CHARACTERIZATION OF THREE MONOCLONAL ANTIBODIES DIRECTED AGAINST A BOVINE CEREBRAL CORTEX CELL SURFACE SIALOGLYCOPEPTIDE

by

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INTRODUCTION

Since the development of the first specific antibody producing hybrid cells in 1975, monoclonal antibodies have become sophisticated and very practical research tools that are presently being employed by a large number of biological research laboratories. This report attempts to describe the advancements that have been made in monoclonal antibody technology in the past decade and to discuss one of the most recent utilizations of monoclonal antibodies, cancer therapy.

Predominantly, however, monoclonal antibodies are used as a method to purify and characterize specific biological proteins. Our laboratory has isolated, from bovine cerebral cortex cells, an 18 kD sialoglycopeptide growth regulator which inhibits cellular protein and DNA synthesis. We have characterized three monoclonal antibodies directed against this growth inhibitor which will eventually enable the rapid purification of the sialoglycopeptide or its parental molecule and allow for the detection of the protein on a variety of cell surfaces. Additionally, these monoclonal antibodies were useful in demonstrating that the sialoglycopeptide is unique from other presently studied growth regulators such as β-transforming growth factor and fibroblast growth regulator and can be depicted as a novel cell growth inhibitor.

DEVELOPMENTS IN MONOCLONAL ANTIBODY TECHNOLOGY

Antibodies are a very important part of the immune system as they have the ability to specifically locate and bind to any ligand which is not recognized as "self." It has been known for decades that introducing a foreign substance or antigen into the body would elicit an antibody response. Antibodies resulting from such responses were found to be present in the sera extracted from animals "immunized" with a particular antigen. These antibodies became useful tools in the laboratory for antigen detection and localization. However, it was evident that sera from immunized animals contained many antibodies directed against other antigen; indeed, antibodies against the desired antigen were of various classes and specificities. This launched the search for methods to obtain substantial quantities of one very specific antibody that would be directed against a single epitope of an antigen. In 1975, Kohler and Milstein (45) published a paper which established the groundwork for the future production of monoclonal antibodies. Their research was based on the observation that only one type of antibody was produced by cloned lymphocytes which had been stimulated by a particular antigen, and that fused cells from two myeloma cell lines resulted in hybrid cells that

synthesized the antibody of each lymphocyte parental cell (8,65). Utilizing Sendai virus, which promoted the fusion of cell membranes, they were able to produce hybridomas from immunized Balb/c spleen cells and a compatible Balb/c myeloma cell line. These hybrids survived in culture and secreted antibodies to sheep red blood cell antigen. Many laboratories soon began to capitalize on the innovative research of Kohler and Milstein.

The next few years saw an explosion of monoclonal antibody technology. For example, improvements were made in the procedures for cell fusion. Sendai virus was replaced by high molecular weight polyethylene glycol as a fusing agent (14,16). which allowed for a greater percentage of hybridoma survival and growth (73). The next improvement came with the development of a Balb/c myeloma cell line that did not secrete antibody prior to cell fusion. P3-X67Ag8 myeloma cells, used for the Kohler and Milstein experiments, produced IgG1(k) antibodies. Hybridomas with these cells and Balb/c lymphocytes secreted not only the desired fusion product, derived from both parental cells, but the P3-X67Ag8 myeloma protein as well. With random association of antibody producing genes from both parental cells, the desired specific antibody would make up only 1/16 of the total immunoglobulins produced by

the hybridomas (73). Therefore, the "non-secreting" Balb/c myeloma cell line Sp 2/0-Ag14 was developed (73) although fusion efficiency with this myeloma cell declined as compared to fusions with P3-X67Ag8. One year later (1979), subclones of P3-X67Ag8 cells were isolated that had lost immunoglobulin expression (40). Fusion efficiency again increased to its previously reported level and the resulting hybrid cells produced pure monoclonal antibodies. The new myeloma cell line was designated X63-Ag8.653 and is the most widely used myeloma line for present hybridization.

It would be impractical to mention every technological advance which has led to the present understanding of monoclonal antibody development. However, in 1980, Fazekas de St. Groth and Scheidegger (14) published a review article on the strategy and tactics of the production of monoclonal antibodies which was instrumental in establishing a means of increasing cell fusion efficiency and clonal stability. They examined the effect of macrophages as a feeder layer for the proliferating hybridomas. A striking increase in the number of culture wells with growing hybrids was observed when macrophages were introduced to the hybridoma cultures. Hybridomas are autotrophic and the addition of macrophages may have

conditioned the selective medium which sustained hybrid vigor and growth. Another benefit of macrophages was their ability to "clean-up" culture wells of bacteria and cellular debris. The optimization of the ratio of spleen cells to myeloma cells that were used during cell fusion was also considered. Most previous hybridizations were carried out using a ten fold excess of spleen cells to myeloma cells. This ratio favored the production of spleen/spleen hybrids which would not survive in culture. Adding a lower number of spleen cells than myeloma cells, however, increased the possibility of a desired fusion product (14). Fusion efficiency and hybridoma survivability and stability are still being extensively studied and improved.

Another change that occurred which remains a part of present techniques was the development of sophisticated assays to detect the presence of monoclonal antibodies in hybridoma culture media. Earliest methods utilized simple plaque assays for antibody detection, as the majority of monoclonal antibodies produced were directed toward large immunogens such as sheep red blood cells (45). However, antibodies produced against non-cellular antigens required the use of radioimmunoassays or enzyme linked immunoabsorbent assays for antibody detection, both of

which serve this purpose. Recently, though, there have been two major advances in monoclonal antibody technology; the hybridization of spleen cells which have been immunized in vitro thus eliminating the time involved in the initial pristine priming, injecting and screening of the mouse (29, 52) and the development of hybridomas using spleen cells from a Robertsonian mouse strain (81) which warrants a more detailed description.

Monoclonal antibody production thus far had dealt with improvements of the tissue culture involved in growing hybridomas. Unfortunately, the fact that hybridomas randomly discard important chromosomes during proliferation was a problem that always hampered hybrid stability (53). In 1983, Taggart and Samloff (76) focused directly on improving the genetics of monoclonal antibody production. Initial studies by Taggart and Samloff showed that the selective media used to sustain hybridoma growth was important for stability. Hypoxanthine, aminopterin, and thymidine (HAT) media, which is a standard hybrid media, preferentially selects for hybrids with the HPRT (hypoxanthine phosphoribosyl transferase) gene located on the X chromosome of spleen cells. The gene coding for the active heavy chain of the immunoglobulin, though, is located on chromosome 12. Antibody producing hybridomas

that discard the X chromosome, therefore, are eliminated by the HAT selection process (51). In a system such as monoclonal antibody production where the probabilities of obtaining cells secreting the desired monoclonal antibody are extremely variable, this event could be detrimental. Taggart and Samloff hybridoma technology involves adenine, aminopterin, and thymidine (AAT) selective culture media which destroys hybrids that do not contain the APRT (adenosine phosphoribosyl transferase) gene. This gene is located on chromosome 8. AAT as an alternative selective media becomes useful when the spleen cells used for cell fusion are from the RBF/Dn strain of mice. These mice carry the Robertsonian 8.12 translocation chromosome. In this system, the genetic information for the immunoglobulin active heavy chain has been translocated from chromosome 12 to chromosome 8 which now also carries the immunoglobulin gene, thus decreasing the possibility of non-producing hybridoma culture.

