Production of Monoclonal Antibodies to Bovine Leukocyte Cell-Surface Components


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ABSTRACT

Fusion of murine myeloma cells with syngeneic spleen lymphocytes has led to development of hybridomas secreting antibodies. Hybrid cells retain the immortality and clonability of myeloma parents as well as the antibody-producing property of lymphocytes. Specificity of monoclonal antibody produced is based on the one lymphocyte-one antibody phenomenon and represents the most effective process for producing specific antisera. Spleen cells from mice immunized with desired antigen are hybridized with nonsecreting mouse myeloma cells. Resulting hybrids are cloned and culture fluids tested for specific antibody activity to the antigen. Positive clones are cultivated in vitro and injected into mice for monoclonal antibody production. This technology has been extended to the bovine species to obtain monoclonal antisera to immunoglobulins and cell-surface components of leukocytes for study of mammary gland immunity. Recent progress in monoclonal research has led to interspecific fusion of murine myelomas with bovine lymphocytes, resulting in hybridomas that produce monoclonal bovine immunoglobulins. Monoclonal antibodies will be useful in investigations applicable to bovine research including purification of immunoglobulins, determining immunoglobulin concentration in colostrum and milk, reference reagents for bovine serology, antibody localization in tissue, gene sequencing, characterizing histocompatibility antigens, distinguishing and quantitating cell types in blood, milk, and udder tissue, and elucidating role of cell subpopulations in the immune response.

INTRODUCTION

Mastitis is a major problem for the dairy industry. The disease causes financial losses by decreased milk yield and quality, increased culling, and higher veterinary costs. A primary objective of immunological research on mastitis has been study of natural defense mechanisms of the mammary gland and development of effective vaccines. These studies have focused on polymorphonuclear leukocytes (neutrophils) and their interactions with both cellular and humoral aspects of immunity. Progress to date has been limited because of inadequate understanding of the bovine immune system. With better knowledge of udder immunology, artificial manipulation of natural defense mechanisms may provide new management tools and practices that could reduce incidence of intramammary infection.

Knowledge of cellular immune responses of the bovine mammary gland is fundamental to understanding pathology of mastitis and mammary immunity. Basic information on the role of leukocytes (neutrophils and cells of lymphocytic and monocytic series) that infiltrate the udder could provide clues to successful development of a vaccine that could stimulate infiltration and phagocytic efficiencies of neutrophils, enhance cell-mediated immune responses, and induce local synthesis of antibody. An effective means for studying activity...
Figure 1. Certain B-lymphocytes (cells 2, 4, 6) in lymphoid tissue respond to antigen-X by synthesizing immunoglobulins (Ig) 2, 4, 6 specific for antigen-X. Each cell line produces one single species of Ig (monoclonal) whose light and heavy chains comprise a single set of variable region sequences. Although Ig's 2, 4, and 6 are directed against antigen-X, the chances are they are specific for different antigenic sites of the 100's or 1000's of sites available; therefore, serum would contain polyclonal antibodies or a mixture of Igs to antigen-X provided by cell lines 2, 4, and 6.

of various leukocyte types that play a part in mammary gland immunity would involve methods to: 1) identify specific cell-surface components involved in antigen recognition, phagocytosis, cell activation, and other functional properties of subpopulations; and 2) determine timely location of specific cell types and subpopulations in tissues of the mammary gland relative to infection status. Such studies require use of labelled, specific antibody.

Until recently, only antisera prepared by conventional methods were available for biological analyses, which often lacked specificity and required elaborate methodologies for purification. Even after extensive absorption, such antisera often crossreact with closely related antigens. Recent development of specific antibody-producing hybrid cell lines (hybridomas) has made it possible to obtain highly specific, pure antiserum. Specific nature of monoclonal antiserum, as opposed to polyclonal antiserum, is based on the one lymphocyte-one antibody phenomenon and represents the most effective means of producing specific reagents (Figure 1).

This paper presents the development and production of monoclonal antibodies, the use of this technology in the bovine system, and the application of monoclonal antibodies to mastitis research.

