Isolation and Characterization of an Antigen of the Bovine C-type Virus

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SUMMARY

By means of gel filtration and isoelectric focusing, an antigen of the bovine C-type leukemia virus was isolated in a highly purified form from extracts of infected cells. The antigen has a molecular weight of approximately 25,000 daltons and an isoelectric point of 6.4 to 6.6. In immunodiffusion experiments, the antigen forms a line of identity with an antigen extracted from highly purified bovine C-type leukemia virus by treatment with ether or Triton X-100. As determined by immunodiffusion analyses, the bovine C-type leukemia virus antigen does not have antigenic determinants in common with the murine or feline leukemia viruses, the foamy-like bovine syncytia virus, or the Mason Pfizer monkey virus.

INTRODUCTION

An indigenous bovine C-type virus has been implicated in the etiology of bovine leukemia by seroepidemiological and transmission studies (5, 7, 8, 26). The virus, tentatively designated as the BLV,2 can be detected consistently in short-term cultures of BC cells of cattle with leukemia or persistent lymphocytosis (23, 33). BLV has also been found in permanent cell cultures (New Bolton Center cell lines) established from peripheral lymphocytes of leukemic cattle (9, 10). More recently, BLV has been transmitted to and propagated in monolayer cell cultures of various origins (15, 31, 36).

Studies from this laboratory have shown that most leukemic cells in soft-term cultures of BC cells of cattle with leukemia or persistent lymphocytosis (23, 33). BLV has also been found in permanent cell cultures (New Bolton Center cell lines) established from peripheral lymphocytes of leukemic cattle (9, 10). More recently, BLV has been transmitted to and propagated in monolayer cell cultures of various origins (15, 31, 36).

A preliminary report of some of these findings has been presented elsewhere (22). Subsequent investigations were concerned with the isolation, purification, and further characterization of the internal BLV antigen. Similar studies in other C-type virus systems were facilitated by the availability of large amounts of purified virus. However, at the time these studies were initiated, our sources of BLV were limited to lymphoid cultures (short-term cultures of bovine BC cells and New Bolton Center cell lines) in which most virus particles are associated with cell membranes. This made it extremely difficult to obtain the amounts of purified virus required to extract enough antigen for characterization. Consequently, we decided to investigate the possibility of isolating and purifying the antigen from BLV-infected cells that contain free antigen in the cytoplasm and that usually have numerous virus particles attached to the cell membrane. Using G-100 Sephadex chromatography and isoelectric focusing, it was possible to obtain a good yield of antigen in a highly purified form from extracts of such BLV-infected cells. The antigen is present on an internal BLV protein with a molecular weight and isoelectric point similar to that of the major internal proteins of other mammalian leukemia viruses. Immunodiffusion studies showed that the BLV antigen is immunologically unrelated to the antigens of MuLV, FeLV, and the foamy-like BSV (20), and the MPMV. A preliminary report of some of these findings has been presented elsewhere (22).

MATERIALS AND METHODS

Cells and Cell Cultures. BLV-infected BC cells were isolated following procedures reported earlier (33) from 2 cows (BF-227 and AJ-147) in multiple-case research Herd BF (5). The cells were cultured in 1-liter spinner culture flasks at a cell concentration of 4 to 6 x 10⁶/ml in Eagle's minimal essential medium containing 10% heat-inactivated (56°, 30 min) fetal bovine serum, 0.25 μg amphotericin B per ml, 200
units of penicillin per ml, and 200 μg streptomycin per ml. After short-term cultivation of the BC cells from these animals, large numbers of BLV particles were found consistently. On the other hand, particles resembling the ubiquitous BSV, the maedi-like R-29 bovine virus (35), Pb3, IBR, or BVD viruses were not detected in these cells despite extensive microscopic studies. IFA tests with specific reference sera failed to detect the antigens of these viruses in cultured or uncultured AJ-147 BC cells. The BF-227 BC cells were tested only for the presence of BSV antigens and were found negative.

As controls, we used BC cells from a normal cow (Bl-196) in leukemia-free herd Bl (5). Neither BLV nor BSV particles nor the antigens of these viruses were found in the Bl-196 cells. The serum of Bl-196 was consistently negative for antibodies against BLV or BSV.