In the past decade, the development of monoclonal antibodies has been one of the most valuable assets to modern research. Such a versatile laboratory tool carries the promise of more novel and exciting research in the near future.

CANCER IMMUNOTHERAPY UTILIZING MONOCLONAL ANTIBODIES

The idea of therapeutically exploiting antibodies directed against tumor cell surfaces is not a new concept. It was proposed as early as 1906 (11). Because of antibody specificity and cytotoxicity, they provided an appealing approach to cell destruction in vivo. With the advent of hybridoma technology (45) unlimited quantities of pure, homogeneous monoclonal antibodies of defined specificity could be produced, thus heightening the potential for antibody therapy. In recent years immunotherapy has gained notoriety in the treatment of cancer and research has been conducted utilizing techniques involving monoclonal antibodies.

Presently, many articles have been published reporting the production of monoclonal antibodies directed against antigens associated with various types of cancer cells and cell lines such as melanomas (30,46), carcinomas (23,50), neuroblastomas (20,42), sarcomas (12,33), and leukemias (17,61). Therapeutically, these types of antibodies have been used in clinical trials, alone and in the presence of complement, as a means of tumor cell eradication. For example, serotherapy, with monoclonal antibodies directed against leukemia or T cell lymphoma antigens (anti-Leu-1) (54), has shown in many studies to clear neoplastic cells

from the blood of patients, at least temporarily (10,55, 59,62). Additionally, complete tumor remission has been observed lasting more than six months after therapy when a patient was given monoclonal antibodies directed against an idiotypic determinant of a B cell lymphoma (56). However, treatment involving the use of a single monoclonal antibody may not be significantly cytotoxic in some cases. Therefore, more than one antibody may be used in conjunction with others to treat a cancer. Effective target cell killing was achieved when antibodies to two epitopes of p97, a human melanoma-associated antigen, reacted synergistically against melanoma cells *in vitro*, whereas neither antibody alone was significantly cytotoxic (27). This synergistic effect also allowed for the use of antibody concentrations of only 2 ng/ml, lower than required dosages for single antibody treatments (27). Interestingly, the synergistic effect was only observed in the presence of a heterologous serum as a source of complement. This creates obvious immunological problems when administered *in vivo*.

Although monoclonal antibodies have been used alone for cancer immunotherapy, they can also be employed as carriers of anti-tumor agents. At least four types of anti-tumor agents can be conjugated to antibodies and these

are: radioisotopes, chemotherapeutic agents, immune response modifiers and toxins (28). First, antibody, Fab or F(ab') fragments, can be labeled with a radioisotope so as to deliver a toxic dose of radiation to the tumor (39). This may be with a beta emitter such as ^{131}I (47) or a gamma emitter like ^{125}I. Unfortunately, it has been discovered that the amounts of radiolabeled tumor associated monoclonal antibodies reaching their target tissues after i.v. administration are sometimes too small to be exploited therapeutically (13). However, these radiolabeled probes do provide an excellent means of identifying and imaging tumor tissue (9,25,47). Secondly, conjugates of monoclonal antibody and chemotherapeutic drugs such as chorambucil, daunomycin or adriamycin have been prepared (18,35) although studies with these conjugates, being utilized for treatment in vivo, have not been published.

A third conjugate which shows therapeutic potential is antibody and a molecule that induces a local delayed-hypersensitivity reaction within the tumor. This conjugate would cause an anti-tumor reaction analogous to that achieved by applications of dinitrochlorobenzene over a skin carcinoma (3) or when B cell growth factor (BCG) is injected into a melanoma nodule (57). Again, no published

information can be found regarding the actual use of this conjugate in therapy.

Of all the previously mentioned means of incorporating monoclonal antibodies as a tool in immunotherapy, the most researched and published method is the fourth conjugate, immunotoxins, which warrant a discussion of more depth. In this conjugate, monoclonal antibodies are linked by chemical reduction of the disulfide bonds of toxin A chain subunits, which result in a thiol group that can be linked to IgG or IgM antibodies by disulfide exchange reaction and average approximately one or two toxin molecules per immunoglobulin (15). Examples of toxins that are conjugated to antibodies are: plant hemitoxins such as pokeweed antiviral protein (36), plant toxins which include ricin (60) and bacterial toxins like diphtheria toxin (64). Ricin, however, appears to be the toxin of choice for most conjugates. In particular, one ricin immunoconjugate, WTI-RTA, directed against T-ALL tumor antigens, demonstrates a ten-fold increase in therapeutic index over other existing conjugates (58) where the therapeutic index is the concentration of immunotoxin required to kill 50% of tumor cells.

Immunotoxins are usually very potent and kill human tumor cells at ng/ml concentrations (1,67). In vitro

efficacies of immunotoxins have been established using standard test systems including protein synthesis inhibition and colony inhibition assays carried out on clonogenic cell lines (6,48). However, of particular interest, is the research performed dealing with immunotoxin efficacy *in vivo*. Weil-Hillman *et al.* (79) have recently demonstrated that 86% of ricin-immunotoxin treated nude mice appeared to have complete tumor regression. It is important, though, to state that treatment with this immunotoxin was accompanied by the cyclophosphamide congener, mafosfamid which aided killing efficiency and persistent tumor regression. Clinical applications of immunotoxins in human cancer patients have been primarily *ex vivo* studies depleting autologous bone marrow grafts of tumor cells in leukemia and lymphoma patients. Ramsey *et al.* (15) used two anti-T-cell antibody ricin conjugates on six leukemia/lymphoma patients who had been conditioned with cyclophosphamide and total body irradiation. Two of these patients were considered disease-free three and eleven months post-transplantation of treated bone marrow.

