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MONOCLONAL ANTIBODIES – PROSPECTS AND APPLICATIONS

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ABSTRACT

It is becoming increasingly important to screen for marine pathogens, using diagnostic tests which are both reliable, quick and inexpensive. Conventional procedures of isolating and characterizing micro-organisms are both time consuming and expensive. It is to be stressed that diagnostic tests for detection of fish pathogen and also for spoilage causing organisms, should be rapid, simple and reliable.

Serology has to a great extent simplified monitoring and detection procedures of most pathogenic micro-organisms. Serological tests are now being used on a routine basis for disease diagnosis all over the world, both for human beings and for animals. Conventional serological tests however used polyclonal antisera which has very little avidity pertaining to immunoglobulin binding. In other words the specific antibodies represent a diverse spectrum of molecules with different avidities (binding capacities), directed against different epitopes (parts) of the antigen. This could lead to several problems viz; cross reactivity, less specificity, batch to batch variation in results etc.

The technique of monoclonal antibodies has revolutionized several areas of modern biological research. Monoclonal antibodies are finding increasing use in disease diagnosis and pathogen detection. They are produced by immortal clones of cells called "Hybridomas". They are identical with respect to several useful parameters viz; IgG subclass, allotype, variable regions, structure, idiotype, affinity and specificity for a given epitope. In using a polyclonal antiserum, the binding of non-specific IgG relative to antigen specific IgG may be uncomfortably high. This problem is greatly reduced with a monoclonal antibody leading to a much higher signal to noise ratio. A monoclonal antibody is capable of being applied in a variety of serological tests viz; ELISA, competiton RIA, dot ELISA, western blot, latex agglutination tests, micro SNT etc. Depending on the nature of antigen used and the monoclonal antibody in question, a diagnostic test can be devised which is convenient, easy to perform and highly specific. Furthermore, a better understanding of the overall molecular makesup of the antigen can be gained using monoclonal antibodies.

Introduction

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Monoclonal antibodies – An overview

Technology for producing monoclonal antibodies was developed nineteen years ago by Milstein and Kohler (1975), this discovery has subsequently revolutionized many areas of biological research. In the past five years there has been tremendous work done in characterizing and producing

monoclonal antibodies against a wide variety of pathogens. The property which makes monoclonal antibodies unique is their single molecular structure, single cells, producing antibody are selected and cloned eventually producing a population of cells, which secrete an identical antibody. The antibody is homogenous, in the sense that the paratopes (combining sites) which bind to the eliciting epitope (antigen site), are identical.

The technique for preparing monoclonal antibodies involves hybridization of an antibody producing cell (spleen Cell) with an immortal cell line (myeloma) to produce a "hybridoma". The hybridoma represents a single clone of antibody producing cell immortalized due to the properties of the myeloma cell line which is basically a tumour cell line. Hence, we have a hybridoma capable of secreting an antibody of known specificity in virtually infinite quantities, viz., the cell line producing it is capable of being cultured in any quantity desirable, hence unlimited quantities of the antibody can be obtained. The antibody thus obtained is highly specific, homogenous and stable with respect to its isotyope, affinity and avidity. Monoclonal antibodies have more affinity and less avidity, when compared to polyclonals, i.e., monoclonal antibodies are more specific in combining with a single epitope than combining with many epitopes. Although in certain cases, a loss in avidity could be disadvantageous. However, in an overall sense the specificity, homogeneity and unlimited availability of monoclonal antibodies are its most important advantages.

Advantages of monoclonal antibodies

Among the various uses of monoclonal antibodies, one of the most important, is its application in diagnostic tests. Rapid diagnostic tests use monoclones in immunoassays. Such tests can yield a high degree of specificity, although it is not always possible to use the monoclonal antibodies as the capture antibody. This is mainly due to differences in affinity of the monoclonal antibodies and avidity of a polyclonal preparation. If the avidity is too high it can remove the trapped antigen by means of antigen based competition (Mc. Cullough 1993). The affinity of monoclonal antibodies is not always a problem, particularly when both capture and detection antibodies are monoclonal. The same monoclonal antibodies can be used for detection and capture, if the antigen in question is large and possesses numerous epitopes for which the monoclonal antibodies are specific. However, it is usual to use two monoclonal antibodies of different specification (Crowther, 1990).

