Antibodies: Globulin (roughly spherical in shape and extractable in saline solutions) glycoproteins (proteins with a carbohydrate content ranging from 3 to 13%), produced by the immune system of an organism in response to exposure to a foreign molecule and characterised by its specific binding to a site, related to an epitope of that molecule; induced response proteins.

The antibodies, like all proteins, are formed of chains of amino acids, which undergo very complex packing, giving the proteins a specific and functionally significant final shape (tertiary configuration), which determines most of the characteristics of the protein.

As globulin proteins are involved in immune reactions, antibodies are called immunoglobulins (abbreviated to Ig).

Antiserum: Blood serum containing antibodies arising out of immunisation or after an infectious disease.

Production of antibodies: Antibodies are produced by the lymphocytes. The process of antibody production and immune response are complex and both the lymphatic and the blood systems are very closely involved.

Autoantibodies: In certain pathological conditions, the thymus may produce antibodies to the body's own endogenous proteins (auto-antibodies), which complicates the immune system.

Antithetic antibodies: Antibodies produced against antibodies; antithetic antibodies have properties similar to those of the antigens.

Polyclonal antibodies: antibodies produced by molecules with several different antigenic determinants (epitopes) and/or several different cell populations.

Monoclonal antibodies: antibodies produced against a single antigenic determinant (epitope) and/or by a single cell population; hence are very specific.

Laboratory tests vary widely in clinical immunology. Some are essential for diagnosis while others are useful in subclassifying disorders. Finally, some are of research interest only but may add to our immunological armamentarium in the future. In this regard, it is important to understand that these tests do vary in their sensitivity and specifi city.

The sensitivity of a test is defined as the number of diseased individuals that are positive for the test compared with those who are negative.

The specificity of a test is the proportion of individuals without a given disease that are negative.

Some assays are quantitative in that they produce precise results. Many of these assays are automated and can be related to international standards. Qualitative assays are less specific and will give answers such as normal abnormal, or positive–negative results.

A. Physiology Immunoglobulins, or antibodies, are glycoproteins present in the gamma globulin fraction of serum.

B. Immunology Immunoglobulins are produced by B lymphocytes (B cells) or plasma cells in response to exposure to as antigen. They react specifically with that antigen in vivo or in vitro and are hence a part of the adaptive immune responsespecifically, humoral immunity.

ANTIBODY PRODUCTION

a. Polyclonal antibodies: Many mammals have been used to produce antibodies, ranging from the horse, sheep, and goat down to mice and guinea pigs. Often an animal species is

selected for antibody production because it will produce less cross-reactive antibodies to a given tissue.
Larger mammals, such as goats and sheep, are used to obtain larger volumes of serum to be used therapeutically in humans.
Polyclonal antibodies are antibodies that are secreted by different B cell lineages within the body . They are a collection of immunoglobulin molecules that react against a specific antigen, each identifying a different epitope.
An **epitope**, also known as **antigenic determinant**, is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells. For example, the epitope is the specific piece of the antigen to which an antibody binds. The part of an antibody that binds to the epitope is called a paratope. Although epitopes are usuallynon-self proteins, sequences derived from the host that can be recognized (as in the case of autoimmune diseases) are also epitopes.
Production
The general procedure to produce polyclonal antibodies is as follows:
1. Antigen preparation
2. Adjuvant selection and preparation
3. Animal selection
4. Injection process
5. Blood serum extraction