Cell line BLV-bat2 was initiated by infecting a bat monolayer cell culture (TbLu) with BLV. Large numbers of BLV particles and high levels of BLV reverse transcriptase activity can be continuously detected in the supernatant fluid of this cell line. As determined by immunological analysis using IFA, IFA absorption, immunodiffusion, and virus neutralization tests, the BLV particles released by this cell line are antigenically indistinguishable from BLV produced by bovine BC cells and New Bolton Center cell lines. The BLV-bat2 cells have been repeatedly tested for BSV, R-29, Pb3, IBR, and BVD viruses and found negative (2, 15).

Antigen Extracts. The BC cells were harvested after 48 to 72 hr in culture and packed by centrifugation at 1,000 x g for 20 min. In some cases, the pellet obtained by centrifugation of the supernatant fluid at 15,000 x g for 10 min was pooled with the cell pack. Cells were lysed by resuspending them in a 1% Triton X-100 (Packard Instrument Co., Downers Grove, Ill.) solution made with Buffer A. Columns were run at room temperature.

Sephadex Chromatography. Antigen extracts were chromatographed on G-100 Sephadex (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) columns (2.5 x 100 cm) equilibrated with Buffer A. Columes were run at room temperature with a flow rate of 12 ml/hr. Fractions of 4 ml were collected and tested by immunodiffusion with reference BLV serum. Positive fractions were pooled, dialyzed against 0.01 M Tris-HCl buffer, pH 7.8, containing 0.1% 2-mercaptoethanol, and concentrated by either lyophiliization or Amicon (Amicon Corp., Lexington, Mass.) ultrafiltration. The final protein concentration was adjusted to 1 to 2 mg/ml. Hereafter, this pool will be referred to as the G-100 antigen fraction.

BLV antigen purified by isoelectric focusing and labeled with 14C (see below) was chromatographed on G-75 Sephadex columns (1 x 100 cm) equilibrated with Buffer A containing 0.01% Triton X-100. The flow rate of the column was 1.3 ml/hr, and fractions of 1 ml were collected. One-tenth ml of each fraction was spotted on Whatman No. 3MM filter discs that were 2.3 cm in diameter (Arthur H. Thomas Co., Philadelphia, Pa.), dried, and then placed in 10 ml of scintillation fluid and counted in a Packard Tri-Carb Model 3320 scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.).

PAG. PAG electrophoresis was performed as described by Maizel (19) using a pH 7 buffer containing 0.1 M sodium phosphate and 0.1% SDS (Sigma Chemical Co., St. Louis, Mo.). Samples (50 to 100 μl) in 0.01 M sodium phosphate buffer, pH 7.0, containing 1% SDS, 1% 2-mercaptoethanol, 3 μl of 0.05% bromphenol blue, and 10% sucrose were heated at 100° for 1 min and then applied to 9-cm gels having an acrylamide monomer concentration of 10%. Gels were run (9 ma/gel) until the tracking dye reached 80% of the gel length. They were then stained with Coomassie Brilliant Blue R by methods described by Maizel (19) and destained in 7.5% acetic acid:5% methanol solution in a Canaco Quick Gel Destainer.

SDS gels were frozen and sliced with a fractionator made with stacked razor blades spaced 1.4 mm apart. Gel slices were placed in wells of a flat-bottomed microtiter plate and eluted with 50 μl of 0.01 M sodium phosphate buffer, pH 7.0, overnight at 25° and then tested by immunodiffusion with reference BLV serum (see below). SDS gel slices containing 14C-labeled proteins were dissolved overnight in 0.2 ml of 30% hydrogen peroxide and counted as previously described.

