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MONOCLONAL ANTIBODIES

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MONOCLONAL ANTIBODIES

Monoclonal antibodies (MAB or MOAB) are identical immunoglobulins, generated from a single b-cell clone. These antibodies recognize unique epitopes, or binding sites, on a single antigen. Derivation from a single b-cell clones and subsequent targeting of a single epitope is what differentiates monoclonal antibodies from polyclonal antibodies.

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, and medicine.

Characters of monoclonal antibodies:

- Monoclonal antibodies (MAB) are single type of antibody that are identical and are directed against a specific epitope (antigen, antigenic determinant) and are produced by b-cell clones of a single parent or a single hybridoma cell line.
- A hybridoma cell line is formed by the fusion of one b-cell lymphocyte with a myeloma cell. Some myeloma cell synthesizes single MAB antibodies naturally.

Production of monoclonal antibodies:

- Monoclonal antibodies are invariably produced from hybridoma clones; whereas each hybridoma clones are meticulously derived by the actual fusion of a myeloma cell together with an antibody producing lymphocytes, and ultimately the hybridoma clone producing the desired antibody is adequately isolated and subsequently identified
- In actual practice the hybridoma cells are mass cultured for the overall production of mabs with the help of one of the following two methods namely:

➢ Culture in peritoneal cavity i.e. in-vivo peritoneal cavity of mice and
➢ Mass in-vitro culture i.e. in-vitro large scale culture vessels

Culture in peritoneal cavity:

- In this developed, tested and tried methodology the hybridoma cells are strategically transplanted into the peritoneal cavity of a suitable and highly purified strain of mice and subsequently the ascetic fluid derived from the animals in duly harvested and the mabs are purified meticulously.

- Importantly, this particular technique positively yields between 50-100 times higher quantum of the desired antibody in comparison to the usual traditional in vitro culture of the hybridomas.

Following characteristic features of this technique, namely:

- Generally the ensuing antibody preparation happen to be a lower purity than those obtained from the corresponding cell cultures, particularly if, serum-free media are employed,
- Methodology involve is predominately a labor-intensive one, and
- Unconditionally and absolutely pathogen-free animals of particular genotypes are essentially required.

Mass in vitro culture:

One may accomplish the large-scale culture of the hybridoma cells by adopting any one of the methodologies namely

- Bioreactor with frequent stirring device
- Aircraft fermenters
- Specific immobilized cells are responsible for the progressive cultivation of the cells at very high densities that markedly increase the production of antibody in vivo

Examples:

- Two typical examples to expatiate the above process i-e, mass in vitro culture, namely:
- Hollow fiber cartridges (i-e. a culture system) – found to yield 40 g mabs per month
- Special ceramic cartridges (i-e. an optical system) – found to yield 50 g mabs per day

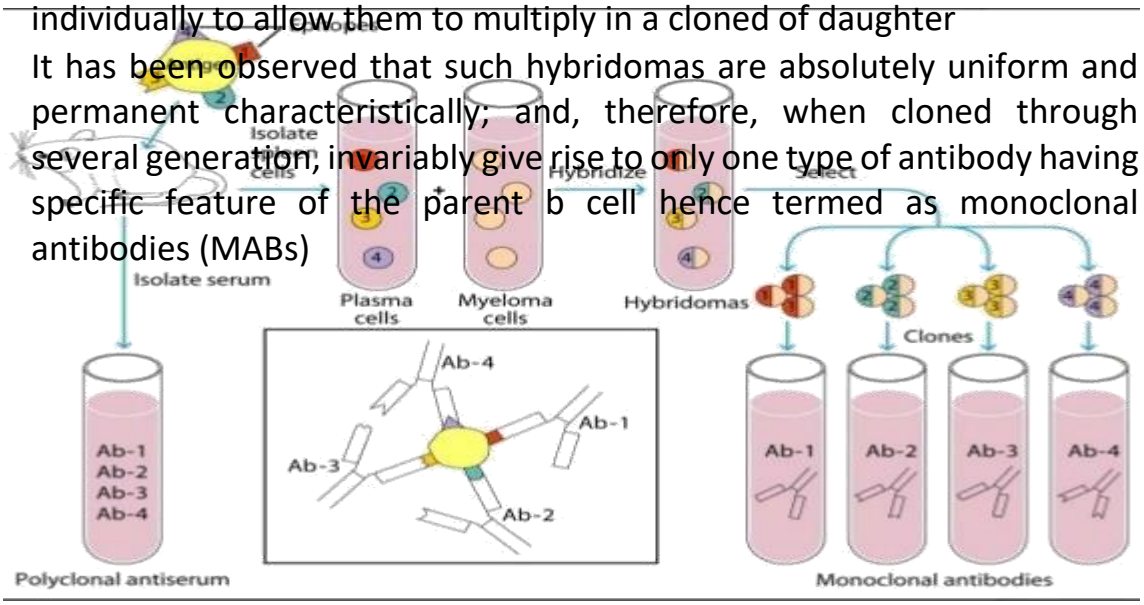
Future scope:

- An extensive and intensive research towards the futuristic developments and progress in the area of immobilized culture systems may ultimately give rise to an increased the production of MABs on in a significant manner and therapy markedly and pronouncedly minimize the cost of their mass production from the cell culture.