Studies much like those previously mentioned using immunotoxins are becoming more prevalent. However, like other forms of cancer treatment, the use of monoclonal

antibodies in immunotherapy has many limitations which must be mentioned. The major problem facing this therapy is the lack of a tumor specific cell surface antigen. Many antibodies are directed against tumor antigens but these may also be associated with non-neoplastic cells as well. A second shortcoming is that although mouse monoclonal antibodies alone may not be cytotoxic to humans, the chemotherapeutic drug or toxin conjugated to the antibody causes very serious side effects. Third, even though most cancer patients have had diseases or other treatments that could impair their immunocompetence, more than 50% make significant antibody responses against the foreign therapeutic antibody (49). Finally, other obstacles include antigenic modulation, free antigen blockade and immunoselection.

Although the actual use of therapeutic antibodies is still a very recent event, much research has been accomplished in this area. Eventually a tumor specific antigen may be discovered. Human-human hybridomas have already been produced. These two events, and the advent of more sophisticated technology, will further enhance immunotherapy.

THE BOVINE SIALOGLYCOPEPTIDE INHIBITOR

Most mammalian cells exist in two physiological states: quiescent and proliferative, which are regulated by various stimulators and inhibitors. Research in the past decade has led to the understanding of the structure and function of polypeptide growth factors that produce cellular proliferation (19,26,37,41). However, much less progress has been made in the study of cell growth inhibitors. The isolation of inhibitors from various cell surfaces has recently been reported (32,66,75,80) but purification to homogeneity and extensive characterization has not yet been demonstrated with many of these inhibitors. One such inhibitor which has been purified and partially characterized, though, is the bovine cerebral cortex cell surface sialoglycopeptide.

The bovine growth inhibitor which is presently being studied was removed from cerebral tissue by a mild pronase treatment followed by ethanol precipitation and chloroform/methanol extraction of the macromolecules (71). This crude preparation was then purified by DEAE ion exchange chromatography and wheat germ agglutinin affinity chromatography. Final purification was by size exclusion and hydroxylapatite HPLC (2). This extensive purification procedure yielded a homogeneous sialoglycopeptide with a

molecular weight of approximately 18 kD and a pI of 3.0 as determined by SDS-PAGE and isoelectric focusing analyses, respectively (72). Other growth inhibitors from chick embryo fibroblasts and bovine mammary gland have only been partially purified (5,82). However, many other inhibitors have been extracted from the conditioned medium of various primary and established cell lines (24,32,34,80) while yet another is considered to be a protein purified from murine serum (21,22).

Initial studies which eventually led to the isolation of the sialoglycopeptide from bovine brain cells, conducted by Kinders et al. (44), described the characterization of glycopeptides from mouse cerebral cortex that inhibited cell growth and protein synthesis. However, the method of isolation and the small amount of cortical tissue that could be removed from a mouse brain limited the supply of inhibitor to minute quantities (5 ug/200 cerebral cortices). Utilizing the bovine brain and a revision of the initial purification procedure, led to production of ug quantities of inhibitor from a single preparation. This material also had a specific activity 46 times greater than the earlier mouse-derived inhibitor (38,44,72).

The growth inhibitory activity of the sialoglycopeptide has been determined to be due to

inhibition of cellular protein and DNA synthesis of specific types of cells (43,72). This activity is not cytotoxic and is readily reversible, traits characteristic of growth inhibitors (78). Specific activity of the sialoglycopeptide, as measured by protein synthesis inhibition, has been reported as 100,000 units/mg protein where 1 unit (50 ng) is equivalent to 25% inhibition. This specific activity is relatively high as compared to, for example, the mouse embryo fibroblast inhibitor protein purified by Wells and Mallucci which requires 30-50 ug of inhibitor protein to produce equivalent inhibition (80). Although the sialoglycopeptide is an effective inhibitor of protein and DNA synthesis of normal cells, its inhibitory activity also blocks proliferation of a wide variety of transformed cells. Notably, all growth inhibitors presently studied are derived from cells that are density-dependent growth arrested. It is logical to assume that density-independent cells would not react to the inhibitory effect of the sialoglycopeptide. However, at least two other inhibitors appear to inhibit the growth of mammary carcinoma cells (4,31) and a neuroblastoma cell line (66).

The sialoglycopeptide inhibitor isolated from bovine cerebral cortex cells, is a potent inhibitor of cell

division of a wide variety of cells isolated from diverse species. Nanogram quantities arrest DNA synthesis of bovine, mouse, rat, human, and even insect species.

Associated with the sialoglycopeptide is a protease activity which cannot be physically separated during purification of the inhibitor to homogeneity and is not a direct result of the purification process (68). This protease activity is unique as it could not be inactivated by a battery of protease inhibitors. Gundersen et al. (21) have recently isolated an inhibitory factor from mouse sera that has protease activity. However, this protease could be deactivated by a serine protease inhibitor. Additionally, the protease activity was demonstrated to be necessary for growth inhibition from the mouse sera inhibitory factor. This is not the case for the sialoglycopeptide. A second unique property of the sialoglycopeptide is its ability to agglutinate transformed or nontransformed cells in solution (74). Although the agglutination reaction of the sialoglycopeptide appears to be discrete from its biological inhibitory activity, the protease activity, associated with the sialoglycopeptide, could be responsible for agglutination at the cell surface (74).

To date, published literature on growth inhibitors is

lacking information as to the mode of action of inhibitory molecules. Initial experiments to determine the interaction of the sialoglycopeptide with its target cells have shown, however, that specific receptors for the sialoglycopeptide exist on target cell surfaces and that there is a direct correlation between receptor occupancy and the inhibition of protein synthesis by the sialoglycopeptide (2). Additionally, Sharifi et al. (69) have demonstrated that this cell surface interaction is sufficient for the biological activity of the sialoglycopeptide. It can be concluded that the sialoglycopeptide does not have to be internalized to inhibit cell proliferation. This information suggests that inhibition may be carried out by a second messenger. It has been shown that the inhibitory activity of the sialoglycopeptide, when bound to its receptor, is antagonized by the calcium ionophore A23187 but not by the sodium ionophore Monensin (70). Therefore, the sialoglycopeptide may have an influence on calcium ion flux, intracellular calcium availability, or subsequent cellular metabolic events that lead to inhibition of protein and DNA synthesis (70). However, the exact nature of the sialoglycopeptide's mode of action and its relationship to calcium ions has yet to be established.

Many interesting aspects of the sialoglycopeptide and its biological activity are still awaiting further investigation. Primary experiments have been initiated to identify and characterize the sialoglycopeptide's target cell receptor. Gangliosides G_{M1}, G_{D1a}, and G_{M3} have been eliminated as possible receptors (co-receptors) for the sialoglycopeptide. Antiidiotypic monoclonal antibodies may eventually prove useful for receptor characterization. Additionally, monoclonal antibodies have already been utilized in studies aimed at comparing the sialoglycopeptide with other growth inhibitors to determine structural homologies. This is an important concept as recently it has been reported that β -transforming growth factor, a growth regulator with a bifunctional role of inhibition and stimulation (63), is closely related or identical to the BSC-1 growth inhibitor described by Holley et al. (32,77). Although the sialoglycopeptide is not structurally related to β -transforming growth factor or fibroblast growth regulator (75), further investigations with other inhibitors are warranted (see manuscript). A final interesting aspect of the sialoglycopeptide that needs further study is the cell cycle phase in which cell growth is arrested. Recent observations have indicated that cell growth arrest was occurring in the phase G1 (7,72).

