

PHARMACOLOGY-III
UNIT-IV
TOPIC: MONOCLONAL ANTIBODIES



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MONOCLONAL ANTIBODIES

Monoclonal antibodies (mAb or moAb) are antibodies that are made by identical immune cells that are all clones of a unique parent cell. Monoclonal antibodies can have monovalent affinity, in that they bind to the same epitope (the part of an antigen that is recognized by the antibody). In contrast, polyclonal antibodies bind to multiple epitopes and are usually made by several different plasma cell (antibody secreting immune cell) lineages. Bispecific monoclonal antibodies can also be engineered, by increasing the therapeutic targets of one single monoclonal antibody to two epitopes.

Outline of production of MABs

The main objective is to produce a homogenous population of MABs against a pre-fixed immunogen. The basic strategy includes

- (i) purification and characterization of the desired antigen in adequate quantity,
 - (ii) immunization of mice with the purified antigen,
 - (iii) culture of myeloma cells which are unable to synthesize hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) enzyme necessary for the salvage pathway of nucleic acids
 - (iv) removal of spleen cells from mice and its fusion with the myeloma cells,
 - (v) following fusion, the hybridomas were grown in hypoxanthine aminopterin thymidine (HAT) medium.
 - The fused cells are not affected in the absence of HGPRT unless their de novo synthesis pathway is also disrupted.
 - In the presence of aminopterin, the cells are unable to use the de novo pathway and thus these cells become auxotrophic for nucleic acids as a supplement to HAT medium. In this medium, only fused cells will grow. Unfused myeloma cell does not have ability to grow in this HAT medium because they lack HGPRT, and thus cannot produce DNA.
 - Unfused spleen cells cannot grow because of their short life spans. Only fused hybrid cells or hybridomas can grow in HAT medium. Hybrid cells have the capacity to grow in the HAT medium since spleen cell partners produce HGPRT.
 - (vi) The hybrid cell clones are generated from single host cells
 - (vii) the antibodies secreted by the different clones are then tested for their ability to bind to the antigen using an enzyme-linked immunosorbent assay (ELISA).
 - (viii) The clone is then selected for future use. Examples of therapeutic monoclonal antibodies
- Monoclonal antibodies for research applications can be found directly from antibody suppliers, or through use of a specialist search engine like CiteAb.

MABs have proved to be extremely valuable for basic immunological and molecular research because of their high specificity. They are used in human therapy, commercial protein purification, suppressing immune response, diagnosis of diseases, cancer therapy, diagnosis of allergy, hormone test, purification of complex mixtures, structure of cell membrane,

identification of specialized cells, preparation of vaccines, and increasing the effectiveness of medical substances

Diagnostic tools in research and laboratory

To detect the presence of this substance/antigen, MAbs can be used. Different technologies in which MAbs are used include Western blot, immunodot blot, ELISA, radioimmuno assay (RIA), flow cytometry, immunohistochemistry, fluorescence microscopy, electron microscopy, confocal microscopy, as well as other biotechnological applications.

Gene cloning

One of the difficulties of gene cloning is identifying the cells that contain the desired gene. If an MAb that recognizes that the gene product is available, it can be used as a probe for detecting those cells that make the product and therapy to detect the gene.

To identify cell types

MAbs contribute to the identification of many different types of cells that participate in the immune response and to unravel interactions occurring during this process. For example, in the lymphocytes with B, T helper (TH) cells and suppressor T, the use of MAbs has established that the various types of T-cells carry cell surface antigens on their surfaces that allow one type to be distinguished from another. The MAbs were also helpful in defining changes in T and B-cells during development.

