAA which is distinct from BLV-induced antigens actively produced in infected cells (25, 26). This antigen was found in both the cytoplasm and membrane of tumor cells. Tumor cells were circulating in the peripheral blood of many cattle with persistent lymphocytosis (25, 26, 28).

Development of the technique to produce monoclonal antibody from hybridoma has opened a new venue of analysis of antigens expressed on virus-induced cells or tumor cells (20). Furthermore, monoclonal antibody which is specific for a single epitope allows a detailed study of the structure and function of antigenic determinants present in complex protein molecules. By using this technique, cell surface antigens of tumor cells were detected and analyzed in experimental animal (6, 8) and human (11, 19, 32) systems.

In order to know the properties of TAA expressed on EBL, we obtained 13 monoclonal antibodies specific for TAA by somatic cell hybridization. Using these monoclonal antibodies, we were able to identify common and individually distinct TAA as well as other different TAAs, expressed on BLV-induced EBL tumors.

MATERIALS AND METHODS

Cells. Neoplastic tissues were obtained from cattle with EBL (Cases T1 to T21) that were positive for BLV antibodies at the time of autopsy. Fresh tumors were collected in Dulbecco’s minimal essential medium with 10% calf serum. Normal kidney cells which were histologically non-neoplastic were also obtained from the cattle with EBL for use in examining the specificities of monoclonal antibodies. Lymphosarcoma was diagnosed by both gross and histological observations. PBL from 31 healthy cattle (negative for BLV antibodies) kidney, and liver cells from 3 individual fetuses and FLK (30) were also used in the present experiments. The nonselecting mouse myeloma cell line P3X63Ag8.653 was used for production of hybridomas (18). The bovine lymphoid cell line EBLC-1, which was established from tumors of cattle with EBL, was used to examine the biological activity of monoclonal antibodies. This cell line has the B-cell surface marker, TAA on the cell surface, and BLV antigen in the cytoplasm.4 The MDCC-MSB1 cell line, derived from a splenic lymphoma of chickens infected with Marek’s disease virus, was also used as a control in the test (2).

Production of Hybridomas. To obtain single tumor cell suspension for immunization, fresh tumors were teased with scissors and forceps. The suspension was filtered through gauze, and cells were washed with cold phosphate-buffered saline (136 mM NaCl:2.7 mM KCl:8 mM Na2HPO4:1.5 mM KH2PO4, pH 7.2). Three-month-old BALB/c mice were immunized s.c. with 1 x 106 viable EBL cells (pooled from 3 to 5 individual EBL cases), followed by injection i.p. of 3 x 106 cells 3 weeks later. The mice were given injections i.p. of the same number of cells after 7 days and then sacrificed 2 to 3 days after the last injection. The spleen cells were fused with P3X63Ag8.653 cells in the presence of 50% Polyethylene Glycol 1000 as described by Nowinsky et al. (24). Hybrid cells were selected by medium containing hypoxanthine, aminopterin, and thymidine (24). After 2 to 3 weeks of incubation, the supernatant of hybrid cells was screened by microcytotoxicity assays (13) for cytotoxic activity against EBL cells and other target cells as described below. Hybridomas that produced antibodies reactive to normal bovine PBL and FLK cells were eliminated. Those which specifically reacted to EBL were cloned 3 times in soft agar (5). Hybridomas were propagated in tissue cultures or in the peritoneal cavity of pristane-primed BALB/c mice. To determine the isotype of the monoclonal antibodies, culture fluid from each hybridoma was concentrated 10 times and then examined by a double-immunodiffusion test using isotype-specific antisera (Miles Laboratories, Inc., Elkhart, IN).