Isoelectric Focusing. Two methods were used in the isoelectric focusing of the G-100 antigen fractions. One was a modification of the micropreparative procedure of Massey and Deal (21). The following solutions were prepared: one contained 1 ml of G-100 antigen fraction in 0.01 M phosphate buffer, pH 7.0, 0.15 ml of pH 3 to 10 ampholytes (40%) (LKB Instruments, Inc., Rockville, Md.), 1.0 g sucrose, 6 μl of 2 mercaptoethanol, 3 μl of 10% Triton X-100, and 2.0 ml of distilled water; the other solution had the same composition as the former except that the volumes of ampholyte and water were 0.05 and 2.57 ml, respectively. Equal volumes of these 2 solutions were mixed with a gradient mixer, and a sucrose gradient was formed in a glass tube (0.6 x 20 cm) with a 5% acrylamide plug. The upper tank of the electrofocusing apparatus contained 0.4% ethylenediamine; the lower compartment was filled with 0.2% phosphoric acid. Isoelectric focusing was carried out at 25° for 15 hr, using 200 V. After the run, the acrylamide plug was pierced, and 0.20-ml fractions were collected in the wells of a microtiter plate. The pH of the fractions was measured at 25° using a Beckman pH meter equipped with combination microprobe.

Electrofocusing was also done in acrylamide-stabilized gradients following the procedure of Wrigley (41). The concentrated gel mixture contained 0.8 ml of a 1% solution of N,N,N',N'-tetramethylethylendiamine, 3 ml of 30:0.8% acrylamide-bisacrylamide solution, and 0.3 ml amphotoles (40%), pH range 6 to 8. Gels (0.5 x 6.5 cm) were prepared by

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D. A. Graves and J. F. Ferrer, manuscript submitted for publication.
Characterization of the Internal BLV Antigen

mixing 0.5 ml of the concentrate with 1.0 ml of distilled water and 10 μl of 10% ammonium persulfate solution. At 25°, the gels polymerized within 1 hr. Fifty-μl samples containing 50 to 100 μg of protein and 5 ml of sucrose in 0.01 M phosphate buffer, pH 7.0-0.1% 2-mercaptoethanol were loaded on top of the gels. A protective layer of ampholytes in 5% sucrose was placed above. The buffers of the upper and lower compartments were 0.4% ethylenediamine and 0.2% phosphoric acid, respectively. Electrophoresis was carried out at 25° using a maximum current of 2 ma/gel. Voltage was increased from 50 to 300 V over a 75-min period and then kept constant for about 60 more min. For pH measurements, gels were frozen and sliced; sets of 2 adjacent slices were eluted with 0.4 ml water overnight at 25°, and the pH of the eluate was measured as described above. Antigen was eluted from individual gel slices overnight at 4° using 100 μl of Buffer A containing 0.01% Triton X-100.

Estimation of Molecular Weight. The following protein standards were used. Pentex crystalline bovine serum albumin (M.W. 68,000) was obtained from Miles Laboratories, Elkhart, Ind.; chymotrypsinogen (M.W. 25,700) and RNase (M.W. 13,700) were obtained from Worthington Biochemical Corp., Freehold, N.J.; and ovalbumin (M.W. 43,000) was obtained from Miles Laboratories, Elkhart, Ind. Bovine serum albumin standards were used. Pentex crystalline bovine serum albumin (New England Nuclear, Boston, Mass.) by means of a reductive alkylation procedure similar to that described by Velicer and Graves (37). Antigen free of ampholytes was dialyzed overnight at 25° against 0.2 M borate buffer, pH 8.9, containing 0.01% Triton X-100 and then concentrated to 200 μl by vacuum dialysis. Two-tenths mCi of [14C]formaldehyde was added to the concentrated antigen. This was followed by 4 successive additions, 30 sec apart, of 10 μl of freshly prepared sodium borohydride (5 mg/ml water). After 1 min, the reaction was terminated by the addition of excess (50 μl) reductant, and the unreacted label was removed by dialysis against Buffer A containing 0.01% Triton X-100.