Obviously, the sialoglycopeptide is a very intriguing molecule. Studies of the sialoglycopeptide and other growth inhibitors as previously discussed, however, will lead to a better understanding of negative regulation of cell growth.

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ABSTRACT

Three monoclonal antibodies, G4, G8 and F10, have been produced and are directed against an 18 kD bovine cerebral cortex cell surface sialoglycopeptide that inhibits cell proliferation and metabolism by blocking the synthesis of DNA and protein molecules. Characterization of these monoclonal antibodies has demonstrated that all three antibodies are of the IgG1 subclass and similarily, all of the antibodies bind to the biologically active polypeptide backbone of the sialoglycopeptide. However, the monoclonal antibodies did not react similarily when tested for their ability to neutralize the biological activity of the sialoglycopeptide. G4 could not block the protein synthesis inhibitory activity whereas G8 and F10 were effective. Upon autohydrolysis of the sialoglycopeptide's protein structure (4 fragments), G8 monoclonal antibody was shown to compete more efficiently for the largest molecular weight fragment. The monoclonal antibody, G8, was also utilized as a tool to compare the sialoglycopeptide with other growth inhibitors presently being studied. It was determined that the sialoglycopeptide contains a region in its amino acid sequence which appears to be unique from fibroblast growth regulator and β-transforming growth factor.

INTRODUCTION

It is now well established that regulation of cell growth involves a complex interaction between growth inhibitors and growth factors (11,21). Various growth factors have been isolated and the interactions of these growth factors with their respective target cells have been investigated in great detail (9,15). There is, however, little information available on the interaction of growth inhibitors. The primary reason for this lack of knowledge concerning growth inhibitors is the difficulty in the purification of these hydrophobic molecules. Our laboratory has purified to homogeniety and partially characterized a bovine cerebral cortex cell surface sialoglycopeptide that inhibits cell proliferation and metabolism (18,16,27,28). The growth inhibitor has been isolated by treating cerebral cortex cells with dilute protease. The released inhibitor was then purified based on charge, carbohydrate composition, size, and hydrophobicity utilizing HPLC techniques (26). The isolated sialoglycopeptide growth inhibitor has a molecular weight of 18 kD, a pI of 3, and when bound to its receptor on the surface of target cells, inhibits cell growth and metabolism in a wide variety of cell lines (2,25). Examination of the growth inhibitor's binding to target

cells suggested that cell surface interaction was sufficient to alter cell metabolism (25). Additionally, results of testing the interaction of the sialoglycopeptide with growth factors such as TPA and EGF, indicated that the sialoglycopeptide acted as an antagonist influencing early events in the signal transduction mechanism of TPA mitogenesis (1,5). Further kinetic studies of the antiprolific activity of the sialoglycopeptide revealed that the inhibitor may influence Ca^{+2} ion fluxes of target cells. This was demonstrated using the calcium ionophore A23187 which antagonized the biological activity of the sialoglycopeptide while monensin, a sodium ionophore, had no inhibitory activity (26).

To investigate further the structure and biological activity of the sialoglycopeptide growth inhibitor, we have developed monoclonal antibodies against the inhibitor. Monoclonal antibodies have been established as practical tools for use in a variety of laboratories. Predominantly, they have been utilized for the purification and identification of specific proteins (12,14,29). Recent investigations, however, have also seen the use of monoclonal antibodies in sophisticated techniques such as molecular probes to localize molecules on cell surfaces (6) and immunotherapy of cancer (17). We have produced three

monoclonal antibodies, G4, G8 and F10, to the sialoglycopeptide. In this report we present evidence that the three monoclonal antibodies belong to the IgG1 subclass, are directed against the polypeptide backbone of the inhibitor, can neutralize the inhibitor's biological activity, bind to individual antigenic determinants, and are unique to the sialoglycopeptide as they do not recognize determinants on other growth inhibitors such as fibroblast growth regulator and β-transforming growth factor.

MATERIALS AND METHODS

PURIFICATION OF THE SIALOGLYCOPEPTIDE: The sialoglycopeptide was prepared as described by Kinders et al. (18) with the modifications of Sharifi et al. (27). Briefly, the cerebral cortex cells from freshly killed cattle were treated at 37° C for 20 min with a pronase solution containing 0.02 units of proteinase/ml bovine cells. The cells were removed by centrifugation and the macromolecules were precipitated with ethanol and extracted with chloroform/methanol. Extracted material was then purified by DEAE ion exchange, wheat-germ agglutinin lectin affinity chromatography, gel permeation, and hydroxyapatite HPLC (27). The purified sialoglycopeptide is an 18 kD protein based on SDS-PAGE analysis and has an isoelectric point of 3.0 (27).

DEGLYCOSYLATION OF INHIBITOR: Deglycosylation of the sialoglycopeptide was carried out essentially by the methods of Manjunath et al. (23). Lyophilized sialoglycopeptide was dissolved in trifluoracetic acid containing 1.0% (v/v) ethyl methyl sulfide. A stream of hydrogen bromide gas was passed through the acidic solution of sialoglycopeptide for 2 hr and then evaporated to dryness under a stream of nitrogen. The deglycosylated

sialoglycopeptide was resuspended in distilled water and dialyzed against water to remove residual acid. Following dialysis, the deglycosylated sialoglycopeptide was concentrated by lyophilization, resuspended in PBS buffer, and passed through a L. polyphemus lectin affinity column where the unbound fractions were collected and assayed for inhibitory activity.

PRODUCTION OF HYBRIDOMAS: Non-immunized Balb/c mice were bled and the sera was tested by solid phase radioimmune assay for the presence of cross reacting antibodies to the sialoglycopeptide antigen. Following preimmune screening, non-reacting mice were injected intraperitoneally with approximately 5 ug of purified sialoglycopeptide in Fruend's complete adjuvant and reinjected similarly one month later with an equal amount of antigen. Ten days after the second injection, mice were bled and the sera were screened by solid phase radioimmune assay for antibody production to the sialoglycopeptide. Positive producers were boosted intervenously with 1 ug of sialoglycopeptide in complete adjuvant and 5 days later were sacrificed for the production of hybridomas.