CDAC Test. The CDAC assays were performed by the trypsin blue dye exclusion method as described previously (13). Single-cell suspensions of tumor, normal kidney from cattle with EBL, and kidney and liver from bovine fetuses were used as target cells. PBL from 31 individual healthy cattle were also used as target cells. Blood was collected with
EDTA as anticoagulant. Lymphocytes were separated from blood by a Ficoll-Conray gradient as described by Bojem (4). Five μl of the target cells (5 × 10⁶ cells/ml), 5 μl of antibody from hybridoma, and 5 μl of rabbit complement were mixed in the wells of a microplate and incubated at 37° for 45 min. After incubation, 10 μl of 1% trypan blue solution were added to each well, and the viability of cells was immediately determined by counting a minimum of 100 cells. The complement and the antibody were replaced by medium for each test to act as control. CI was calculated by the following formula:

\[
CI = \frac{\% \text{ of viable cells in control well} - \% \text{ of viable cells in test sample}}{\% \text{ of viable cells in control well}} \times 100
\]

To know the maximum nonspecific cytotoxicity of the monoclonal antibodies, PBL from 133 cattle negative for BLV antibodies were examined by the CDAC test. The mean CI was 13.3 ± 7.8 (S.D.). A CI value greater than 31.7 (more than 1.5 times that of negative controls) was considered to be positive in this test system.

For the titration of monoclonal antibody by the CDAC test, the microcytotoxicity test was used as described previously (27). Briefly, 2 μl of the target cells (5 × 10⁶ cells/ml), 2 μl of serial 2-fold dilutions of culture supernatants or ascitic fluid, and 2 μl of rabbit complement were injected under mineral oil into wells of a Terasaki microtiter plate and incubated at 37° for 45 min. After incubation, 1 μl of 0.5% trypan blue solution was added, and the viability of the cells was compared with those in the control wells. In this test system, antibody which reduced the viability of target cells by less than 50% was considered to be positive for cytotoxicity. The titer of monoclonal antibody was determined by cytotoxicity test and expressed as the reciprocal of the highest dilution of antibody giving a positive reaction.

FA Test. Indirect FA tests for acetone-fixed and unfixed living cells were performed as described previously (26) using monoclonal antibodies and fluorescein isothiocyanate-conjugated anti-mouse IgG goat serum (Cappel Laboratories, West Chester, PA).

Adsorption Test. To examine the specificity of monoclonal antibody, culture supernatant of c453 hybridoma was separately adsorbed twice with tumor (T8 and T10), normal kidney from an EBL case (T10), or culture supernatant of c453 hybridoma was separately adsorbed twice with rabbit anti-mouse immunoglobulin serum by precipitation with 50% ammonium sulfate, followed by gel filtration through a column of Sephacryl S 300 (Pharmacia Fine Chemical Uppsala, Sweden).

Biotinylation of IgG Fractions. Biotinyl-N-hydroxysuccinimide ester (E-Y Laboratories, Inc., San Mateo, CA) was used to covalently bind biotin to purified rabbit IgG. One ml of the purified IgG (1 mg/ml), equilibrated previously with 0.1 M NaHCO₃, was mixed with 60 μl of a 1-mg/ml solution of biotin dissolved in dimethyl sulfoxide and incubated at 37° for 4 hr. After incubation, the mixture was dialyzed against phosphate-buffered saline.

Lab-ELISA. Lab-ELISA, as described previously (7), was used to titrate the potency of monoclonal antibodies. Partially purified TAA was used as antigen. All ELISAs were carried out in rigid, flat-bottomed 96-well polystyrene plates (Flow Laboratories, Inc., Hamden, CT). After dilution of the TAA in 0.05 m phosphate buffer (pH 8.0) containing 0.5 m NaCl, 50 μ of a predetermined concentration of the antigen (Chart 1) were adsorbed to the individual wells at 37° for 3 hr. The plate was washed 3 times with 0.1% Tween 20 in 0.05 m phosphate buffer containing 0.5 m NaCl. ELISA was performed in the following steps. (a) Fifty μl of ascitic fluid or IgG, diluted with 0.05 m phosphate buffer containing 0.5 m NaCl, were incubated in each of the TAA-adsorbed wells at 37° for 40 min. Unbound immunoglobulins were removed from the wells by washing 3 times with 0.1% Tween 20 in 0.05 m phosphate buffer containing 0.5 m NaCl. ELISA was performed in the following steps. (a) Fifty μl of ascitic fluid or IgG, diluted with 0.05 m phosphate buffer containing 0.5 m NaCl, were incubated in each of the TAA-adsorbed wells at 37° for 40 min. Unbound immunoglobulins were removed from the wells by washing 3 times with 0.1% Tween 20 in 0.05 m phosphate buffer containing 0.5 m NaCl. (b) Fifty μl of rabbit anti-mouse immunoglobulin serum was added to each well, and the plate was incubated at 37° for 40 min. The wells were washed as described above. (c) Fifty μl of peroxidase-labeled avidine (E-Y Laboratories, Inc., San Mateo, CA) were added to each well and further incubated at 37° for 40 min; then, wells were washed as described above. (d) Two hundred μl of a substrate

\[
\text{CI} = \frac{\% \text{ of viable cells in control well} - \% \text{ of viable cells in test sample}}{\% \text{ of viable cells in control well}} \times 100
\]