Protein purification

MAB affinity columns are readily prepared by coupling MAbs to a cyanogen bromide-activated chromatography matrix, eg, Sepharose. Since the MAbs have unique specificity for the desired protein, the level of contamination by unwanted protein species usually is very low. Since the MAB-antigen complex has a single binding affinity it is possible to elute the required protein in a single, sharp peak. The concentration of the relative protein relative to total protein in a mixture can ever be very low. This method also has limitations. Achieving 100% pure protein is difficult because there is always a tendency for small amounts of immunoglobulin to leak off the immune-affinity column. Additionally, MAB do not distinguish between intact protein molecules and fragments containing the antigenic site.

Recombinant

The production of recombinant monoclonal antibodies involves repertoire cloning, CRISPR/Cas9, or phage display/yeast display technologies. Recombinant antibody engineering involves antibody production by the use of viruses or yeast, rather than mice. These techniques rely on rapid cloning of immunoglobulin gene segments to create libraries of antibodies with slightly different amino acid sequences from which antibodies with desired specificities can be selected. The phage antibody libraries are a variant of phage antigen libraries. These techniques can be used to enhance the specificity with which antibodies recognize antigens, their stability in various environmental conditions, their therapeutic efficacy and their detectability in diagnostic applications. Fermentation chambers have been used for large scale antibody production.

Chimeric antibodies

While mouse and human antibodies are structurally similar, the differences between them were sufficient to invoke an immune response when murine monoclonal antibodies were injected into humans, resulting in their rapid removal from the blood, as well as systemic inflammatory effects and the production of human anti-mouse antibodies (HAMA).

Recombinant DNA has been explored since the late 1980s to increase residence times. In one approach, mouse DNA encoding the binding portion of a monoclonal antibody was merged with human antibody-producing DNA in living cells. The expression of this "chimeric" or "humanised" DNA through cell culture yielded part-mouse, part-human antibodies.

Human antibodies

Ever since the discovery that monoclonal antibodies could be generated, scientists have targeted the creation of fully human products to reduce the side effects of humanised or chimeric antibodies. Two successful approaches have been identified: transgenic mice and phage display. Applications

Diagnostic tests

Once monoclonal antibodies for a given substance have been produced, they can be used to detect the presence of this substance. Proteins can be detected using the Western blot and immuno dot blot tests. In immunohistochemistry, monoclonal antibodies can be used to detect antigens in fixed tissue sections, and similarly, immunofluorescence can be used to detect a substance in either frozen tissue section or live cells.

Analytic and chemical uses

Antibodies can also be used to purify their target compounds from mixtures, using the method of immunoprecipitation.

Therapeutic uses

Main article: Monoclonal antibody therapy

Therapeutic monoclonal antibodies act through multiple mechanisms, such as blocking of targeted molecule functions, inducing apoptosis in cells which express the target, or by modulating signalling pathways

Cancer treatment

One possible treatment for cancer involves monoclonal antibodies that bind only to cancer cell-specific antigens and induce an immune response against the target cancer cell. Such mAbs can be modified for delivery of a toxin, radioisotope, cytokine or other active conjugate or to design bispecific antibodies that can bind with their Fab regions both to target antigen and to a conjugate or effector cell. Every intact antibody can bind to cell receptors or other proteins with its Fc region Monoclonal antibodies for cancer. ADEPT, antibody directed enzyme prodrug therapy;

ADCC: antibody dependent cell-mediated cytotoxicity; **CDC**: complement-dependent cytotoxicity; **MAB**: monoclonal antibody; **scFv**, single-chain Fv fragment. MABs approved by the FDA for cancer include

Alemtuzumab

Bevacizumab

Cetuximab

Gemtuzumab ozogamicin

Ipilimumab

Ofatumumab

Panitumumab

Pembrolizumab

Ranibizumab

Rituximab

Trastuzumab

Autoimmune diseases

Monoclonal antibodies used for autoimmune diseases include infliximab and adalimumab, which are effective in rheumatoid arthritis, Crohn's disease, ulcerative colitis and ankylosing spondylitis by their ability to bind to and inhibit TNF- α . Basiliximab and daclizumab inhibit IL-2 on activated T cells and thereby help prevent acute rejection of kidney transplants.