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Monoclonal Antibodies in Fish and Shellfish Health Management in India

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Abstract

The paper describes the superiority of monoclonal antibodies (MAb) over conventional polyclonal antisera. Studies undertaken indicate that *Aeromonas hydrophila* isolates are highly heterogenous and variation exists even between isolates from a farm, requiring a large number of MAbs for classification and use of information in vaccine development. However, some of the MAbs could be used for detection of homologous isolates in fish kidney by immunodot assay and evaluation and standardization of biofilm of *A.hydrophila* for oral vaccination in carps.

Introduction

Fish and shellfish aquaculture has been established as an industry in India in the last decade. Indian major carps *(Catla catla, Labeo rohita and Cirhinus mrigala)* are cultured on a large scale particularly in the states of Andhra Pradesh, Uttar Pradesh, Punjab and Haryana. In Andhra Pradesh alone, more than 40,000 tonnes of Indian major carp are raised in farms annually, valued at more than Rs. 600 million[1]. Shrimp are cultured in all maritime states of India, covering about 100 700 ha. Due to intensive culture of fish and shellfish, diseases of microbial etiology of economical significance have surfaced in rearing and growout ponds. Annual losses due to diseases in carp culture in the state of Andhra Pradesh are estimated at about Rs. 40 million. Viral disease in shrimp culture has become a stumbling block and the economic loss was estimated at about Rs. 9 450 million in 1995-96. Hence, to sustain the aquaculture industry, appropriate health management measures involving development of diagnostics and vaccines are necessary. Development of specific, sensitive and rapid diagnostic methods are essential to detect the various stage of disease such as peracute, acute to sub acute and chronic infection. In addition, there is a need to develop simple and sensitive methods for stereotyping pathogens for epidemiological study. At present diagnosis/stereotyping is performed in many laboratories using the conventional biochemical methods which are tedious, expensive and not sufficiently sensitive to differentiate the large number of heterogeneous isolates. Another area in health management, which needs greater attention, is the development of effective vaccines for disease prevention. Since a large number of isolates of different virulence and stereotype exists in a disease situation, there is a need to detect common immunogenic antigens shared by several isolates to develop an appropriate polyvalent vaccine.

[1] US$ 1= Rs 47 (May 2001)

Antibodies in health management

The importance of antibodies in health management measures such as diagnosis, identification, stereotyping, antigen characterization, epidemiology and vaccine development is well known. Conventionally, polyclonal antibodies and antigen preparations raised in rabbits are used in disease diagnosis, stereotyping and other biological applications. However, due to the limitation of polyclonal antisera such as cross reaction, limited quantity, unwanted background reactions and inability

Table 1. Comparison between conventional antiserum and monoclonal antibody.

	Conventional antiserum	Monoclonal antibody
Determinant	Several	Single
Specificity	Variable with animal and bleed Partial cross-reactions with common determinants Seldom too specific	Standard Unexpected cross reactions may occur; May be too specific for requirements
Affinity	Variable with bleed	May be specific during cloning
Yield of useful antibody	Up to 1 mg/ml	Up to 100 mg/ml in tissue culture; up to 20 mg/ml in acidic fluid
Contaminating immunoglobulin	Up to 100%	None in culture; 10% in acidic fluid
Purity of antigen	Either pure antigen or serum absorption	Some degree of antigen purification desirable but not essential

to discriminate antigen at epitope level, monoclonal antibodies (MAb) are becoming increasingly popular. The monoclonal antibodies are superior in a number of aspects to conventional polyclonal antisera (Table 1).

Since their initial description by Kohler and Mielstein in 1975, MAbs have made a profound impression in all areas of biology and biotechnology. MAbs production involves obtaining antibody producing lymphoid cells from immunized mice. These cells are then immortalized by hybridization with an established myeloma cell line followed by separation of individual hybridoma clones. The clones obtained are grown in tissue culture, so that the MAbs produced by them can be harvested. A schematic illustration of the production of monoclonal antibodies is given in Fig. 1.