Essentially, hybridoma production and cell maintenance were performed as described by Fazekas de St. Groth and Scheidegger (8). Antigen stimulated splenocytes or the

non-secreting Balb/c myeloma cell line P3-X63-Ag8.6.5.3 (Ag8) (American Type Culture Collection, Rockville, MD) were centrifuged at 350 x g and resuspended in serum-free Dulbecco's modified Eagle medium, recentrifuged at 350 x g and counted. Cell viability was determined by Trypan blue exclusion and viable splenocytes and Ag8 myeloma cells were mixed at a ratio of 1:2, respectively, and pelleted by slow speed centrifugation. The supernatant fluid was removed and 1 ml of a 50% (v/v) polyethylene glycol 4000 solution in DMEM, with 5% (v/v) dimethylsulfoxide, was slowly added to the pellet with constant mixing. Cells were then concentrated by centrifugation at 100 x g for 6 min to enhance fusion. The cell pellet was resuspended in serum-free DMEM, recentrifuged and then the cells were resuspended in selective hypoxanthine, aminopterin, and thymidine medium (HAT) (20). Hybridomas were plated at 1 x 10^5 cells/well over a macrophage feeder layer and maintained with additions of fresh HAT medium every three days to the culture wells. Culture wells producing anti-sialoglycopeptide antibody, as determined by solid phase radioimmune assay, were cloned by limiting dilution and then expanded to increase production of monoclonal antibody.

SOLID PHASE RADIOIMMUNE ASSAY: The solid phase radioimmune assay was carried out as described by Tsu and Herzenberg (31) with slight modifications. 1 ug/well goat anti-mouse immunoglobulins [IgM, IgG, and IgA] (Cooper Biomedical, Malvern, PA) in 0.005 M phosphate buffered saline, pH 7.2 (50 ul/well) were absorbed to Immulon 1 Removawells (Dynatech, Alexandria, VA) overnight at 4° C. Assay wells were washed three times with 0.05% (v/v) Tween 20 (Sigma Chemical Co., St. Louis, MO) in phosphate buffered saline (washing solution). Non-specific binding sites were blocked with 1% (w/v) egg ovalbumin (Sigma Chemical Co.) solution at room temperature for 4 hr. Wells were again washed with washing solution and 50 ul of either purified monoclonal antibody or cell culture supernatant containing the monoclonal antibody were added to each well. Assays were incubated overnight at 4° C, washed, and again incubated overnight at 4° C with 50,000 cpm/well of radiolabeled sialoglycopeptide. All antigen dilutions were made in blocking solution and after the final incubation, each well was again washed three times and the bound radioactivity determined.

PURIFICATION OF MONOCLONAL ANTIBODY: Hybridoma culture supernatant was passed through a Cibracon blue affinity

column (Bethesda Research Laboratories, Gaithersburg, MD). Eluent containing the monoclonal antibody, as determined by spectrophotometry, was collected and the immunoglobulins in the fluid from the Cibracon blue column were then precipitated with a 40% (v/v) ammonium sulfate solution overnight at 4° C. The precipitated IgG fraction was then pelleted by centrifugation at 5,000 rpm for 10 min and resuspended with phosphate buffered saline. Monoclonal antibody concentration was obtained by Coomasie blue protein determination.

RADIOIODINATION OF PROTEINS: Proteins were radioiodinated by the chloramine T method of Greenwood et al. (10) with the following modifications. Proteins were incubated with I and chloramine T and then the reaction was quenched with 200 ul of a saturated tyrosine solution (3). No reducing agent was used. The free ^{125} I was separated from the proteins by gel filtration, and after dialysis of the radioiodinated sample against 0.1 M KI overnight, the radiolabeled proteins were dialyzed extensively against phosphate buffered saline.

SIZE-EXCLUSION HPLC: The sialoglycopeptide and its autohydrolyzed peptide fragments were purified by HPLC using the methods described by Sharifi et al. (27). Briefly, biologically active fractions were injected into a

G3000 TSK column equilibrated with 0.2 M potassium phosphate (pH 6.5) and the flow rate was 1.0 ml/min. Samples from the column were dialyzed against deionized water, lyophilized, and tested for biological inhibitory activity and protein content. Fractions with biological activity were pooled and kept frozen at -80° C.

PROTEIN SYNTHESIS INHIBITION ASSAY: Methods to determine materials that can inhibit protein synthesis were described by Kinders et al. (19). 25 ul of material in Dulbecco's modified Eagle medium (DMEM)/N-2-hydroxy-ethylpiperazine-N'-2-ethanesulfonic acid (HEPES) was placed in 13 x 100 mm tubes, 5 x 10 3T3 cells in 100 ul of DMEM/HEPES (pH 7.1) were added, and the tubes were covered and incubated at 37° C for 45 min. Following incubation, 2.0 uCi of [^{35}S] methionine in 10 ul of DMEM/HEPES (pH 7.1) was added and the tubes were reincubated at 37° C for 1 hr. Cells were then pelleted by centrifugation and lysed with deionized water. Macromolecules were precipitated by addition of an equal volume of 20% ice-cold trichloroacetic acid. The precipitates were collected by centrifugation and the supernatant discarded. The pellet was resolubilized in 0.1 M NaOH and reprecipitated with 20% trichloroacetic acid and the macromolecules were collected by centrifugation. The

precipitated material was again resuspended in distilled water and resolubilized in 0.1 M NaOH. An aliquot of the resolubilized material was taken for liquid scintillation counting.

RESULTS

Conditioned medium from hybridoma cultures producing anti-sialoglycopeptide monoclonal antibodies were titrated against the sialoglycopeptide, utilizing a solid phase radioimmune assay, to determine an approximate antibody avidity. From data shown in Fig. 1, the monoclonal antibody G8 sustained an antibody titer of 9600, signifying the midpoint between the maximum and minimum radioactive cpm of bound sialoglycopeptide detected. Results seen in Fig. 1 were also representative of the approximate antibody titer of the monoclonal antibodies G4 and F10 as well.

After determining the avidity of the monoclonal antibodies, it was imperative to isotype them before carrying out further experiments. Established mouse immunization protocols designed to elicit antibody responses typically result in secondary immune responses from which high titers of IgG antibodies are produced having a high affinity for the immunogen. Therefore, we suspected that the anti-sialoglycopeptide monoclonal antibodies were IgG antibodies. Utilizing the anti-mouse antibodies of several classes and subclasses provided by a commercial isotyping kit, we prepared an ELISA assay. Visual inspection of the assay wells revealed, correlating

with antibody titer, that again the three monoclonal antibodies were similar as they were all of the IgG1 subclass (Table 1). Determination of monoclonal antibody subclass was accomplished by comparing experimental assay wells to positive control wells containing all classes of mouse antibodies (normal mouse serum) and negative control wells without second antibody.