CI had the highest antigenic activity against c453 monoclonal antibody when examined by ELISA. Therefore, the concentrated 0.6 m KCl eluate was used as partially purified TAA. Protein concentration of this TAA was 6 mg/ml as determined by the method of Lowry et al. (22).

For control, materials were also prepared from normal bovine lymph nodes by the same procedures as described above.

Cytotoxicity Inhibition Test. The cytotoxicity inhibition test was performed to determine the antigenicity of the solubilized TAA. One volume of serial 2-fold dilutions of the antigen was mixed with one volume of diluted asciatic fluid of clone c453. After incubation at 37° for 45 min, the residual cytotoxic activity of clone c453 was tested against tumor cells.

Isolation of IgG Fractions of Antibody. Rabbit IgG was fractionated from rabbit anti-mouse immunoglobulin serum by precipitation with 50% ammonium sulfate, followed by gel filtration through a column of Sephacryl S 300 (Pharmacia Fine Chemical Uppsala, Sweden).

Chart 1. Reactivities of monoclonal antibody c453 with TAA. Serial 2-fold dilutions of partially purified TAA or normal extracts prepared from normal bovine lymph nodes were reacted with c453 (1:100) or culture fluid of myeloma cells by ELISA. The reactivity of c453 with TAA; A, reactivity of c453 with normal extracts; O, reactivity of myeloma culture fluids with TAA; △, reactivity of myeloma culture fluids with normal extracts.
solution containing 0.2 mM 2′-azino-di(3-ethylbenzthiazoline sulfonic acid) (Sigma Chemical Co., St. Louis, MO) and 0.004% H2O2 in 0.05 M citrate buffer, pH 4.0, were added to each well, and the plate was incubated at 37° for 1 hr. A540 was scored by a Microelisa automatic reader (Flow). The working dilution of both biotinylated rabbit IgG with anti-mouse immunoglobulin and the peroxidase-labeled avidine was 1:800.

ELISA end point titer was estimated from the dose-response curve of serial dilutions of monoclonal antibodies. P3X63Ag8.653 growth medium was used as control, and its A540 was always less than 0.15. The ELISA end point titer was taken as the reciprocal of the maximum dilution of antibody giving A540 > 0.2.

To examine the reactivity of monoclonal antibodies to BLV, purified and solubilized BLV antigen (10 μg/ml) was prepared and tested by ELISA.

Effect of Monoclonal Antibodies on Cell Growth. EBLC-1 and MDCC-MSB1 cells were used to examine the effect of the antibodies on cell growth. EBLC-1 cells (1 x 10⁶ cells/well) or MDCC-MSB1 cells (5 x 10⁶ cells/well) were suspended in 1 ml of DMEM, containing 10% fetal calf serum plus either 50 μg of IgG from c453 or 50 μg of IgG mixture composed of 25 μg each from c453 and c164, and plated in a 24-well plate (Nunc, Roskilde, Denmark) in triplicate wells. The cells and IgG mixtures were incubated at 37° for 30 to 3 hr for EBLC-1 cells and at 41° from 12 to 54 hr for MDCC-MSB1 cells. After incubation, the number of live cells was determined by the trypan blue dye exclusion test. For control, culture fluid from P3X63Ag8.653 or 50 μg of IgG from a monoclonal antibody (clone 1) against paramyxovirus (4) were used in place of c453 and c164.

RESULTS

Production of Hybridoma Cells. A total of 207 clones was obtained in 4 fusion experiments, and 23 of them secreted antibodies against tumors from EBL. Ten of the 23 hybridoma cells secreted antibodies reactive with normal bovine PBL as well as tumor cells. Thirteen hybridomas secreting antibodies against EBL, but not against normal PBL nor FLK cells, were cloned 3 times in soft agar.