Production:

- The various step that are intimately involved in the production of monoclonal anti (MABs) are represented sequential as follows
- A very specific antigen (immunogen) comprising of four epitopes was injected into mice where b cells have already commenced generation antibodies against that antigen.

- The same mice (pure strain of albino mice), received another 'booster dose' of the same antigen so as to accomplish a much desired ' secondary response'
- The spleen of the treated mice was duly removed after a gap of 3-4 days that essentially comprised of b cells active enough in the process of synthesizing 'specific antibodies'.
- The isolated spleen was adequately macerated and the resulting spleen cells thus obtained in the form of a suspension consisting of b cells giving rise to four distinct cell lines i.e., one cell line representing a specific antigenic determinant (epitope).
- The resulting spleen cells were meticulously mixed with the myeloma cells of the mice derived from the bone marrow and incubated in a culture medium containing polyethylene glycol (peg).
- Quite a few of the spleen cells were adequately fused with neoplasm (tumor) cells to result into the formation of hybrid myeloma cells.
- The spleen cells thus obtained ate hypoxanthine phosphoribosyl transferase (HPRT)- positive and fuse with myeloma cells to gives rise to hybridomas besides utilize hypoxanthine categorically to generate purine and pyrimidines.
- The hybrid myeloma cell does survive and continue to multiply indefinitely thereby producing a good number of specific antibodies against specific antigens
- Each hybridomas cell is isolated meticulously and duly cultured individually to allow them to multiply in a cloned of daughter
- It has been observed that such hybridomas are absolutely uniform and permanent characteristically; and, therefore, when cloned through several generation, invariably give rise to only one type of antibody having specific feature of the parent b cell hence termed as monoclonal antibodies (MABs)



List and types of monoclonal antibodies (FDA approved):

List of examples some FDA-approved monoclonal antibody drugs.

- Abciximab (reopro)
- adalimumab (humira, amjevita)
- alefacept (amevive)
- Alemtuzumab (campath)
- Basiliximab (simulect)
- belimumab (benlysta)
- Each monoclonal antibody listed above has a role in treating a targeted disease (for example, basiliximab treats transplant rejection while belimumab treats systemic lupus erythematosus

Side effects of monoclonal antibodies:

These side effects are compiled from side effects listed for several monoclonal antibodies. Each type of monoclonal antibody has its own side effect profile and may or may not cause some of the side effects listed here.

Common side effects of monoclonal antibodies include:

- Allergic reactions
  - Chills
  - Weakness
  - Diarrhea
  - Vomiting
  - Rash
  - Itching
  - High blood glucose levels
  - Cough

Other side effects of monoclonal antibodies include:

- **Shortness of breath**
  - Peripheral edema
  - Headache
  - Fever
  - Muscle aches and pain
  - Decreased of appetite
  - Insomnia
  - Abdominal pain
  - Back pain

Serious side effects of monoclonal antibodies may include one or more of the following:

- **Serious infections**

- Cancer
- Serum sickness
- CHF
- Generation of antibodies
- Enterocolitis
- Gastrointestinal perforation
- Mucositis
- Stomatitis
- Anemia
- Reduced white blood cell counts
- Hypothyroidism

Drugs or other compounds interact with monoclonal antibodies:

- Serious infections are more likely to occur when monoclonal antibodies are combined with other drugs that suppress the immune system (for example, steroids).
- Another example of drug interaction is that the drug methotrexate reduces the absorption of adalimumab (monoclonal antibody) by 29%-49%, but no adjustments to the dose of adalimumab need to be made when methotrexate is given concomitantly.
- Monoclonal antibodies may interfere with the effectiveness of vaccines. Live vaccines, including attenuated vaccines, should not be used while patients are being treated with monoclonal antibodies. Patients should complete all recommended immunizations prior to receiving monoclonal antibodies

Formulations of monoclonal antibodies are available:

Monoclonal antibodies are designed to be administered by injection. They are supplied as:

- Lyophilized powder for reconstitution
- Solution for injection

Advantages of using Monoclonal Antibodies:

- Though expensive, monoclonal antibodies are cheaper to develop than conventional drugs because it is based on tested technology.
- Side effects can be treated and reduced by using mice-human hybrid cells or by using fractions of antibodies.
- They bind to specific diseased or damaged cells needing treatment.
- They treat a wide range of conditions.