Overall it is clear that monoclonal antibodies can be applied to all types of immunoassays, depending on the type of assay and the characteristics being sought of the test material. Monoclonal antibodies can be used as capture and/or detection reagents. The wide application of hybriodoma technology to diagnostic tests, in particular to immunoassays, has greatly increased the potential to provide detailed characterization and more applicable information (Mc. Cullough, 1993).

Methodology

Antibody producing lymphoid cells from immunized animals have a very short life when cultured under in-vivo conditions. Myeloma cell lines are derived from a cancerous unchecked proliferation of antibody producing cells. As the myeloma begin as a single cell, all of its progeny constitute identical lymphocytes. The antibody produced by these lymphocytes is homogenous because it is all derived from a single clone of cells. Such an antibody could be theoretically called as a monoclonal antibody. However, since the antibodies have no predefined specificities, the antigen involved is an unknown one. It had not yet been possible to induce myelomas specific for a given antigen as a solution to this problem. However, a technique for combining the growth characteristics of myeloma cells and the predetermined antibody specificity of normal immune spleen cells has been developed. In doing so, a 'hybrid' cell was developed which expressed the special properties of both the cell types. This was done by fusing a myeloma cell with an antibody secreting cell from the spleen of an immunized mouse. The result is an artificially created cell called a 'hybridoma', which is essentially a specific antibody producing factory. In such cells, the myeloma portion provides 'immortality' (unrestricted rapid proliferation) and thus production of large quantities of monoclonal antibodies; the immune lymphocytes portion provides the information for the specificity of the antibody (Avidity/Affinity).

This procedure involves several important steps, some of which are shown schematically in Fig.1. Basically, mice are immunized in a usual manner with an antigen against which specific antibodies are required. This antigen need not be specially purified to contain only the desired epitopes, each of the epitopes on the antigen stimulates a specific clone of B-lymphocytes. Thus a single