The antibody titers of the 13 monoclonal antibodies were determined by CDAC test using T16 tumor cells and by ELISA using partially purified TAA (Table 1). The CDAC titers of culture fluids of Group 1 clones were between 1:32 and 1:128, and those of ascitic fluids were between 1:512 and 1:8192. The CDAC antibody titers of culture fluids of clones 885 and 903 were 1:32, and those of ascitic fluids were 1:512. The other clones (2064, 2065, 4134, and 4366) belonging to Group 2 did not react with T16 tumor cells. Also, clone 311 of Group 3 did not show any cytotoxic activity against T16 tumor cells.

Determination of the immunoglobulin isotype showed that all of the antibodies secreted by Group 1 clones were IgG2b (λ). Antibodies of Group 2 were found to be IgG1 (κ), IgG1 (λ), or IgG2b (κ), and the antibody of the Group 3 was IgG1 (κ) (Table 1).

Reactivities of Monoclonal Antibodies with Tumors, Normal Bovine Cells, and BLV Antigens. The reactivities of the 13 monoclonal antibodies with 19 individual tumors from EBL were examined by CDAC and FA tests. According to the reactivities of their culture fluids, the 13 clones were divided into 3 groups (Tables 1 and 2). The first group consisted of hybridomas that secrete antibodies reactive with all EBLs tested. The second group comprised hybridomas whose culture fluids reacted with several, but not with all, of the EBLs tested. The antibody from the third group reacted only with the tumor cells used for immunization (Table 2). The CI of positive reaction varied from 40 to 94.2. These results indicate that Group 1 monoclonal antibodies recognize common TAA expressed on all of the tumor cells from EBL, and Group 2 monoclonal antibodies recognize other TAAs that differ from those recognized by Group 1. The Group 3 monoclonal antibody may recognize individually distinct TAA expressed on the EBL tumors.

To ascertain the specificity of the monoclonal antibodies to TAA, the reactivities of the 13 monoclonal antibodies with various bovine or sheep cells were examined by CDAC and FA tests (Table 3). The Cls and the S.D.s of the 13 antibodies with PBL from 31 individual normal cattle varied from 0.9 ± 1.2 to 12.8 ± 12.2. Since CI less than 31.7 was considered negative, these reactivities were negligible. In CDAC and FA tests, none of the antibodies reacted with normal kidney tissues from 3 individual cattle with EBL whose tumor cells were lysed by Group 1 antibodies, nor with 4 individual bovine fetuses. All antibodies did not react with FLK cells which expressed BLV antigen on their cell surface. Furthermore, none of the antibodies reacted with the soluble BLV antigens that showed positive reaction to anti-BLV serum by ELISA. Hemagglutination activity of the antibodies to bovine, sheep, and horse RBC could not also be detected (data not shown).

Acetone-fixed tumor cells from 11 EBL cases showed a positive reaction in an FA test with the monoclonal antibodies (Table 2). The positive cells showed TAA in the cytoplasm (Fig. 1A). In unfixed living cells, specific membrane fluorescence was observed in tumor cells from all EBL tested (Fig. 1B).

Adsorption Test Using c453 Clone. Adsorption tests were also done using c453 monoclonal antibody to confirm further its specificity. After adsorption, its residual cytotoxic activity against 5 individual tumors from EBL was examined (Table 4). Adsorption of c453 with T8 tumor abolished its reactivity with cells from T8 as well as other tumors (T7, T10, T15, and T17). Adsorption of c453 with T10 abolished its reactivity with all tumors tested. However, adsorption of c453 with normal kidney from T10 or normal bovine PBL did not diminish its reactivity. Adsorption experiments were done using other hybridomas from Group 1, and similar results were obtained (data not shown).

Inhibitory Effect of Monoclonal Antibody on Growth of EBLC-1 Cells. To know the biological activity of the monoclonal antibodies to TAA, EBLC-1 cells were cultured in medium containing Group 1 monoclonal antibody. The number of live cells was reduced in cultures containing either IgG of c453 or IgG mixture of c453 and c164 as compared with cultures containing either P3X63Ag8.653 or IgG from a clone against paramyxovirus (Chart 2A). Both c453 and C164 showed no cytotoxicity to the EBLC-1 cells in the absence of rabbit complement. The specificity of this inhibition for cell growth was further tested by using MDCC-MSB1 cells that are negative for TAA of bovine lymphosarcoma. No inhibitory effect on growth of MDCC-MSB1 cells was observed in the presence of IgGs from c453 and c164 (Chart 2B).