Upon identification of the subclass of the monoclonal antibodies, we were then interested in determining if the monoclonal antibodies were directed towards the polypeptide or the oligosaccharide portions of the sialoglycopeptide. The purified sialoglycopeptide growth inhibitor is an 18 kD glycoprotein which has sidechains of low molecular weight carbohydrate moeities but the biologically active portion of this molecule is the polypeptide backbone (24). To determine if the monoclonal antibodies are directed against the biologically active polypeptide backbone, we performed a competitive solid phase radioimmune assay. The competitive solid phase radioimmune assay enabled the detection of monoclonal antibody binding to the polypeptide portion of the sialoglycopeptide by evaluating the binding competition produced by adding deglycosylated and radiolabeled native sialoglycopeptide simultaneously to the

solid phase assay. Results shown in Table I indicate that the three anti-sialoglycopeptide monoclonal antibodies bound to the biologically active polypeptide backbone of the growth inhibitor. Bound radiolabeled glycosylated sialoglycopeptide is shown to be reduced 65% by 16 ng of deglycosylated sialoglycopeptide when simultaneously incubated in assay wells containing the monoclonal antibody, G4. Similar results were obtained with G8 and F10.

The ability of the three monoclonal antibodies to neutralize the inhibitory action of the sialoglycopeptide was tested by incubating each of the monoclonal antibodies with sialoglycopeptide overnight at 4°C. A constant molar concentration (3 inhibitory units) of growth inhibitor was incubated with an equal or increasing molar concentration of purified monoclonal antibody. The solution containing the antibody-antigen complex was then incorporated into an assay to detect protein synthesis inhibition of Swiss 3T3 fibroblasts. The monoclonal antibodies G8 and F10 were able to neutralize 100% of the biological activity of the sialoglycopeptide with a 1:10 or 1:1 molar equivalent, respectively (Table 2). Opposingly, G4 could only achieve 46% neutralization at a 100 fold molar excess of G4 to the growth inhibitor. Control assays revealed that without

preincubation of the antibodies with the sialoglycopeptide, the antibodies did not significantly affect the biological activity of the inhibitor (data not shown). Although, in experiments previously described in this report all three monoclonal antibodies reacted to the polypeptide backbone of the sialoglycopeptide, G4 appeared to be a distinct monoclonal antibody as determined by neutralization assay data.

An interesting property of the purified sialoglycopeptide is that it carries an unique protease activity as well as inhibitory properties. As determined by Sobieski et al. (30), this protease activity is not responsible for the biological inhibitory activity. Because of the associated protease activity, the growth inhibitor molecule is subject to autohydrolization at 37°C and this provided a means to further characterize the three monoclonal antibodies. When purified to homogeneity, the sialoglycopeptide inhibitor elutes from gel permeation HPLC as a single peak (Fig. 2). However, when incubated at 37°C for either 1 hr or 24 hr, four discrete peaks elute from the HPLC column (Fig. 3). Three of these peaks had molecular weights of 16 kD (I), 10 kD (III), and < 4 kD (IV) using HPLC molecular weight control standards.

Unfortunately, peak II could not be separated sufficiently from peak I, therefore, it was not used for further experiments.

Utilizing the polypeptide fragments associated with peaks, I, III, IV, and the native sialoglycopeptide, a competitive radioimmune assay was performed to detect fragment binding by the three monoclonal antibodies. Data from Figs. 4 and 5 showed that all three monoclonal antibodies bound to fragments I and III. However, G8 produced a binding competition between fragment I and radiolabeled sialoglycopeptide which was equivalent to the competition produced by G8 between labeled and non-labeled native sialoglycopeptide. This competition was not evident with G4 or F10. Accordingly, all three monoclonal antibodies also bound to fragment III but did not produce the binding competition that was seen between G8 and fragment I. Conclusions drawn from this information established that at least epitopes for all three monoclonal antibodies are located on fragments I and III of the sialoglycopeptide. The lowest molecular weight fragment resulting from autohydrolysis of the sialoglycopeptide, fragment IV, was not bound by any of the monoclonal antibodies (data not shown).

Aside from characterization of the anti-

sialoglycopeptide monoclonal antibodies, G8 was used as a tool to compare the sialoglycopeptide with other growth regulators that are presently being studied. A limited supply of growth regulators allowed for the use on only one monoclonal antibody for this experiment. In this experiment fibroblast growth regulator (FGR) and β-transforming growth factor (TGF) were used because they have biological properties similar to that of the sialoglycopeptide. This suggested that the sialoglycopeptide might contain regions in its amino acid sequence that are shared with these other growth regulators. However, as seen in Fig. 6, G8 did not recognize epitopes on either FGR or TGF. In a competitive solid phase radioimmune assay, radiolabeled sialoglycopeptide and either TGF and FGR were simultaneously added in equimolar concentrations to assay wells containing bound monoclonal antibody. Neither of the growth regulators could compete with the sialoglycopeptide for the antibody's binding sites even at concentrations to assay wells containing bound monoclonal antibody. Neither of the growth regulators could compete with the sialoglycopeptide for the antibody's binding sites even at concentrations of 100 fold excess of TGF and FGR. Therefore, it can be concluded that at least the amino acid

sequence acting as the epitope for G8 is different from the other known growth inhibitors. These data confirm that the sialoglycopeptide is not similar to either of these previously identified growth regulatory molecules.

DISCUSSION

Three monoclonal antibodies specific for a sialoglycopeptide cell growth inhibitor, G4, G8, and F10 have been isolated and purified. These monoclonal antibodies were isotyped using a standard ELISA assay utilizing anti-immunoglobulin typing antisera and were shown to be IgG1 antibodies. This was not an unusual finding as the IgG1 subclass makes up 70% of the IgG proteins elicited by an immune response (7). The IgG antibody is also a very stable molecule with a high affinity for its specific ligand (7). This enabled indefinite storage of the antibodies for future experiments and will be beneficial for future use in affinity chromatography purification of the sialoglycopeptide.

Further characterization of these monoclonal antibodies was carried out using a competitive solid phase radioimmune assay to establish the approximate location of the antibody epitopes on the sialoglycopeptide molecule. The competitive solid phase radioimmune assay has been used successfully in similar studies characterizing antibodies directed against creatine kinase and lactate dehydrogenase and has been determined to be more sensitive than traditional ELISA assays (32). Therefore, we utilized this assay to compete glycosylated native sialoglycopeptide with

its deglycosylated counterpart (27). It was possible that the monoclonal antibodies were specific for one of the carbohydrate sidechains associated with the native sialoglycopeptide and this would render the antibodies unsuitable for future investigations such as amino acid sequencing of the growth inhibitor. However, data from this experiment allowed the conclusion that all three monoclonal antibodies had epitopes located on the biologically active polypeptide backbone of the sialoglycopeptide (Table 1).