Labeling and Purification of BLV. BLV was labeled and purified following essentially the procedure of Robinson et al. (30). Semiconfluent monolayers of BLV-bat1 cells growing in 75-cm Falcon flasks (Falcon Plastics, Oxnard, Calif.) were incubated at 37° for 48 to 72 hr with 25 ml of complete medium containing 2.5 μCi of [3H]uridine per ml (26 Ci/m mole) (New England Nuclear, Boston, Mass.). The supernatant fluids were collected, pooled, and clarified by centrifugation at 10,000 × g, 10 min. The virus was then either pelleted by ultracentrifugation (54,000 × g, 90 min) or precipitated with a saturated solution of ammonium sulfate. The pelleted or precipitated virus was resuspended in a small volume of TNE buffer containing 5% sucrose and centrifuged (275,000 × g for 3 hr) in a 50.1 Beckman rotor (Beckman Instruments, Inc., Fullerton, Calif.) through a discontinuous sucrose gradient (20 and 60%) made in TNE buffer. The opaque band at the interphase was collected, incubated at 37° for 30 min with 5 μg of pancreatic RNase per ml (Worthington Biochemical Corp.), and layered onto a preformed linear sucrose gradient (15 to 55%). After centrifugation at 275,000 × g for 4 hr, 0.25-ml fractions were collected. The density of each fraction was determined by refractometry. Fraction samples (0.1 ml) were spotted on Whatman No. 3MM filter discs (2.3 cm in diameter), dried, precipitated with 5% cold trichloroacetic acid, and counted in a Packard Tri-Carb liquid scintillation spectrometer.

Unlabeled virus was concentrated and purified following the same procedure but omitting the RNase treatment of the opaque band obtained from the discontinuous sucrose gradient. In some experiments, the visible virus-containing band corresponding to a density of approximately 1.15 g/cm³ of the linear gradient was collected and centrifuged through a 2nd linear gradient. For electron microscopic examination, pools of 2 to 3 adjacent fractions (0.25 ml from each fraction) were prepared, diluted, and centrifuged at 275,000 × g, 60 min. The pellets were fixed with 3% glutaraldehyde, postfixed with 1% osmium tetroxide, stained en bloc with 0.5% uranyl acetate, dehydrated in a graded series of ethanol, and embedded in Maraglas. Thin sections were stained with uranyl acetate and lead citrate and studied with a Siemens-Elmiskop IA (Siemens Corp., Iselin, N.J.). Chemicals used in the preparation of electron microscopic samples were obtained from Polysciences, Inc., Warrington, Pa.

Serological Procedures. Immunodiffusion tests were carried out in 2% Noble agar (Immunoplates, Pattern C; Hyland Laboratories, Los Angeles, Calif.) as described previously (3). Immunoelectrophoresis, as described by Williams (39), was performed on microscope slides (1 x 3 inches) coated with 1% Noble agar in barbital buffer (pH 8.2; ionic strength, 0.04).

The origin and characterization of the reference bovine sera for BLV and BSV have been reported elsewhere in detail (2). Reference serum 27-125, obtained from a regression case of bovine leukemia, reacts up to a dilution of 1:256 to 1:512 in IFA tests with BLV-infected target cells, and in immunodiffusion tests, forms a strong precipitin line with ether-treated BLV. This serum also contains fluorescent antibodies against BSV. IFA, IFA absorption, and virus neutralization tests have clearly demonstrated that BLV and BSV are antigenically unrelated. Reference BLV serum Ser-276 was collected from a leukemic cow. The IFA titer of this serum on BLV-infected cells is 1:256, whereas at a dilution of 1:8 it is completely negative on BSV-infected cells. Ser-276 also has a strong precipitation activity against disrupted BLV. Reference BSV serum Se-354 was collected from a normal cow (BH-128) in leukemia- and BSV-free herd BH (5). This serum reacts up to a dilution of 1:256 in IFA tests with acetylene-fixed BSV-infected cells and in immunodiffusion experiments forms 2 precipitin bands with Triton X-100-disrupted BSV. Se-354 is completely negative in IFA tests against acetylene-fixed cells infected with BLV, R-29, IFR, or BVD viruses (2). The other antisera used in this study were obtained from Huntington Laboratories (Baltimore, Md.) through the courtesy of Dr. Jack Gruber (Resource and Logistics Program of the National Cancer Institute, NIH). Serum (Lots IX-580 and IX-1621) was collected from rats.
bearing syngeneic tumors induced by inoculation of MSV-MuLV. Serum IS-8 was prepared by immunization of a goat with Tween-ether-disrupted FeLV. These antisera are known to react strongly in immunodiffusion, as well as in other serological tests, with both interspecies- and intraspecies-specific antigenic determinants of MuLV and FeLV (4, 24, 27, 32, 40). Serum 2-5-0752 was collected from a goat immunized with detergent-disrupted MPMV and absorbed in our laboratory with lyophilized fetal calf serum. This serum reacts both in immunodiffusion and in radioimmunoassays with the p.26 and p.15 polypeptides of the MPMV (34).