An antigen/adjuvant conjugate is injected into an animal of choice to initiate an amplified immune response. After a series of injections over a specific length of time, the animal is expected to have created antibodies against the conjugate. Blood is then extracted from the animal and then purified to obtain the antibody of interest.
Inoculation is performed on a suitable mammal, such as a mouse, rabbit or goat.Larger mammals are often preferred as the amount of serum that can be collected is greater. Anantigen is injected into the mammal. This induces the B-lymphocytes to produce IgG immunoglobulins specific for the antigen. This polyclonal IgG is purified from the mammal'sserum.
The primary goal of antibody production in laboratory animals is to obtain high titer, high affinity antisera for use in experimentation or diagnostic tests. Adjuvants are used to improve or enhance an immune response to antigens. Most adjuvants provide for an injection site, antigen depot which allows for a slow release of antigen into draining lymph nodes.
Many adjuvants also contain or act directly as:
1. surfactants which promote concentration of protein antigens molecules over a large surface area, and
2. immunostimulatory molecules or properties. Adjuvants are generally used with soluble protein antigens to increase antibody titers and induce a prolonged response with accompanying memory.

Such antigens by themselves are generally poor immunogens. Most complex protein antigens induce multiple B-cell clones during the immune response, thus, the response is polyclonal. Immune responses to non-protein antigens are generally poorly or enhanced by adjuvants and there is no system memory.

Animal selection

Animals frequently used for polyclonal antibody production include chickens, goats, guinea pigs, hamsters, horses, mice, rats, and sheep. However, the rabbit is the most commonly used laboratory animal for this purpose. Animal selection should be based upon:

1. the amount of antibody needed,
2. the relationship between the donor of the antigen and the recipient antibody producer (generally the more distant the phylogenetic relationship, the greater the potential for high titer antibody response) and

Goats or horses are generally used when large quantities of antisera are required. Many investigators favor chickens because of their phylogenetic distance from mammals. Chickens transfer high quantities of IgY (IgG) into the egg yolk and harvesting antibodies from eggs eliminates the need for the invasive bleeding procedure. One week's eggs can contain 10 times more antibodies than the volume of rabbit blood obtained from one weekly bleeding. However, there are some disadvantages when using certain chicken derived antibodies in immunoassays. Chicken IgY does not fix mammalian complement component C1 and it does not perform as a precipitating antibody using standard solutions.

Although mice are used most frequently for monoclonal antibody production, their small size usually prevents their use for sufficient quantities of polyclonal, serum antibodies. However, polyclonal antibodies in mice can be collected from ascites fluid using any one of a number of ascites producing methodologies.

When using rabbits, young adult animals should be used for primary immunization because of the vigorous antibody response. Immune function peaks at puberty and primary responses to new antigens decline with age. Female rabbits are generally preferred because they are more docile and are reported to mount a more vigorous immune response than males. At least two animals per antigen should be used when using outbred animals. This principle reduces potential total failure resulting from non-responsiveness to antigens of individual animals.

Antigen preparation

The size, extent of aggregation and relative nativity of protein antigens can all dramatically affect the quality and quantity of antibody produced. Small polypeptides (<10 ku) and non-protein antigens generally need to be conjugated or crosslinked to larger, immunogenic, carrier proteins to increase immunogenicity and provide T cell epitopes. Generally, the larger the immunogenic protein the better. Larger proteins, even in smaller amounts, usually result in better engagement of antigen presenting antigen processing cells for a satisfactory immune response. Injection of soluble, non-aggregated proteins has a higher probability of inducing tolerance rather than a satisfactory antibody response.

Advantages

1-They're inexpensive to make and can be generated fairly quickly, taking up to several months to produce.

2-PAbs are heterogeneous, which allows them to bind to a wide range of antigen epitopes. Because PAbs are produced from a large number of B cell clones, they're more likely to successfully bind to a specific antigen.