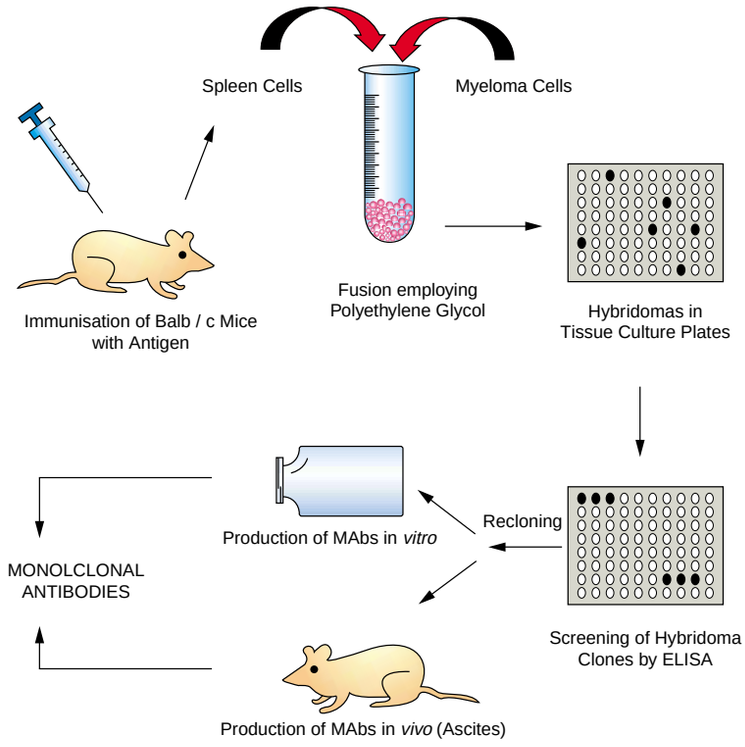

Fig. 1. Schematic illustration of production of monoclonal antibodies.

MAbs are widely used in the diagnosis, stereotyping and analysis of antigens in microbial pathogens in medical and veterinary sciences. Currently, they are also used in disease research and health management in aquaculture systems of developed countries. However, their application in commercial aquaculture systems in developing countries is yet to be implemented at any significant level. The Fish Pathology and Biotechnology Laboratory of the Department of Aquaculture, College of Fisheries, Mangalore, established the first hybridoma laboratory in fisheries in India in 1993 with funding support from the Department of Biotechnology, Government of India, and initiated a project on monoclonal antibodies to *Aeromonas hydrophila*. Among the fish bacterial diseases in freshwater, bacterial hemorrhagic septicemia caused by *A.hydrophila* is a major concern, causing a mortality rate of 30-70% in rearing ponds. Several isolates of the pathogen are also associated with human and other aquatic vertebrate diseases. The pathogen is highly heterogeneous biochemically, phenotypically,

serologically and in virulence. Under this project, a panel of five MAbs recognizing various epitomes of *A.hydrophila* on lipopoly-saccharides of the cell wall was raised. These antibodies were employed first for development of an ELISA/immunodot for epitope analysis of the isolates collected from several hosts/environments of the country. Epitopes shared or otherwise by the different isolates were then identified for serotyping and determining the antigenic relationship between isolates from different regions and hosts. The result indicated that the *A.hydrophila* isolates are highly heterogeneous and variation exists even between isolates from a farm. Hence a battery of a large number of MAbs is required for classification. The information should then be used in vaccine development. Although a MAb recognizing a common epitope of *A.hydrophila* could not be obtained, some of the MAbs could be used for detection of homologous isolates in fish kidney by immunodot assay (Fig. 2). Further, MAbs could be conveniently used for evaluation and standardization of biofilm of *A.hydrophila* for oral vaccination in carps. A biofilm preparation of *A.hydrophila* was found to be superior to a conventional free cell preparation (Azad et al. 1997, in press) which was also confirmed by localization of the vaccine antigen by MAb based immunoflouresence.

Fig. 2. Detection of a* A.hydrophila *in kidney of Indian major carp by MAb based immunodot assay.

Conclusion

Understanding a pathogen itself is a main step in disease management. Therefore, there is scope for the application of MAbs in fish and shellfish health management in the Indian subcontinent. At present, MAbs of white spot syndrome virus (WSSV) and *Aphanomyces invadan* associated with EUS are being produced mainly for diagnosis and epidemiological analysis.

Acknowledgement

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