Initial characterization of the monoclonal antibodies, thus far, indicated that the antibodies were of identical subclass and were similar in their ability to bind to the biologically active sialoglycopeptide. However, when the growth inhibitor was autohydrolyzed and the fragments separated by HPLC (Fig. 3), the monoclonal antibodies appeared to bind the peptide fragments differently (Figs. 4 and 5). For example, G8 produced substantial competition of binding between the native sialoglycopeptide and peptide fragment I whereas G4 and F10, although having the ability to bind fragment I, did not produce equal competition. These data may reflect the possibility that the epitope for G8 is made more available to the antibody as a result of the tertiary structure of fragment I. All three monoclonal

antibodies revealed similar competition of binding for fragment III. Although the molecular weights of fragments I and III are very close (16 and 10 kD, respectively), the tertiary structure of fragment III may have left the G8 epitope less available for binding.

Apparent differences between monoclonal antibodies, G4, G8, and F10 were again noted in studying the ability of the antibodies to neutralize the biological activity of the sialoglycopeptide. Antibodies have previously been utilized in neutralization assays of biologically active molecules to identify proteins (4,14) and in experiments neutralizing growth inhibitors for the purpose of discriminating between antibodies (29). In our neutralization studies G4 lacked the ability to block the inhibitory properties of the sialoglycopeptide as compared to G8 and F10 which abolished the activity (Table 2). G8 and F10's neutralizing ability, and the knowledge that the sialoglycopeptide does not need to be internalized to produce a biological response (25), suggests that they may be directed against antigenic determinants at or near the inhibitor's receptor binding site. Opposingly, G4 which binds to the biologically active sialoglycopeptide, as previously discussed, may have an epitope distal to the site of binding.

Initial experiments utilizing the monoclonal antibodies for other than characterization was to establish the sialoglycopeptide as a novel growth inhibitor. Cell growth inhibitors with biological properties similar to the sialoglycopeptide have been reported (4,13). Two such growth regulators, fibroblast growth regulator (FGR) (33) and β-transforming growth factor (TGF) (22), were incorporated into a competitive solid phase radioimmune assay to detect similarities to the sialoglycopeptide. Data from Fig. 6 indicated that the monoclonal antibody, G8, would not bind to either FGR or TGF. Thus, the sialoglycopeptide contains, at least, an unique amino acid sequence which is acting as a determinant for G8. In addition, Bascom et al. (1) have reported that FGR and TGF do not competitively bind with the sialoglycopeptide for its cell surface receptor. However, this does not imply that homologies to FGR and TGF do not exist. Comparisons to other growth inhibitors have not yet been completed, but to date, it appears that the sialoglycopeptide is a novel growth inhibitor.

Experiments have been initiated using the anti-sialoglycopeptide monoclonal antibodies as aids in the purification of the growth inhibitor by affinity chromatography. Fab fragmentation of the monoclonals would

also allow the probing of cell surfaces to detect the sialoglycopeptide on a variety of established and primary cell cultures. Aside from the practicality of the monoclonal antibodies as laboratory tools, further characterization of the antibodies would be useful. The results of this study suggest that G4, G8, and F10 may be directed against individual antigenic determinants. Eventually, these antibodies may aid in the localization of the specific amino acid sequence which is responsible for the sialoglycopeptide's biological activity and enable the growth inhibitor to be synthesized in the laboratory.

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^{125}I-Sialoglycopeptide Bound (CPM x 10^{-3})
0
1
2
3
4
5
2
4
8
16
32
64
128
256
512
Antibody Dilution (x 10^2)

Fig. 1. Anti-sialoglycopeptide monoclonal antibody titration curves. Antibody titer of G4 (Δ), G8 (●) and F10 (□) antibodies were determined by solid phase radioimmune assay as described in the materials and methods. Following initial absorption of goat anti-mouse immunoglobulins to polystyrene wells, serially diluted samples of each antibody were added to the wells. 50,000 cpm radiolabeled sialoglycopeptide was then incubated in each well. After washing, bound radioactivity was measured. Each assay was performed in triplicate.

MONOCLONAL ANTIBODY	ISOTYPE	10.5 ng NATIVE SIALOGLYCOPEPTIDE (cpm)	DEGLYCOSYLATED SIALOGLYCOPEPTIDE (ng) / NATIVE SIALOGLYCOPEPTIDE (cpm)	COMPETITION (%)
G4	IgG1 [a]	15020 ± 620	2 / 12152 ± 1250 [b]	19
			4 / 11232 ± 990	25
			8 / 9244 ± 1072	33
			16 / 5264 ± 308	65
G8	IgG1	13252 ± 1408	2 / 11910 ± 138	10
			4 / 10796 ± 122	19
			8 / 9074 ± 1094	32
			16 / 6312 ± 180	52
F10	IgG1	16386 ± 1392	2 / 14380 ± 556	12
			4 / 12134 ± 746	26
			8 / 8922 ± 798	46
			16 / 6030 ± 312	63

Table 1. Isotyping and binding of the monoclonal antibodies to the biologically active sialoglycopeptide.

a. Isotyping was accomplished using a commercial ELISA isotyping kit. Briefly, polystyrene microtiter wells were coated with goat anti-mouse immunoglobulins and incubated overnight at 4°C. Following, each experimental assay received hybridoma supernatant, anti-immunoglobulins typing antisera, peroxidase conjugated goat anti-rabbit IgG, and o-phenylene diamine (a chromophore substrate), respectively. Assay wells were repeatedly washed with phosphate buffered saline and allowed to incubate at room temperature for approximately 1 hr between each antibody addition.

b. Deglycosylated sialoglycopeptide assay was carried out using a solid phase competitive radioimmune assay as described in the materials and methods. Following initial plating of anti-mouse immunoglobulins, assay wells received G4, G8, or F10 monoclonal antibodies. Radiolabeled glycosylated and increasing concentrations of non-labeled deglycosylated sialoglycopeptide were then added simultaneously. After final washes, bound radioactivity was determined.

MONOCLONAL ANTIBODY	MOLAR RATIO SIALOGLYCOPEPTIDE TO MONOCLONAL ANTIBODY	ACID PRECIPITABLE COUNTS (cpm)	INHIBITORY ACTIVITY (%)
G4	NO ANTIBODY	7388 ± 1088	100
	1 : 1	6267 ± 559	85
	1 : 10	6774 ± 312	92
	1 : 100	4730 ± 184	64
G8	NO ANTIBODY	8690 ± 50	100
	1 : 1	6992 ± 384	80
	1 : 10	8740 ± 392	0
	1 : 100	8613 ± 832	0
F10	NO ANTIBODY	8057 ± 154	100
	1 : 1	9670 ± 639	0
	1 : 10	9704 ± 313	0
	1 : 100	10324 ± 1769	0

Table 2. Neutralization of the biologically active sialoglycopeptide using the monoclonal antibodies. Following incubation of G4, G8 and F10 monoclonal antibodies with the sialoglycopeptide overnight at 4°C, the antibody-ligand complexes were added to 3T3 cells. [^{35}S] methionine was then introduced into each assay tube. Cells were pelleted, lysed, precipitated with trichloroacetic acid and resolubilized as described in the materials and methods. Aliquots of each assay were taken for liquid scintillation counting and the percent protein synthesis inhibition calculated. Control assays contained only sialoglycopeptide.