Extraction of TAA from Tumors and Its Reactivity with Monoclonal Antibodies. Analysis of the sodium deoxycholate extracts from tumor cells after centrifugation at 105,000 x g showed that the lower one-third fraction of the supernatant had a CDAC inhibition titer of 1:84, and the upper two-thirds fraction had a titer of 1:16. The lower one-third of the supernatant was further purified by DEAE-cellulose column chromatography and
Table 1

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<th>Group</th>
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<th>Asitic fluid</th>
<th>Culture fluid</th>
<th>ELISA titer with ascitic fluid</th>
<th>Binding activity of ascitic fluid with soluble TAA</th>
<th>Immunoglobulin class and subclass</th>
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<td>ND</td>
<td>1280</td>
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<td>IgG1a, x</td>
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a Microcytotoxicity test was performed using T16 as target cells. Although 2064, 2065, 4134, 4366, and 311 antibodies reacted with several of the tumors tested (Table 2), these clones did not react with T16 tumor. Therefore, antibody titer of the clones was not determined.

b ELISA was performed using partially purified TAA as antigen. For preparation of TAA, 6 individual tumors (T1, T2, T6, T8, T10, and T14) were used. All of the monoclonal antibodies from Groups 1 and 3 reacted with TAA. Monoclonal antibodies from Group 2, 885, 903, 4134, and 4366, reacted with the TAA, while 2064 and 2065 did not.

c Binding activity was expressed as high or low when the specific absorbance at the plateau level was > or < 0.8 in the ELISA, respectively.

d ND, not determined.

Table 2

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<th>T14</th>
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a Mean ± S.D.

b +, results positive for specific fluorescence; -, negative results for same.

c —, CI less than 31.7.

used as ELISA antigen.

Reactivities of monoclonal antibody with the partially purified TAA were examined by ELISA as follows. Serial 2-fold dilutions of TAA (0.6 M KCl eluate) or normal extracts (0.6 M KCl eluate from normal bovine lymph nodes) used as control were reacted with a single dilution (1:100) of c453 monoclonal antibody (Chart 1). A dose-response curve was obtained by serial dilutions of TAA with c453. c453 antibody showed a slightly nonspecific reaction with normal extracts at high concentrations (> 1.5 mg/ml). Control myeloma culture fluid showed no reactivity with either TAA or normal extracts. Therefore, a 1-mg/ml concentration of TAA was used for the titration of monoclonal antibodies by ELISA.

The ELISA titers of the 13 monoclonal antibodies with partially purified TAA are shown in Table 1. The titers of antibodies of Groups 1 and 2 varied from 320 to 1280 and from 320 to 640, respectively. Monoclonal antibodies of 2064 and 2065 did not react with the TAA prepared from pooled tumors of T1, T2, T6, T8, T10, and T14. The titer of Group 3 antibody was 1280.

The 11 monoclonal antibodies reactive with the TAA were compared for their binding activities using the antigen that eluted with 0.6 M KCl. Serial 2-fold dilutions of ascitic fluids from the 11 antibodies were tested by ELISA using a 1-mg/ml concentration of TAA. The results showed that the binding activities of Group 1 antibodies were of high affinity (A40s > 0.8), but those of antibodies from Groups 2 and 3 were of low affinity (A40s < 0.8) (Table 1).

DISCUSSION

We have isolated 13 clones secreting monoclonal antibodies to tumor cells from cattle with EBL by somatic hybridization. These monoclonal antibodies were directed against TAA ex-
pressed on tumor cells from cattle with EBL but not against histocompatibility antigen nor BLV antigens, because they did not react with normal bovine PBL (B- and T-cells), normal tissues from cattle with EBL, or BLV antigens (Table 3). These antibodies were also not directed against heterophile antigen, because hemagglutination activities of these antibodies against bovine, sheep, and horse RBC could not be demonstrated. The cytotoxic activity of the monoclonal antibodies against tumor cells was abolished by adsorption of the antibodies with homologous and heterologous tumor cells but not with normal cells, including normal tissues from cattle with EBL (Table 4). Since c453 monoclonal antibody did not react with normal bovine fetal liver and kidney cells and bovine PBL activated by phytohemagglutinin and pokeweed mitogen in a preliminary experiment, it may not be directed against an embryonic antigen or a differentiation antigen, although further studies will be needed to characterize the antigen that anti-TAA antibody defines.