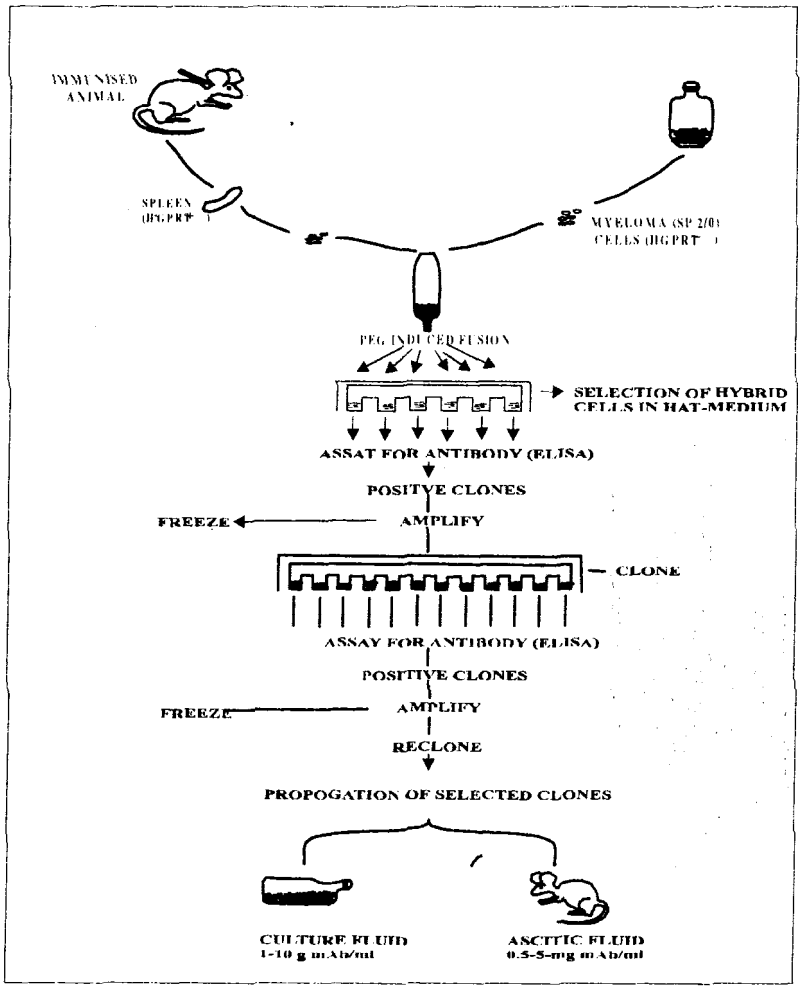

Fig.1 Protocol for generation of hybridcells producing monoclonal antibodies

antigen can stimulate a number of B-cell clones. The mouse spleen is removed and the spleen cell suspension is mixed with suspension of mouse myeloma cells. Polyethylene glocol is added to the mixture, which causes some of the lymphocytes to fuse with the cancer cells to produce hybridoma. East hybridoma cell is selected in a specific selective media called HAT (Hypoxanthine, Aminopterin and Thymidine) has 2 pathways of nucleic acid metabolism viz., the denovo pathway and the salvage pathway. The selection for the growth of hybrid cells only is based on the facts that 1) spleen cell lines that serve for fusion are mutants lacking the enzyme HGPRT (Hypozanthine guanine phosphoribosyl

transferase) which are established by growing the cells in presence of 8-azaguanine or 8-azaserine. The cells could be deficient in the enzyme thymidine kinase also (i.e., TK) these cells are obtained by growing in the presence of the thymidine analogue 2-bromodeoxy uridine. Such mutants die in the presence of aminopterin which blocks the salvage pathway (as they are deficient of the enzyme HGPRT). Only the hybrid cells between the spleen and myeloma will survive in the presence of aminopterin, but for this they require hypoxanthine and thymidine that are essential base precursors of the alternate/salvage pathway. Hence after incubating in selection media, i.e., HAT media, only viable hybridomas can grow and replicate. Hybridoma are then selected and separated into individual cells of plastic dishes and allowed to grow into clones. Each hybridoma clone may produce monoclonal antibody; the antibody is secreted into the culture media. Each hybridoma is screened for the antibody of interest and is identified accordingly. Since a purified antigen preparation is not used initially, it is possible that a lot of hybridomas will secrete non-specific for unimportant antibodies, hence screening for the right hybridoma is identified, it may be recloned by cell culture, or it may be maintained invivo by injection into the peritoneal (abdominal) cavity of the mouse. It is also important to note that cell culture supernatants yield about 100 microgram or more of monoclonal antibody per milliliter, in vivo culture yields about 1000 microgram or more of monoclonal antibody from the peritoneal cavity, such propagation is also called as ascitic propagation, the hybridomas could also be cryopreserved in liquid nitrogen in cryopreservation media, i.e., media containing dimethyl sulfoxide (DMSO).

Conclusion

Monoclonal antibodies are important because they are homogenous, highly specific and produced readily in large quantities. They bind to one and only one antigen and hence are monospecific. Monoclonal antibodies have become very important tools in biomedical research, in 1981 for example, they allowed the investigation to determine the type of immune system cell that was being destroyed by the AIDS virus. Monoclonal antibodies have become very important in diagnostic and therapeutic medicine; in a proposed human cancer therapy, monoclonal antibodies are being used experimentally to attach and destroy tumor cells. They are also being used as 'biological missiles', with a payload to specific cancerous or tumor cells, leaving normal cells intact. They are being used in rapid diagnostic kits for various diseases, allergies, pregnancy detection etc. Also monoclonal antibodies have become very useful in differentiation of the myriad strains of micro-organisms.

Among the recent advances in the field of hybridoma research, the technique of in vitro immunization is a very important one. The technique of invitro immunization involves circumventing the need for immunizing live mice. Hence the time needed for preparing monoclonal antibodies is considerably reduced. Here the spleen cells are maintained in a special supplement added to media, viz. Poke weed nitrogen soup. The splenocytes are primed with the antigen while they are in the media, immunization done in this manner has several advantages over conventional in vivo procedures.

Monoclonal antibodies can be used for rapid detection, characterization and isolate confirmation of the causative agents of Epizootic Ulcerative Syndrome (EUS), white spot disease in shrimps, aeromonas infections, etc. The therapeutic and prophylactic potential of monoclonal antibodies in several important marine species also needs to be worked upon.

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