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Review

Monoclonal antibodies in clinical diagnosis: A brief review application

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Monoclonal antibodies (mAb) have been an invaluable tool that has added to our biological knowledge for over a decade. mAb are important diagnostic reagents used in biomedical research, microbiological research in diagnosis of Hepatitis, AIDs, influenza, herpes simplex, Chlamydia infections and in treatment of such diseases as infections and cancer. The worldwide clinical diagnostics industry is valued at approximately $19 billion, with a growth rate of nearly 5% per year. Kohler and Milstein have first developed the means for the production of monoclonal antibodies. The first mAb production, a whole new era in the study of biotechnology has been opened. Further this hybridoma technology has been improved over the years, particular by pre-selection of antigen-binding B cells and by screening with antigen-coating filters. The modern popularity of the immunoassay is almost directly related to the development of recombinant mAb technology advancement. Hybridoma-derived or bacterially cloned monoclonal antibody technology has enabled the mass production of highly specific probes for antigenic sites, whether on enzymes, receptors, hormones, or microbial products. The great utility of such antibody assays is in their ability to be easily automated and standardized, primarily through an adaptation of the enzyme-linked immunosorbent assay. Monoclonal antibody diagnostic kits being increasingly used to identify communicable diseases including transfusion transmissible infections. More than 100 different monoclonal antibody diagnostic products are currently available. These monoclonal antibodies are produced by *in vitro and in vivo* method but have advantages and some disadvantages. The aim of present mini review articles is to demonstrate the monoclonal antibodies for the diagnosis of viral disease, their application and current market in clinical sciences.

Key word: Monoclonal antibody application, diagnostic tool.

REVIEW

Biotechnology drugs can broadly be grouped into four categories. There are two mature sectors that are set to generate >95% of total biotech sales from 2004 to 2010 (based on Datamonitor forecasts): recombinant protein therapeutics (rDNA proteins) and monoclonal antibodies (mAbs). There are also two early-stage industries: nucleic acid therapeutics and therapeutic vaccines, which are unlikely to launch many products with significant revenue-generating potential over the short- to mid-term.

The worldwide clinical diagnostics industry is valued at approximately $19 billion, with a growth rate of nearly 5% per year. In the worldwide market, the biggest areas of bulk sales are for immunoassays ($7.2 billion yearly), clinical chemistry ($3.1 billion), hematology and blood gases (>$2.0 billion), and routine microbiology ($1.3 billion). Although a biochemical assay exists for almost every disease, diabetes is the largest single disease diagnostic category at $2.8 billion yearly (Hasulo, 2001). Historically, antibiotic and drugs in these therapy areas have driven biotechnology market evolution, and together they make up a significant proportion of total biotech market sales (Pijpers and Belsey, 2006: Churchill and Belsey, 2006).

Antibodies have become common and essential research tools for many applications, including western blotting, immunohistochemistry, immunocytochemistry, enzyme-linked immunosorbant assay (ELISA), immuno-

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precipitation and flow cytometric analysis. In addition, antibodies are now being designed for therapeutic applications, including suppression of the immune system after organ transplantation (Koch et al., 2002; Bumgardner et al., 2001) treatment of cancers such as leukemia and inhibition of angiogenesis (Stephan et al., 2004).

In 1984, Kohler and Milstein were awarded the Nobel Prize in recognition of the importance of their contribution to the development of means for the production of monoclonal antibodies. Their breakthrough occurred in 1975, when they fused normal antibody-producing B lymphocytes with myeloma tumor cells; resulting in clones of cells they termed hybridomas (Kohler and Milstein, 1975). Since their first production, a whole new era in the study of biotechnology has been opened. The technique-'hybridoma technology'-proved to be general, and a wide range of Monoclonal antibodies have been made which bind to protein, carbohydrate, nucleic acid and hapten antigens, and which even have catalytic activities (Pollack et al., 1986) leading to many practical application for monoclonal antibodies in research and human health care (Knapp, 1989) and to patent disputes (Ekins, 1989). The technology has been improved over the years, particular by pre-selection of antigen-binding B cells and by screening with antigent-coating filters (Gheradi et al., 1990).