DEVELOPMENT OF ANTIBODY-PRODUCING HYBRIDOMAS

In the early 1970's, Milstein and coworkers (8) studied somatic cell mutations, gene modification, and combining specificity of antibodies. These scientists produced structural mutants of a mouse myeloma protein secreted by a cultured cell line; however, detection of mutant cells was difficult and laborious because proteins synthesized by parent cells often lacked recognizable antibody activity. They hypothesized that changes of antibody binding activity would
have been the best indicator of minute differences resulting from mutation, but no myeloma cell lines existed that secreted an antibody that could be assayed easily. Concurrent with this work, Cotton and Milstein (7), while studying genetic control of antibody synthesis, fused two myeloma cells to determine whether allelic exclusion could be modified. In addition to demonstrating molecular consequences of gene splicing, their work more importantly showed that there was no allelic exclusion by hybrids; information from both parent cells was codominantly expressed, and hybrids synthesized all immunoglobulin (Ig) chains produced by both parents. This finding suggested a possible source for an antibody-producing cell required in the mutation experiment. Because it had not been possible to generate myelomas producing antibody against specified antigens nor continuous lymphoid cell lines producing specific antibody, these workers considered the possibility of fusing a normal lymphoid cell with a myeloma cell. Theoretically, expression of the plasma cell’s specific antibody-producing property would be immortalized by reproducing ability of the hybrid. Through their attempts to apply this theory, Kohler and Milstein (16) successfully fused spleen cells (lymphocytes) with myeloma cells and produced the first monoclonal antibodies of lymphocyte origin. The general procedure followed is outlined in Figure 2. Resulting hybridomas maintained the antibody-producing capability of spleen cells and the immortal character of myeloma cells. Such hybrids also secreted Ig’s produced by parent myelomas; however, a nonsecreting variant subsequently was produced (34) that could be fused with spleen cells and yield a hybrid expressing only antibody synthesized by the lymphocyte parent. Hence, a hybridoma could be cultivated in which the antibody-producing ability of the spleen lymphocyte was immortalized.

**PRODUCTION OF INTRASPECIFIC ANTIBODY-PRODUCING HYBRIDOMAS**

**Immunization of Spleen Cell (Lymphocyte) Donors**

Balb/cj mice are primed (i.p.) at 8 to 12 wk of age with antigen. Whole cells used as the immunizing agent are concentrated to 1 to 2 x 10^7, emulsified, and injected in a total volume of .2 ml. Soluble protein antigens are precipitated, and 100 to 200 μg is emulsified in Freund’s complete adjuvant (FCA) and injected as described. Booster injections are given approximately 4 wk later without FCA. Multiple boostings are optional.

**Hybridization and Cloning of Hybridomas**

Four days after the final boost is administered, spleen cells are harvested aseptically by grinding spleen tissue between frosted glass slides. Approximately 5 to 10 x 10^7 cells/spleen are recovered and washed in growth medium (RDGS-50:50 mixture of RPMI-1640 and Dulbecco’s Modified Eagle’s Medium, 10% fetal calf serum, 2mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin).
Figure 3. Production of hybrid cells. Myeloma cells are fused with spleen B-cells with PEG. Resulting heterokaryons, each containing two or more nuclei, undergo one division forming daughter cells (hybridomas) that secrete monoclonal antibody.

Concentration of spleen cells is adjusted to 1 to $4 \times 10^8$ and mixed with myeloma cells in a range from 1:1 to 1:10 (myeloma:spleen cells). The established Sp 2/0 myeloma cell line (34) is one of several current lines of choice. The Sp 2/0, of Balb origin, is a nonsecreting, 8-aza-guanine-resistant hybridoma variant derived from the myeloma line P3-X63-Ag8. Spleen cells and myeloma cells currently are fused with polyethylene glycol (PEG). A small percentage of cells will fuse successfully, however, depending on the immunizing agent. For example, frequency of fusion of spleen cells of mice immunized against sheep red blood cells is approximately 25 antibody-producing hybrids/spleen, whereas fusion of spleen cells from mice immunized against H-2b haylotype is about .25/spleen (47). Resulting hybrid-myelomas or hybridomas express both the immortal characteristic of myeloma cells and the specific antibody-producing property of spleen cell lymphocytes (Figure 3). After fusion, cells are diluted in growth medium containing 100 mM hypoxanthine, $2 \times 10^{-3}$ M aminopterin, and 30 mM thymidine (HAT-medium) to select for hybridomas and eliminate spleen and myeloma cells that did not fuse (21). See Figure 4. Aliquots of cell suspension (.1 ml) are distributed to wells of a 96-well microtiter plate with a cloning apparatus (11) and incubated at 37°C in a 10% CO$_2$ atmosphere. Optimal cell concentration in each aliquot is one hybrid cell/well; however, cloning efficiencies are such that it is more desirable to distribute culture dilutions containing different numbers of cells to wells (limit dilution) to assure one hybrid per well. Clones become visible 5 to 15 days after fusion. An alternate cloning method involves use of semi-soft agar (15). Instead of using 96-well microtiter plates, hybrids are suspended in soft agar and layered over a firm layer of agar in culture medium in a petri dish. Individual colonies then are transferred to liquid culture and supernatants tested for antibody activity as described below.