Using polyclonal antibodies, it was demonstrated previously in our laboratory that all the tumors induced by BLV shared cross-reacting TAA on the cell surface and in the cytoplasm (25, 26, 28). We produced monoclonal antibodies to TAA to confirm this finding. The 13 monoclonal antibodies described in Tables 1 and 2 were tentatively divided into 3 groups: Group 1 (clones c453, c444, c432, c164, c153, and c143), which reacted with all the tumors tested; Group 2 (clones 885, 903, 2064, 2065, 4134, and 4366), which reacted with several, but not all, of the tumors tested; and Group 3 (clone 311), which reacted only with the homologous tumors. By using Group 1 clones, the presence of a common TAA on tumor cells from cattle with EBL was confirmed. In Rous sarcoma virus-induced mouse tumors, an individually distinct tumor antigen in addition to a common antigen was demonstrated by using monoclonal antibodies (21). We know of no reports regarding the presence of individually distinct TAA on different tumors induced by BLV. Since monoclonal antibody of Group 3 (clone 311) reacted only with T2 tumor and not with normal tissues from the same host that was used for immunization, this antibody may recognize individually distinct TAA on EBL tumor. As shown in Table 2, the antigenic determinants of TAA recognized by the Group 2 antibodies may differ from those of the common and individually distinct TAs. Further characterization of the TAs recognized by antibodies of Groups 2 and 3 will be described in a separate paper (1).
The inhibitory effect of IgGs from c453 on the growth of EBLC-1 cells appeared to be specific, because this effect was not observed in MDCC-MSB1 cells. Moreover, monoclonal antibodies against paramyxovirus did not show any inhibitory effect on the EBLC-1 cell line. Although many monoclonal antibodies have been observed to exhibit in vitro growth inhibition of cells, the precise mechanism is not entirely clear (9, 10). However, there is a possibility that this growth inhibition is caused by inhibition of DNA or protein syntheses.

In the present experiment, TAA was extracted from cells. All of the monoclonal antibodies except clones 2064 and 2065 reacted with the partially purified TAA that was eluted with 0.6 M KCl in DEAE-cellulose column chromatography. Clones 2064 and 2065 did not react with each of the 6 individual tumors used for extraction of TAA. The relatively lower binding activities of monoclonal antibodies of Groups 2 and 3 to partially purified TAA as compared with those of Group 1 may be due to the low affinity of the former, the lack of purity of TAA used, or the different antigenic determinant recognized by the monoclonal antibodies. The competitive binding assay among these groups of antibodies using TAA purified by isoelectric focusing and the identification of the antigenic molecule recognized by these antibodies using Western blot techniques are presented in a separate report (1).

Monoclonal antibodies directed against the common TAA should be useful as a diagnostic tool for detecting the tumor cell in mass screening. There are many BLV-infected cattle in enzootic areas in Japan, but it is not known whether the infection will progress to lymphosarcoma or not. In the preliminary experiments, we detected the antigens on PBL from cattle with EBL using monoclonal antibodies of c453 and c143. Furthermore, PBL from cattle with persistent lymphocytosis also showed a positive reaction against the antibodies. These monoclonal antibodies may be useful tools for the diagnosis of lymphosarcoma and for determining whether or not the BLV-infected cattle will progress to exhibiting the tumor in future.

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REFERENCES

Fig. 1. Indirect immunofluorescence test of acetone-fixed (A) and living (B) tumor cells. The monoclonal antibody used was the undiluted culture fluid of c453 clone. A, cells showing cytoplasmic fluorescence; B, cells showing membrane fluorescence. × 1600.
Tumor-associated Antigens on Bovine Leukemia Virus-induced Bovine Lymphosarcoma Identified by Monoclonal Antibodies

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