Hybridoma technology was first extended by somatic cell genetic, which allowed antibody mutants to be selected (Buggemann et al., 1982), their functional properties to be changed by switching heavy chain constant regions and antibodies to be made with dual specificity (Milstein and Cuello, 1983). Gene technology later revolutionized this potential antibody genes, and can now be altered to order. New vistas appeared, reviving the forgotten excitement of the old discipline, immunochemistry of antibodies. Initially antibody genes were obtained than from hybridoma, cloned into plasmid vectors, and expressed as complete antibody in mammalian cells (Neuberger, 1983) or segment in bacteria (Better et al., 1988). The ready manipulation of genes cutting and pasting of restriction fragments, or by site mutagenesis, has allowed the construction of new antibody reagents and fine mapping of antibody structure-function relationships.
A new approach has been proposed with the identity to bypass hybridoma (Orlandi et al., 1989). Antibody gene are cloned directly from lymphocytes of immunized animals and expressed in bacteria and the antibody produced is screened for binding antigens (Ward et al., 1989). As does hybridoma technology, the process relies on animal immunization to give rise to many antigens-specific cells. In the animal, antibodies of low affinity first produced antigen-induced proliferation of cells, and then high affinity variants are generated by point mutation and selection. Hybridoma technology can immortalized these cells; gene technology can immortalize their genes. In both cases however, it is animal that 'invent' the new molecule.

The *in vitro* tissue-culture method is now widely used for mAb production as compared to the mouse ascites method. But *in vitro* methods have been expensive and time-consuming relative to the costs and time required by the mouse ascites method and often failed to produce the required amount of antibody even with skilled manipulation. Modern *in vitro* methods have increased the success rate to over 90% and have reduced costs (Marx et al., 1997). *In vitro* approaches are driven by advances in antibody library design (Fun, 2007) and these antibodies library is subsequently used for selecting and increasing the affinity of human antibodies displayed on ribosome, phage, bacterial and yeast and mammalian cell derived from human b cells.

More than 100 different monoclonal antibody diagnostic products are currently available (Janis, 1996). These larger quantities are used for routine diagnostic procedures and for therapeutic purposes. The use of monoclonal antibodies (mAb) in biomedical research has been and will continue to be important for the identification of proteins, carbohydrates, and nucleic acids. Their use has led to the elucidation of many molecules that control cell replication and differentiation, advancing our knowledge of the relationship between molecular structure and function. These advances in basic biological sciences have improved our understanding of the host response to infectious-disease agents and toxins produced by these agents, to transplanted organs and tissues, to spontaneously transformed cells (tumors), and to endogenous antigens (National Research Council, 1999).

Monoclonal antibodies (mAb) are important reagents used in biomedical research, microbiological research, in diagnosis of Hepatitis, AIDs, influenza, herpes simplex, and in treatment of such diseases as infections and cancer (Hawkers, 2006). These antibodies are produced by cell lines or clones obtained from animals (*in vivo* and *in vitro*). *In vitro*, monoclonal antibodies form the basis of a number of diagnostic tests. For example, monoclonal antibodies against a hormone can detect pregnancy only 10 days after conception. Specific monoclonal antibodies are used for rapid diagnosis of hepatitis, influenza, herpes simplex, and Chlamydia infections. "Monoclonal antibodies are playing a valuable role in diagnostic medicine in tests to determine the concentration of specific proteins in the blood or urine. For example, an unusually high blood level of a prostate-specific antigen, which is measured by its interaction with a monoclonal antibody, provides an early warning that a man may have developed prostate cancer. Antibodies can also be used in protein purification. When a purified antibody is added to a crude mixture of proteins, the specific protein being sought selectively combines with the antibody and precipitates from solution (Karp, 1996).

PERSPECTIVE

It has been nearly 30 years since Kohler and Milstein

described a technique for immortalizing antibody producing cells, identifying their products and cloning the cells to obtain monoclonal antibodies. Monoclonal antibodies are important diagnostic reagents used in biomedical research and diagnosis of viral and bacterial diseases. The production of monoclonal antibodies is not straight forward or easy but the common production steps are: immunization of mice and selection of mouse donors for generation of hybridoma cells, screening of mice for antibody production, preparation of myeloma cells fusion of myeloma cells with immune spleen cells, cloning of hybridoma cell lines by “limiting dilution” or expansion and stabilization of clones by ascites production purification of monoclonal antibodies, antibody engineering for ligand binding and diagnostic kits preparation. These monoclonal antibodies are produced by *in vitro* and *in vivo* method.

The modern attractiveness of monoclonal antibodies is almost directly related to the advancement of recombinant mAb technology. There are about 100 different diagnostic kits based on mAb technology available in the market. The worldwide markets of the clinical diagnostics are approximately $19 billion, with annual growth rate of nearly 5% per year.

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