Screening for Antibody-Producing Hybrids

When clone diameter approaches 2 to 3 mm, culture fluid is tested in a cell binding assay for antibody directed against the cell type used to immunize donor mice. Screening assays commonly used are indirect cell-binding, radio-immunoassay (RIA; Figure 5), enzyme-linked immunosorbant assay, or fluorescence-activated

Figure 4. Hybridomas are selected using the hypoxanthine-aminopterin-thymidine (HAT) selection system. a) Major biosynthetic pathway for purines and pyrimidines in cells is blocked by folic acid antagonist aminopterin. b) Cells can still synthesize DNA via salvage pathways utilizing preformed nucleotides, providing enzymes thymidine kinase (TK) and hypoxanthine guanine phosphoribosyl transferase (HGPRT) are present; if one or the other enzyme is absent, DNA synthesis ceases. Spleen cells (TK+, HGPRT+) die in culture but when fused with myelomas (lacking TK or HGPRT) only hybrid cells will grow in HAT medium.

CELL BINDING RADIOIMMUNOASSAY

Figure 5. Procedure for indirect radioimmunoassay of monoclonal antibody in culture supernatants of hybrid clones.

cell sorter. For antibodies directed against soluble antigens, polyvinyl plates are coated with soluble protein antigen by addition of 20 to 50 µl of antigen (50 to 500 µg/ml) to wells, incubated at room temperature for 1 h, and washed (3x) with PBS containing .1 to 1% bovine serum albumin and .1% NaN₃ to remove unbound antigen. Supernatants of hybrid clones are tested for presence of Ig directed against antigen by 20 to 100 µl supernatant added to wells. After 1 h, wells are washed as described, and radioiodinated anti-Ig is added, followed by incubation and washing as described. Individual wells then are counted in a gamma counter. Wells with more than 2 to 3x control (culture medium) are considered positive. Positive clones are recloned several times and tested for antibody production to insure stability of hybrids. During initial clonings, a portion of hybrid cells may undergo either chromosome rearrangement or loss and lose genes necessary for antibody production. Overgrowth by contaminating clones or variants that arise during cloning also attribute to instability.

Freezing of Positive Clones and Production of Antibody-Producing Tumors

Clones producing desired antibody (up to 10 to 100 µg/ml) can be maintained in culture for long periods; however, they require intensive observation and feeding. Hybrids proliferate so rapidly that they must be subcultured frequently, and occasionally nonproducing variants arise and predominant over antibody-producing clones. Long culture also increases chance of contamination by antibiotic-resistant microorganisms. Therefore, to insure the future of clones, it is necessary either to freeze clones or to inject them into syngeneic mice for tumor
production. For frozen storage, clones are
grown in culture to 6 to 8 x 10^7/ml, con-
centrated by centrifugation, and resuspended in
freezing medium containing phosphate-buffered
saline (70%), fetal calf serum (20%), and
dimethylsulfoxide (10%) and placed in a −80°C
freezer or liquid nitrogen. For tumor production,
approximately 1 x 10^6 cells are injected
subcutaneously into 3- to 4-mo-old mice. When
tumors become visible (approximately 10 days)
sera are tested for antibody by a cell binding
assay. Mice with positive tumors are bled to
obtain monoclonal antiserum, but they soon
succumb to the tumor. Therefore, before
reaching the critical stage, they are sacrificed
and tumor cells grown in culture for antibody
production or frozen for later use or transferred
to other mice. Ability of frozen tumor cells to
produce a solid antibody-producing tumor or
ascites after thawing and injection into mice
can be used as a criterion to determine suc-
cessful passage and stability of the monoclonal.
Sera from tumor-bearing mice contain from 3
to 15 mg/ml monoclonal antibody.

USE OF MONOCLONAL ANTIBODY
TECHNIQUES IN BOVINE RESEARCH

Basic procedure developed by Kohler and
Milstein (16, 17) for producing monoclonal
antibodies has been adopted in a variety of
disciplines as a research tool in genetics, de-
velopmental biology, virology, and especially
immunology. Monoclonal antibodies are used in
characterization of cell-surface components
during growth and development and the manner
in which surfaces change during differentiation,
identification of viruses and detection of exact
differences between variants, distinguishing
compatibility in donor/host grafts, immuno-
therapy, radioimmunoassay of hormones and
drugs, purification of molecules by affinity
chromatography, and identifying new antigens
that distinguish subpopulations of cells.