MPMV antigen was prepared by ether treatment of MPMV recovered from human lymphoblastoid (NC-37) cells and purified by 2 successive sucrose density centrifugations. This virus was kindly supplied by Dr. M. Ahmed (Pfizer Laboratories, Maywood, N. J.).

The standard reference BLV antigen was an ether-treated extract of cells and high-speed pellets of culture fluid from BLV-infected cell line NBC-13. Extensive and repeated studies have demonstrated that this cell line is free of other bovine viruses and their antigens (2).

BSV antigen was prepared by Triton X-100 treatment of an extract of cells and high-speed virus pellets of supernatant fluids from bovine embryo spleen cell cultures heavily infected with BSV (2). In immunodiffusion tests, this antigen preparation forms 2 distinct precipitation bands with reference BSV serum Se-354.

Tween-ether-disrupted MuLV and FeLV preparations were obtained from Flow Laboratories, Inc. (Rockville, Md.), and Dr. F. Noronha (Cornell University, Ithaca, N. Y.), respectively.

Determination of Protein Content. The estimation of protein concentration was performed according to the procedure of Lowry et al. (18) using bovine serum albumin as the standard.

RESULTS

Isolation of the Antigen from Homogenates of Infected Cells. Fig. 1 shows the typical results obtained when extracts of BLV-infected cells were subjected to electrophoresis and then reacted with reference BLV serum. The BLV antigen migrates toward the cathode as a single precipitin band.

Chart 1 shows the typical profile obtained when an extract of BLV-infected cells was chromatographed on a G-100 Sephadex column. As determined by immunodiffusion analysis of the fractions with reference BLV serum, the BLV antigen eluted consistently at about 1.5 times the void volume of the column. When compared with protein standards run separately in the same columns, the antigen-containing fractions were found to elute after ovalbumin and before chymotrypsinogen.

Typical results of the analysis of G-100 BLV antigen fractions by SDS-PAG electrophoresis are presented in Fig. 2. Gels 3 and 4 correspond to G-100 antigen fractions of 2 different extracts of BLV-infected BC cells. As expected, these gels show numerous bands, most of which probably correspond to cellular proteins. Duplicates of these gels were examined by immunodiffusion with reference BLV sera and reference BLV antigen. The antigen was consistently and exclusively found in the region of the gels indicated by a in Fig. 2. When the material of region a was isolated and rechromatographed, it showed the same mobility (Gel 5). By comparison with gels containing the reference proteins (Gel 1), the molecular weight of the polypeptide of region a was estimated to be approximately 25,000 daltons.

G-100 antigen fractions were also analyzed by isoelectric focusing. Acrylamide-stabilized gradients with a pH range of 6 to 8, gave pI values of 6.4 to 6.6 for the antigen. Since artifacts are known to occur rather frequently with electrophoresis in gels, the antigen preparations were focused in sucrose-stabilized gradients with a pH range of 3 to 10 for a more accurate estimate. The pI value obtained (6.4 to 6.7) was similar to that obtained by the previous procedure.

BLV antigen fractions isolated by isoelectric focusing in acrylamide gels were pooled, labeled with 14C, and analyzed by SDS-PAG electrophoresis (Fig. 3). Compared to the original G-100 antigen fraction (Gel 2), the antigen preparation purified by isoelectric focusing (Gel 3) has considerably fewer protein bands and retains the protein with a molecular weight of 25,000. Judging from the appearance of the band, the integrity of the protein was not affected by the 14C labeling procedure. In addition, immunodiffusion analysis showed that the 14C-labeled protein retained its specific antigenic activity.