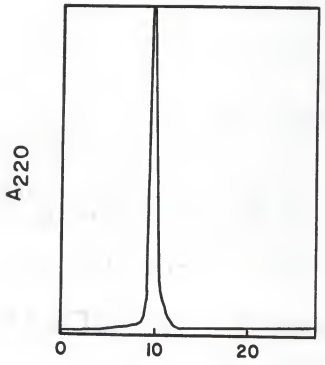
A220
0
10
20
Retention Time (min)

Fig. 2. Homogeneity of the sialoglycopeptide as defined by gel permeation HPLC. Purified sialoglycopeptide was applied to a gel permeation HPLC column equilibrated with 0.2 M phosphate buffer, pH 6.5. Flow rate of the column was 1.0 ml/min. The fractions at approximately 10 min were tested for inhibition of 3T3 cell protein synthesis.

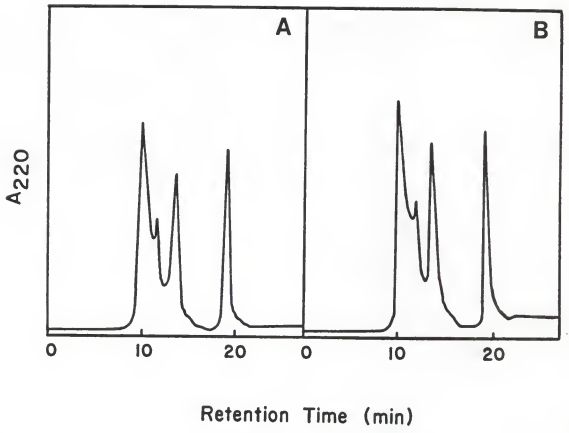
A
B
A_{220}
0
10
20
0
10
20
Retention Time (min)

Fig. 3. Autohydrolyzed sialoglycopeptide as defined by gel permeation HPLC. Briefly, purified sialoglycopeptide was incubated for 1(A) or 24(B) hr at 37°C and subsequently applied to a HPLC gel permeation column equilibrated with a 0.2 M phosphate buffer, pH 6.5. Flow rate was 1.0 ml/min. All fractions were tested for inhibitory activity.

125I-Sialoglycopeptide Bound (CPM x 10^-3)
0
2
4
6
1
10
50
100
Fragment I (ng)

Fig. 4. Binding of the monoclonal antibodies to sialoglycopeptide fragment 1. Following initial plating of goat anti-mouse immunoglobulins to microtiter wells, assays received either G4 (Δ), G8 (●) or F10 (□) monoclonal antibody. After incubation, wells received 10 ng radiolabeled sialoglycopeptide and increasing concentrations of nonlabeled fragment 1, simultaneously. Radioactivity was measured following final washes of assay wells.

^{125}I-Sialoglycopeptide Bound (CPM x 10^{-3})
0
2
4
6
1
10
50
100
Fragment 3 (ng)

Fig. 5. Binding of the monoclonal antibodies to sialoglycopeptide fragment 3. Following initial plating of goat anti-mouse immunoglobulins to microtiter wells, assays received either G4 (Δ), G8 (●) or F10 (□) monoclonal antibody. After incubation, wells received 10 ng radiolabeled sialoglycopeptide and increasing concentrations of nonlabeled fragment 3, simultaneously. Radioactivity was measured following final washes of assay wells. Each assay was performed in triplicate.

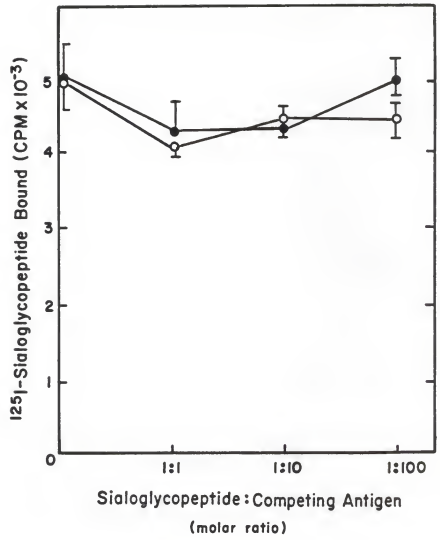
125I-Sialoglycopeptide Bound (CPM x 10-3)
0
1
2
3
4
5
1:1
1:10
1:100
Sialoglycopeptide : Competing Antigen
(molar ratio)

Fig. 6. Comparison of the sialoglycopeptide to fibroblast growth regulator (FGR) and B-transforming growth factor (TGF). After initial coating of microtiter wells with goat anti-mouse immunoglobulins, each assay well received monoclonal G8. Following incubation, 2 ng (1 mole) radiolabeled sialoglycopeptide and increasing molar concentrations of either FGR (○) or TGF (●) were added simultaneously. Each assay was performed in triplicate.

CHARACTERIZATION OF THREE MONOCLONAL ANTIBODIES DIRECTED AGAINST A BOVINE CEREBRAL CORTEX CELL SURFACE SIALOGLYCOPEPTIDE

by

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B.S., Kansas State University, 1981

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ABSTRACT

Since the development of the first specific antibody producing hybrid cells in 1975, monoclonal antibodies have become sophisticated and very practical research tools that are presently being employed by a large number of biological research laboratories. This report attempts to describe the advancements that have been made in monoclonal antibody technology in the past decade and to discuss one of the most recent utilizations of monoclonal antibodies, cancer therapy.

Predominantly, however, monoclonal antibodies are used as a method to purify and characterize specific biological proteins. Our laboratory has isolated, from bovine cerebral cortex cells, an 18 kD sialoglycopeptide growth regulator which inhibits cellular protein and DNA synthesis. We have characterized three monoclonal antibodies directed against this growth inhibitor which will eventually enable the rapid purification of the sialoglycopeptide or its parental molecule and allow for the detection of the protein on a variety of cell surfaces. Additionally, these monoclonal antibodies were useful in demonstrating that the sialoglycopeptide is unique from other presently studied growth regulators such as β-transforming growth factor and fibroblast growth regulator and can be depicted as a novel cell growth inhibitor.