More recently, production of monoclonals
has been extended to the bovine for use in
studying milk secretion and mammary gland
immunology. Monoclonals may be useful in
determining distribution and function of
proteins involved in Ig binding and the synthesis
and secretion of milk components. For example,
monoclonal antibodies to antigens of bovine
milk-fat-globule membrane (22), a component
originating from the plasma membrane of milk
secretory cells, will be useful for distinguishing
this cell type from others in milk, identifying
membranes in cell fractionation studies, identi-
fication of specific Ig receptors on the surface
of mammary secretory cells, and study of Ig
transport. Surface proteins may also be im-
portant in cellular aspects of mammary gland
immunology. For instance, it has been con-
tended that homing of lymphocytes to the
udder involves recognition of histocompatibility
antigens on mammary epithelial cells (41).

There is a great demand for highly specific
antisera to various classes and subclasses of
bovine Ig's (10). Use of monoclonal antibody
will provide information on antibody production
and transport and aid our understanding of the
bovine immune system in mastitis. Srikumaran
(37) and Srikumaran et al. (39) reported
production and characterization of monoclonal
antibodies to bovine Ig's. By cultivating murine
hybridoma cell lines secreting antibovine Ig's,
these workers were able to isolate hybridomas
that secreted antibodies specific to IgG2
(Figure 6a) and IgM (Figure 6b). Mass pro-
duction of these antibodies will be used in: 1)
affinity chromatography for preparation of
highly purified bovine IgG2 and IgM from
whole serum; 2) estimating bovine Ig con-
centrations in serum, colostrum, and milk; 3)
determining components involved in binding
and uptake of bovine Ig by mammary secretory
epithelium and complement binding; and 4)
preparation of ferritin- or peroxidase-labelled Ig
for localization in udder tissue. Work is in
progress by these same scientists to obtain
monoclonals to IgA and other subclasses of
IgG. In addition to studying humoral aspects of
immunity in the bovine by monoclonal anti-
bodies, a study of cellular factors involved is
needed to gain better insight to mechanisms of
immune response of mammary gland.

MONOCLONAL ANTIBODIES TO
BOVINE LEUKOCYTE
CELL-SURFACE COMPONENTS

Monoclonal antibodies have been employed
successfully as probes to identify specific
cell-surface antigens for characterization of
leukocyte populations and subpopulations in
humans (1, 2, 9, 12, 18, 24, 30, 32, 43) and
laboratory animals (3, 19, 36, 40, 42, 44). Only
recently has the monoclonal technique been
Figure 6. Radioimmunoassay of affinity-purified monoclonal antibody specific for a) IgG₂ and b) IgM.

Figure 7. Initial screening of single clones for antibovine neutrophil activity. RDGS-growth medium as described in text.

extended to the bovine species for studying cell-surface components on leukocytes.

Monoclonals to Polymorphonuclear Leukocytes (Neutrophils)

Neutrophils are an essential protective mechanism against infection and represent the second line of defense against invading mastitis pathogens. These leukocytes attempt to eliminate bacteria via phagocytosis followed by intracellular kill. Although the mammary gland can mobilize large numbers of neutrophils from circulating and storage pools, phagocytic ability and efficiency of intracellular kill are reduced in milk. Various aspects of neutrophil structure and function are involved in these processes, i.e., neutrophil surface receptors that bind Ig and complement that facilitate recognition of opsonized bacteria. Because such receptor activity is reduced in milk, we sought to produce monoclonal antibodies to neutrophil cell-surface components by the technology pioneered by Kohler and Milstein (16, 17). It was our objective to obtain monoclonals that would be useful to study the nature and number of antibody binding sites that may be instrumental in distinguishing surface differences between blood and milk cells and in identification of neutrophil subpopulations important to mastitis resistance.