![Chart 1. Sephadex G-100 chromatography of an extract of BLV-infected BC cells from Cow AJ-147. The extract (70 mg protein) was applied to the column and individual fractions were checked for BLV antigen (BLVA) by immunodiffusion with reference BLV serum. The total amount of protein in the antigen-containing fractions (71 through 75) was 1.1 mg. The reference proteins used to calibrate the column were bovine serum albumin (B, M.W. 68,000), ovalbumin (O, M.W. 43,000), and RNase (R, M.W. 13,700). The void volume (Vo) was measured with blue dextran 2000.](image-url)
For further purification, BLV antigen isolated by isoelectric focusing and labeled with \(^{14}\text{C}\) was chromatographed on G-75 Sephadex columns. The fractions corresponding to the radioactive peak having antigen activity in immunodiffusion testing were then analyzed by SDS-PAG electrophoresis. The gel fractions were digested and analyzed in the scintillation counter. As shown in Chart 2, at least 75% of the radioactivity of the sample was present in a major sharp peak that had the molecular weight of the BLV antigen.

**Detection of the Antigen in Purified BLV.** Virus obtained from the supernatant fluids of \(^{3}\text{H}\)uridine-treated BLV-bat cultures was concentrated and purified by centrifugation through a discontinuous gradient followed by centrifugation in a linear sucrose gradient. As shown in Chart 3, a distinct peak of radioactivity was obtained at the buoyant density of 1.15 g/ml. No radioactivity was observed elsewhere in the gradient. Electron microscopic examination of a pellet obtained from pooled fractions, corresponding to the density of 1.13 to 1.16 g/ml, showed large numbers of typical C-type virus particles relatively free of contaminant materials (Fig. 4). Virus particles were not seen in the other fractions of the gradient. Radioactive peaks at buoyant densities of 1.12 to 1.18 g/ml were not seen in sucrose gradients of BLV pellets obtained from BLV-bat\(_2\) cultures incubated with \(^{3}\text{H}\)thymidine or in pellets obtained from supernatant fluids of uninfected bat TbiLu cultures treated with \(^{3}\text{H}\)uridine.

Virus recovered from the 1.15-g/ml density fraction of the gradient was ether treated and tested by immunodiffusion against reference BLV serum. As shown in Fig. 5, this preparation (Well 1) formed a single line of precipitation showing reaction of complete identity with the antigen isolated from the homogenates of BLV-infected BC cell (Well 5) and with the reference BLV antigen obtained from cell line NBC-13 (Well 4).

**DISCUSSION**

This study shows that an internal BLV antigen can be partially purified from extracts of infected cells by Sephadex chromatography, followed by isoelectric focusing. Further purification of the antigen was obtained by chromatography in Sephadex G-75. The antigen has a molecular weight of about 25,000 daltons, as determined by SDS-PAG electrophoresis analysis, and a pl of 6.4 to 6.6, as estimated by isoelectric focusing in acrylamide gel. Similar values for the molecular weight and pl have been reported for the major internal antigens of other C-type viruses (1, 12, 14, 25) and MPMV (34).

As determined by immunodiffusion experiments, the BLV antigen isolated in this study is the same as the antigen detected previously in ether-treated preparations of unpurified BLV and in acetone-fixed, infected cells (3, 5–7). It could be argued that the antigen is a virus-coded cellular antigen rather than a virion component. However, for this it would be necessary to postulate that BLV codes for a type of antigen that has not been detected in other C-type virus systems. Indeed, the only known cellular antigen coded for by C-type virus genomes are located at the cell membrane and are sensitive to ether treatment and acetone fixation.