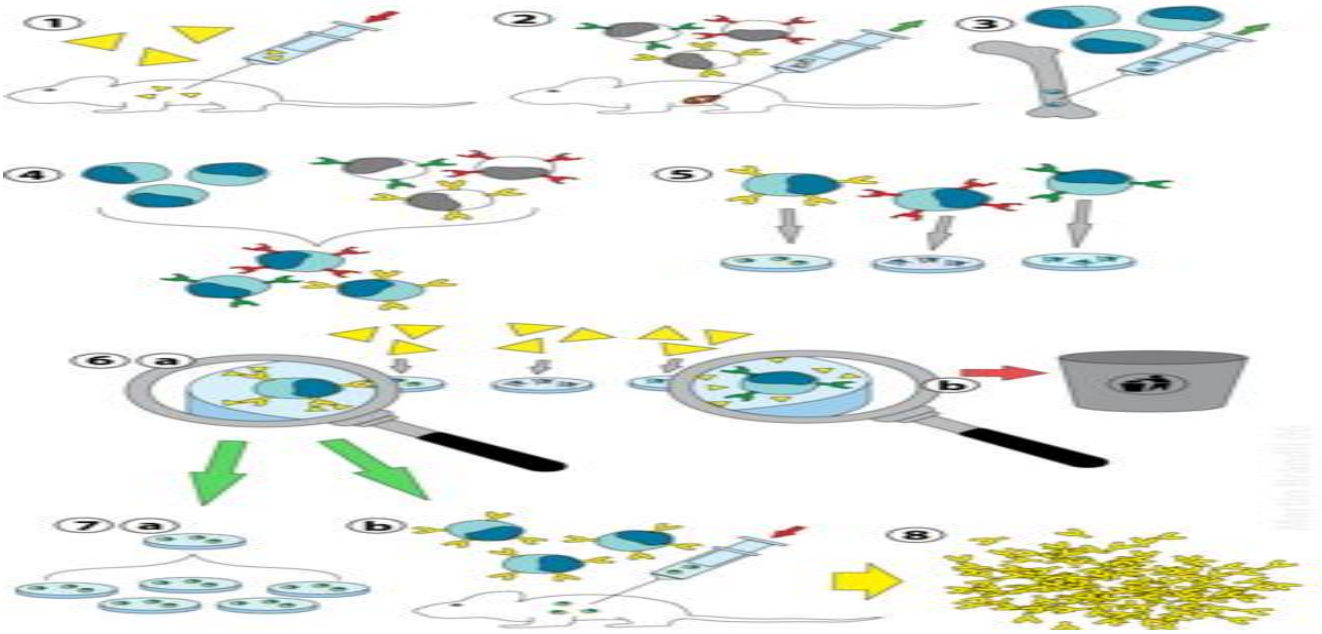
3-PAbs remain stable in different environments, such as a change in pH or salt concentration, which allows them to be more applicable in certain procedures.
4-Additionally, depending on the amount needed, PAbs can be made in large quantities in relation to the size of the animal used.

Monoclonal antibodies are homogenous 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 anantigen 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. Given almost any substance, it is possible to produce monoclonal antibodies that specifically bind to that substance; they can then serve to detect or purify that substance. This has become an important tool in biochemistry, molecular biology, andmedicine. When used as medications, non-proprietary drug names end in **-mab** (see "Nomenclature of monoclonal antibodies") and many immunotherapy specialists use the word **mab** anacronymically.

Hybridoma technology is a method for producing large numbers of identical antibodies (also called monoclonal antibodies). This process starts by injecting a mouse (or other mammal) with an antigen that provokes an immune response. A type of white blood cell, the B cell that produces antibodies that bind to the antigen are then harvested from the mouse. These isolated B cells are in turn fused with immortal B cell cancer cells, a myeloma, to produce a hybrid cell line called a**hybridoma**, which has both the antibody-producing ability of the B-cell and the exaggerated longevity and reproductivity of the myeloma. The hybridomas can be grown in culture, each culture starting with one viable hybridoma cell, producing cultures each of which consists of genetically identical hybridomas which produce one antibody per culture (monoclonal) rather than mixtures of different antibodies (polyclonal). The myeloma cell line that is used in this process is selected for its ability to grow in tissue culture and for an absence of antibody synthesis. In contrast to polyclonal antibodies, which are mixtures of many different antibody molecules, the monoclonal antibodies produced by each hybridoma line are all chemically identical.