Spleen cells from mice immunized with bovine peripheral blood leukocytes (PBL) were hybridized with Sp 2/0 myeloma cells. Subsequent cloning resulted in isolation of 261 hybrid clones (Figure 7). Of these, 82 clones secreted antibody specific to neutrophils but not to bovine erythrocytes, lymphocytes, gamma globulin, or whole serum. These 82 were recloned. Twenty-eight of the 82 clones produced anti-neutrophil Ig and were recloned, cultured in 2 ml media in wells of 24-well
plates, and supernatants screened for binding activity to neutrophils. Of the 28 clones retained, 16 maintained production of neutrophil-specific antibody after subsequent cloning and growth in culture (Figure 8). Hybridomas cultured in roller bottles en masse, frozen, and subsequently recultured, continued to synthesize and secrete neutrophil-specific antibody. After injection into mice, 15 hybrids resulted in solid tumors and one resulted in ascites. Neutrophil-specific antibody was detected in tumor-bearing mice at dilutions of 1:256,000 (Figure 9). Tumors were excised and frozen, and upon subsequent passages through mice, all continued to produce antineutrophil antibody.

Indirect immunofluorescence was used to detect differential binding of monoclonal antibodies from various clones to identify subpopulations of neutrophils. Bovine blood neutrophils were collected, and a 50 μl suspension containing 6 × 10^6 cells/ml mixed with a dilution 1:40 of serum-derived monoclonal antibody for 96-well microtiter plates; normal Balb/cJ serum served as control. Mixture was
incubated on ice for 20 min followed by washing (2x) in PBS-bovine serum albumin-azide. Cells were resuspended in 100 μl fluorescein-labelled sheep antimouse Ig (protein concentration 600 μg/ml), incubated on ice for 20 min, and washed (3x) as described. Wet mounts were examined by a Leitz microscope equipped with a Ploemopak vertical illuminator with an HBO 200 W mercury lamp. Cells were examined for fluorescent surface-staining at a magnification of 1000x. Preliminary testing of monoclonal antibodies from two clones indicated differential binding to two subpopulations of neutrophils. Monoclonal antibody 5C4 bound to approximately 50% of peripheral blood neutrophils whereas antibody 3G2 bound to less than 10% of blood neutrophils. Work is in progress to determine if the 14 remaining monoclonal antibodies identify other subpopulations of neutrophils and to determine their respective properties. Each monoclonal antibody will be tested in functional assays to determine the functional nature of cell-surface component to which it is directed. For example, monoclonals will be included in a neutrophil phagocytosis assay to determine whether antibody binds to receptors for opsonized bacteria in an effort to quantitate such cell-surface proteins on blood and milk neutrophils.

Application of Monoclonal Antibody for Study of Bovine Neutrophils

As stated previously, the next step in characterization of the 16 neutrophil-specific antibodies is to elucidate nature of surface antigens to which they are directed and to identify existence of neutrophil subpopulations and their functional properties. Applications of neutrophil-specific monoclonals in the determination of functional properties of subpopulations may include: 1) determining surface receptors for casein and fat, which competitively interfere with bacteria for recognition by neutrophils; 2) inducing neutropenia to study effects of blood neutrophil infiltration on milk secretion and pathology of mastitis by excluding effects of neutrophils without exclusion of other leukocytes (lymphocytes, monocytes) that infiltrate the udder; 3) determining variation in subpopulations of neutrophils under various conditions, i.e., among cows (25, 28), stage of lactation (14), and stress (13, 26) to phagocytose bacteria; 4) determining mechanisms involved in diapedesis and migration of neutrophils into milk and their effect on neutrophil surface structure and function; and 5) distinguishing differences between surface components of milk and blood neutrophils as affected by milk components.

MONOCLONAL ANTIBODIES TO OTHER LEUKOCYTE TYPES

Induction and regulation of cell-mediated immune (CMI) responses involve complex interactions between T- and B-lymphocytes and macrophages that bear surface markers and receptors. The T-lymphocytes, in addition to exerting their own effect on microorganisms, develop into memory and effector cells upon contact with antigen. The later influence function of other leukocytes by releasing lymphokines, which regulate the CMI response. The B-lymphocytes have specific cell-surface antigens and Ig molecules that bind antigen leading to proliferation and development of memory cells and antibody-producing plasma cells. Helper T- and B-lymphocytes and macrophages also interact for optimal antibody production. Studies of mice have demonstrated that lymphoid cells committed to specific Ig synthesis arise from Peyers patches and home to the mammary gland where they are responsible for local production of antibody (31). Analysis of the distribution pattern of T- and B-lymphocytes in blood, mammary tissue, and milk of the bovine would facilitate greatly understanding immune responses to udder pathogens.

Population frequencies and properties of lymphocytes in bovine blood and milk have been studied (4, 5, 6, 20, 23, 35, 45), but the role of these cells in preventing infection and their activity during progress of inflammation remains unclear. Investigations indicate need for further characterization of T- and B-lymphocytes, as well as null cells, to obtain a clearer understanding of the local immune response in cattle.