Disadvantages of using Monoclonal Antibodies:

- Time consuming project - anywhere between 6 -9 months.
- Very expensive and needs considerable effort to produce them.
- Small peptide and fragment antigens may not be good antigens-monoclonal antibody may not recognize the original antigen.

- Hybridoma culture may be subject to contamination.
- System is only well developed for limited animal and not for other animals.
- More than 99% of the cells do not survive during the fusion process – reducing the range of useful antibodies that can be produced

APPLICATIONS OF MONOCLONAL ANTIBODIES:

Diagnostic Applications:

- Monoclonal antibodies have revolutionized the laboratory diagnosis of various diseases. For this purpose, MAbs may be employed as diagnostic reagents for biochemical analysis or as tools for diagnostic imaging of diseases.

A. MAbs in Biochemical Analysis:

- Diagnostic tests based on the use of MAbs as reagents are routinely used in radioimmunoassay (RIA) and enzyme-linked immunosorbent assays (ELISA) in the laboratory. These assays measure the circulating concentrations of hormones (insulin, human chorionic gonadotropin, growth hormone, progesterone, thyroxine, triiodothyronine, thyroid stimulating hormone, gastrin, renin), and several other tissue and cell products.

(A) Hormonal disorders:

Hormonal disorders analysis of thyroxine, triiodothyronine and thyroid stimulating hormone for thyroid disorders.

(B) Infectious diseases:

Infectious diseases by detecting the circulatory levels of antigens specific to the infectious agent e.g., antigens of Neisseria gonorrhoeae and herpes simplex virus for the diagnosis of sexually transmitted diseases.

(C) Cancers:

Cancers estimation of plasma carcinoembryonic antigen in colorectal cancer, and prostate specific antigen for prostate cancer. Besides diagnosis, estimation of tumor markers is also useful for the prognosis of cancers.

(D) Pregnancy:

Pregnancy by detecting the urinary levels of human chorionic gonadotropin.

(B) MAbs in Diagnostic Imaging:

- Radiolabeled—MAbs are used in the diagnostic imaging of diseases, and this technique is referred to as immunoscintigraphy. The radioisotopes commonly used for labeling MAb are iodine—131 and technetium—99. The MAb tagged with radioisotope are injected intravenously into the patients.
- These MAbs localize at specific sites (say a tumor) which can be detected by imaging the radioactivity. In recent years, single photon emission computed tomography (SPECT) cameras are used to give a

more sensitive three dimensional appearance of the spots localized by radiolabeled— MAbs.

- Immunoscintigraphy is a better diagnostic tool than the other imaging techniques such as CT scan, ultrasound scan and magnetic resonance. For instance, immunoscintigraphy can differentiate between cancerous and non-cancerous growth, since radiolabeled—MAbs are tumor specific. This is not possible with other imaging techniques. Monoclonal antibodies are successfully used in the diagnostic imaging of cardiovascular diseases, cancers and sites of bacterial infections.

Therapeutic Applications:

- Monoclonal antibodies have a wide range of therapeutic applications. MAbs are used in the treatment of cancer, transplantation of bone marrow and organs, autoimmune diseases, cardiovascular diseases and infectious diseases.

1. **MAbs in drug delivery:**
   The drugs can be coupled with MAb (directed against a cell surface antigen of the cells, say a tumor) and specifically targeted to reach the site of action
2. **MAbs in the dissolution of blood clots:** Tissue plasminogen activator (tPA) can be used as a therapeutic agent to remove the blood clots. A monoclonal antibody directed against fibrin can be coupled to tPA and used for degradation of blood clots.
3. **Protein Purification:**
   Monoclonal antibodies can be produced for any protein. And the so produced MAb can be conveniently used for the purification of the protein against which it was raised. MAbs columns can be prepared by coupling them to cyanogen bromide activated Sepharose (chromatographic matrix). The immobilized MAbs in this manner are very useful for the purification of proteins by immunoaffinity method.
4. **Autoantibody Fingerprinting:**
   The occurrence of autoantibodies and their involvement in certain diseases is well known (e.g. rheumatic arthritis). A new category of individual specific (IS) autoantibodies have been discovered in recent years. These IS-autoantibodies are produced after birth and reach maximum in number by 2 years, and then remain constant for the later part of life.
5. Monoclonal antibodies produced against IS-autoantibodies can be used for their detection, and identification of individuals. This technique referred to as autoantibody fingerprinting, is particularly useful for the detection of criminals, rapists etc. The autoantibodies collected from samples such as blood, saliva, semen and tears can be used