Laboratory animals (mammals, e.g. mice) are first exposed to the antigen that an antibody is to be generated against. Usually this is done by a series of injections of the antigen in question, over the course of several weeks. These injections are typically followed by the use of in vivo electroporation, which significantly enhances the immune response. Once splenocytes are isolated from the mammal's spleen, the B cells are fused with

immortalised myeloma cells. The fusion of the B cells with myeloma cells can be done using electrofusion. Electrofusion causes the B cells and myeloma cells to align and fuse with the application of an electric field. Alternatively, the B-cells and myelomas can be made to fuse by chemical protocols, most often using polyethylene glycol. The myeloma cells are selected beforehand to ensure they are not secreting antibody themselves and that they lack the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) gene, making them sensitive to the HAT medium (see below).
Fused cells are incubated in HAT medium (hypoxanthine-aminopterin-thymidine medium) for roughly 10 to 14 days. Aminopterin blocks the pathway that allows for nucleotide synthesis. Hence, unfused myeloma cells die, as they cannot produce nucleotides by the salvage pathways because they lack HGPRT. Removal of the unfused myeloma cells is necessary because they have the potential to outgrow other cells, especially weakly established hybridomas. Unfused B cells die as they have a short life span. In this way, only the B cell-myeloma hybrids survive, since the HGPRT gene coming from the B cells is functional. These cells produce antibodies (a property of B cells) and are immortal (a property of myeloma cells). The incubated medium is then diluted into multi-well plates to such an extent that each well contains only one cell. Since the antibodies in a well are produced by the same B cell, they will be directed towards the same epitope, and are thus monoclonal antibodies.
The next stage is a rapid primary screening process, which identifies and selects only those hybridomas that produce antibodies of appropriate specificity. The first screening technique used is called ELISA. The hybridoma culture supernatant, secondary enzyme labeled conjugate, and chromogenic substrate, are then incubated, and the formation of a colored product indicates a positive hybridoma. Alternatively, immunocytochemical screening can also be used.



(1) Immunisation of a mouse **(2)** Isolation of B cells from the spleen **(3)** Cultivation of myeloma cells **(4)** Fusion of myeloma and B cells **(5)** Separation of cell lines**(6)** Screening of suitable cell lines **(7)** *in vitro* **(a)** or *in vivo* **(b)** multiplication **(8)** Harvesting

The B cell that produces the desired antibodies can be cloned to produce many identical daughter clones. Supplemental media containing interleukin-6 (such as briclone) are essential for this step. Once a hybridoma colony is established, it will continually grow in culture medium like RPMI-1640 (with antibiotics and fetal bovine serum) and produce antibodies.
Multi well plates are used initially to grow the hybridomas, and after selection, are changed to larger tissue culture flasks. This maintains the well-being of the hybridomas and provides enough cells for cryopreservation and supernatant for subsequent investigations. The culture supernatant can yield 1 to 60 µg/ml of monoclonal antibody, which is maintained at -20 °C or lower until required.
By using culture supernatant or a purified immunoglobulin preparation, further analysis of a potential monoclonal antibody producing hybridoma can be made in terms of reactivity, specificity, and cross-reactivity.

Applications

Diagnostic tests

Once monoclonal antibodies for a given substance have been produced, they can be used to detect the presence of this substance. The Western blot test and immuno dot blottests detect the protein on a membrane. They are also very useful in immunohistochemistry, which detect antigen in fixed tissue sections and immunofluorescence test, which detect the substance in a frozen tissue section or in live cells.

Analytic and chemical uses

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

Therapeutic treatment 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.[38][39]

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.

Autoimmune diseases Monoclonal antibodies used for autoimmune diseases 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. Omalizumab inhibits human immunoglobulin E (IgE) and is useful in moderate-to-severe allergic asthma.

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. Below are examples of clinically important monoclonal antibodies.