Subpopulations of T- and B-lymphocytes in cows have been demonstrated by a number of cell-surface markers (antigens and receptors) identified by immunofluorescence and rosetting techniques. Such identification schemes require elaborate methodologies for separation and
purification of cell populations. Results have been variable and identification encumbered by lack of reliable techniques to enumerate and assess functions of subpopulations accurately.

Monoclonal Antibodies Directed Against Bovine Peripheral Blood Lymphocytes

Feasibility of classifying subpopulations of bovine lymphocytes by monoclonal antibodies has been demonstrated. Pinder et al. (29) studied the immune system of cattle with microbial infections. To evaluate changes of lymphocyte subpopulations, monospecific antisera were required as markers for cell-surface antigens. Using bovine peripheral blood lymphocytes (PBLy) they immunized mice, collected spleens, and performed three independent fusions of B-lymphocytes with myeloma cells. Supernatants from clones were tested in a cell-binding RIA for presence of anti-PBLy antibody, and positive hybrids that secreted antibody specific for bovine lymphocytes were cloned doubly. Monoclonal antibody of five hybrids bound to the majority of bovine lymphocytes; two of these antibodies were specific for polymorphic antigens. One antibody bound to B-lymphocytes and serum IgM. Antibodies of the five remaining hybrids bound to subpopulations of lymphocytes. Four monoclonal antibodies bound only to bovine lymphocytes, six bound to lymphocytes from other bovidae, but none bound to human cells. After further characterization, these monoclonals will provide useful means of studying the immune system of cattle and evaluating changes of lymphocyte subpopulations during the course of disease.

More recently, hybrid cell lines secreting monoclonal antibodies to bovine lymphocyte surface antigens were cultivated (33). Four hybridomas secreting antibody to the cell-surface protein beta-2 microglobulin (β2M) were stabilized and passaged through mice providing high titer serum antibody (1/192,000). Monoclonal antiovine β2M antibody will be useful in purification of histocompatibility antigens as well as bovine β2M and will provide means for genetic analyses and characterization of major histocompatibility antigens important to bovine research.

RECENT PROGRESS IN MONOCLONAL RESEARCH

Monoclonal Antibody Production by Interspecific Hybrids

Most antibody-secreting hybridomas have been produced by fusion of murine myeloma cells with murine B-lymphocytes, a process referred to as intraspecific fusion. However, interspecific fusion of murine myeloma cells with nonmurine B-lymphocytes also has been employed successfully to produce hybridomas (27, 46). The successful interspecific fusion of murine myelomas with normal bovine spleen cells resulting in viable, antibody-producing hybrids was reported by Srikumaran et al. (38). Bovine lymphocytes were isolated from the spleen of a mature Holstein cow, fused with myeloma cells, and distributed to wells of microtiter dishes. Supernatants of single hybrids were assayed for production of bovine IgG1, IgG2, and IgM by competitive RIA. Initial screening of 63 single hybrid clones indicated that 21 clones produced bovine Ig, and hybridomas were stabilized after subcloning three times. Three clones (LHRB1, LHRB2, LHRB3) were selected for antibody characterization and were able to secrete 5 to 10 μg Ig/ml per 5 × 10^5 cells per 24 h over 6 mo. After adaptation for growth as antibody-producing tumors or long-term culture, the monoclonal Ig's were isolated from serum or culture fluid and purified by affinity chromatography. Monoclonal antibodies synthesized by LHRB1 and LHRB3 were composed of entire molecules of IgG1 and IgM, respectively, whereas Ig produced by LHRB2 was characterized as IgG2 heavy chains only. These monoclonals will provide 1) monoclonal Ig for sequencing studies, 2) reference reagents for bovine Ig serology, 3) antigens for antisera production, and 4) mRNA for cloning bovine Ig genes. Further production and characterization of hybridomas of bovine × murine will facilitate investigations of bovine immune system with monoclonal antibodies.

Although production of monoclonal antibodies important to the study of mammary gland immunity is still in its infant stage, progress has been made in development of monoclonals to various bovine Ig molecules and to cell-surface components that distinguish leukocyte types and their subpopulations. Recent successful cultivation of interspecific
hybrids producing bovine Ig's has exposed novel areas of investigation into the bovine immune system and will be particularly useful for sequence studies and in preparation and testing of class-specific antisera.

REFERENCES