On the other hand, the only known C-type virus antigen with a molecular weight of 25,000 to 30,000 daltons that is resistant to ether and acetone is the major internal virion antigen.
tory (3), we found that the BLV antigen did not react in immunodiffusion tests with antisera having high titer of antibodies against interspecies- and intraspecies-specific antigens of MuLV and FeLV. In addition, immunodiffusion analyses failed to demonstrate antigenic determinants in common between the BLV antigen and the antigens of BSV and MPMV. Samples of BLV antigen and/or purified virus were sent to Dr. C. Sherr (Viral Leukemia and Lymphoma Branch, National Cancer Institute, NIH, Bethesda, Md.), Dr. S. Aaronson (Viral Carcinogenesis Branch, National Cancer Institute, NIH, Bethesda, Md.), and Dr. W. Parks (Viral Carcinogenesis Branch and Viral Leukemia and Lymphoma Branch, National Cancer Institute, NIH, Bethesda, Md.). Applying highly sensitive competitive radioimmune assays, these investigators failed to find any cross-reactivities between BLV and MuLV, FeLV, RD-114, Woolly monkey virus, MPMV, or the endogenous murine, porcine, and baboon C-type viruses. It has been established that both interspecies- and interspecies-specific antigenic determinants are present in the major internal protein of other C-type viruses (13, 28). Thus, it is conceivable that the internal BLV antigen may have antigenic specificities other than that detected by the reference bovine serum. Recently, a C-type virus has been isolated from sheep and found to share with BLV the ether-resistant antigen detected by serum from BLV-infected cows (29). This suggests that the antigen represents an interspecies-specific determinant characteristic of a family of C-type viruses which differ from that to which most of the other known mammalian C-type viruses belong. In an attempt to detect other antigenic specificities in the BLV protein, antisera against this protein are being prepared in heterologous species. The information that a BLV antigen can be obtained in relatively pure form from extracts of infected cells may be of potential value with regard to other systems where C-type viruses may be incompletely expressed or strongly cell-associated.

ACKNOWLEDGMENTS

We thank Dr. Robert Marshak for his help. The skilled technical assistance of Vicki Baliga and Ann Brundige is appreciated. We also thank Barbara Blevis and Betty Thompson for help in the preparation of the manuscript.

REFERENCES

Characterization of the Internal BLV Antigen


Fig. 1. Immunelectrophoresis of a cell extract (200 μg protein) prepared from BLV-infected cultures of BC cells from Cow AJ-147. The trough was filled with reference BLV serum 27-125.

Fig. 2. SDS-PAGE electrophoresis of the G-100 Sephadex fraction of cell extracts prepared from infected and noninfected cultures of BC cells. Gel 1 contains the reference proteins ovalbumin (O, M.W. 43,000), chymotrypsinogen (C, M.W. 25,700), and RNase (R, M.W. 13,700). Gel 2 contains 12 μg of the G-100 fraction (Tubes 70 through 76) from extracts of uninfected BC cultures. Gel 3 contains 19 μg of a G-100 antigen fraction from an extract of infected BC cell culture of AJ-147. Gel 4 contains 14 μg of a G-100 antigen fraction from extracts of infected BC cells of BF-227. In parallel gels, the BLV antigen was detected only in the area marked a on Gels 3 and 4. Gel 5 shows the results obtained after rechromatographing the BLV antigen fraction isolated by SDS-PAGE electrophoresis from 65 μg of G-100 antigen extract.

Fig. 3. SDS-PAGE electrophoresis of BLV antigen partially purified by isoelectric focusing and labeled with [14C]formaldehyde. Gel 1 contains the reference proteins bovine serum albumin (B, M.W. 66,000), chymotrypsinogen (C, M.W. 25,700), and RNase (R, M.W. 13,700). Gel 2 shows the chromatographic profile of the G-100 antigen fraction used. Gel 3 shows the chromatographic profile of the BLV antigen isolated from the same G-100 fraction by isoelectric focusing. Gels were stained with Coomassie Brilliant Blue R.

Fig. 4. Electron micrograph of sucrose gradient purified BLV. × 84,000.

Fig. 5. Immunodiffusion analysis of BLV antigen preparations. Well 1 contained ether-disrupted BLV purified by density gradient centrifugation. Wells 2 and 3 were empty. Well 4 contained the standard reference BLV antigen obtained from cell line NBC-13. Well 5 contains antigen isolated by isoelectric focusing from an extract of BLV-infected BC cells. The center well contained reference BLV serum 27-125.
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