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DEVELOPMENT OF AN ANTIBODY: A REVIEW

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Abstract:

In a normal human body a balance environment is present. It is because of healthy immune system present in the body. Immune system is a complex network of cells and proteins which have ability to defend the infection acquired in body. As a result a healthy body has the ability to defeat the invading micro-organism. The invasion of microorganism is recognized with the help of developed antigens in body. In order to defend these foreign substances, antigens stimulates the production of antibodies. Monoclonal antibodies have approved applications in different types of critical and acute illness. It act as a promising treatment in the critical illness like cancer of any organ. Nowadays they are also used in treatment of infectious disorders like bacterial, viral and fungal infection. Antibodies can be prepared by different methods but large scale production of antibodies is done with the help of Hybridoma technology. In hybridoma technology clones of antibodies are prepared. Antigen firstly fused with B lymphocyte cell and then an hybrid cell is prepared in collaboration with myeloma cell to produce a defense mechanism in the form of antibodies. The main focus of writing this review is to access knowledge about what antibodies are and how they are developed for treatment of critical disorders in current scenario.

Keywords: Antigen, Antibody, Monoclonal Antibody, Hybridoma technique.

Introduction:

Nowadays there is wide variety of critical illness available which cannot be treated effectively with the help of drugs. As a result patient compliance towards such disorders is very less. In the early 19th century if a person suffers from such type of critical illness like cancer, some pandemic disorders etc then chance of recovery was very less⁽¹⁾

But in last three decades that is around 1986 an effective treatment in the form of monoclonal antibody was developed. In 1986, the first monoclonal antibody is approved by US FDA which gave a boom to antibody development industry. Nowadays treatment of cancer is done effectively with the help of various types of approved antibodies. As a result a new way to treat such types of critical illness has been approved. ⁽²⁾

Antibodies are regarded as immunoglobulin which is produced as protective proteins by body in response to invasion of foreign particles called antigen. For every antigen, a particular antibody is produced. An antigen can be of different types like it could be bacteria, virus, fungi, venom or toxin ⁽³⁾

Antigen- Antibody Interaction:

When a foreign substances attacks human body in the form of antigen, antibody is produced in body with the help of specialized white blood cells called B lymphocytes (B cells). Antigen binds to the surface of B cells and help to stimulate antigen to multiple and produce clone. On multiplication mature b cells are produced which are regarded as plasma cells. These plasma cells finally secrete millions of antibodies in order to activate defense mechanism towards a particular disease. When antibodies circulate in blood stream then it attacks the similar antigen and neutralizes them in order to make the infected body healthy as soon as possible⁽⁴⁾

Isotopes of Antibody:

There are various types of antibody isotopes produced in response to invasion of different types of foreign particles. Some of them are as follows:

1. IgA: It is found in mucosal areas such as gut, respiratory tract etc.
2. IgD: It is used to stimulate basophile and mast cells.
3. IgE: It is used to bind allergens in body. It is used to protect against parasite.
4. IgG: It is available in four forms and all of them helps in protection against pathogens.
5. IgM: It tries to eliminate pathogens at an early stage before IgG gets activated in the body⁽⁵⁾

Functions of Antibody in body:⁽⁶⁾

- Neutralization: These antibodies work by blocking the bacterial body to render its attack ineffective.
- Agglutination: These antibodies try to attract foreign particles for Phagocytosis.
- Precipitation: They attack foreign particles by clumping it into serum antigen solution.
- Lysis of foreign cell
- Induction of inflammation by attracting inflammatory cells.

Monoclonal Antibodies:

The antibodies which are made from identical immune cells which are clones to unique parent cell are called monoclonal antibodies. They are regarded as monoclonal because they bind to single epitope means they try to bind only a particular surface of antigen which can be easily recognized by antibody. Due to this reason nowadays special types of antibodies are being developed which bind to specific target in order to cure disease.

Monoclonal antibodies play an important role in branch of biochemistry, molecular biology and medicine. They are different from polyclonal antibodies because polyclonal antibodies have the ability to bind with multiple epitopes of antigen at the same time⁽⁷⁾

Evolution of monoclonal antibodies:

- At the beginning of 20th century, Paul Ehrlich invented selectively targets magic bullets named monoclonal antibodies.
- In 1970, the structure of antigen and antibodies are studied for treatment of cancer.
- In 1973, Jerrold Schaber, first introduced complex human-mouse monoclonal antibodies for treatment of cancer.
- In 1975, Cesar milestone, introduced the hybridoma technique for fusion of myeloma cells with B lymphocytes.
- In 1988, humanized monoclonal antibodies are developed.
- In 2018, James P Alision received Nobel Prize for discovery of cancer treatment with the help of hybrid monoclonal antibodies⁽⁸⁾

Development of Antibody:

There are various methods to develop monoclonal antibodies on large scale. Some of the methods are Hybridoma technology, Novel Mab development technology, antibody heterogeneity, purification, recombinant technology, chimeric antibodies, human antibodies etc.

In all the above techniques hybridoma technology is the most widely used and efficient techniques for large scale production of antibodies⁽⁹⁾

Steps involved in hybridoma technique:

Following steps are involved in production of monoclonal antibodies by hybridoma technique:

1. Cell fusion:

In this step polyethylene glycol or electro-fusion is used to induce cell fusion. Polyethylene glycol fuses with the single cell membrane of myeloma which leads to formation of single cell with two nuclei. This membrane dissolves before mitosis. In another method of electro-fusion, the adjacent membranes of cells are fused with the help of pulsative electric field.

2. Hybridoma screening:

After efficient fusion with the help of polyethylene glycol, only 1 in 100000 cells fuse properly to prepare perfect hybridoma. As a result large number of unfused cells is present in the culture.

As compared to other cells only myeloma cells have the tendency to survive on HAT (Hypoxanthine Aminopterin Thymidine) medium because they have tendency to replicate as much as possible. The myeloma cells contain an enzyme named HGPRT (Hypoxanthine guanine phosphoribosyl transferase).by presence of this enzyme the myeloma cells survive in HAT medium.

3. Monoclonal antibody Production:

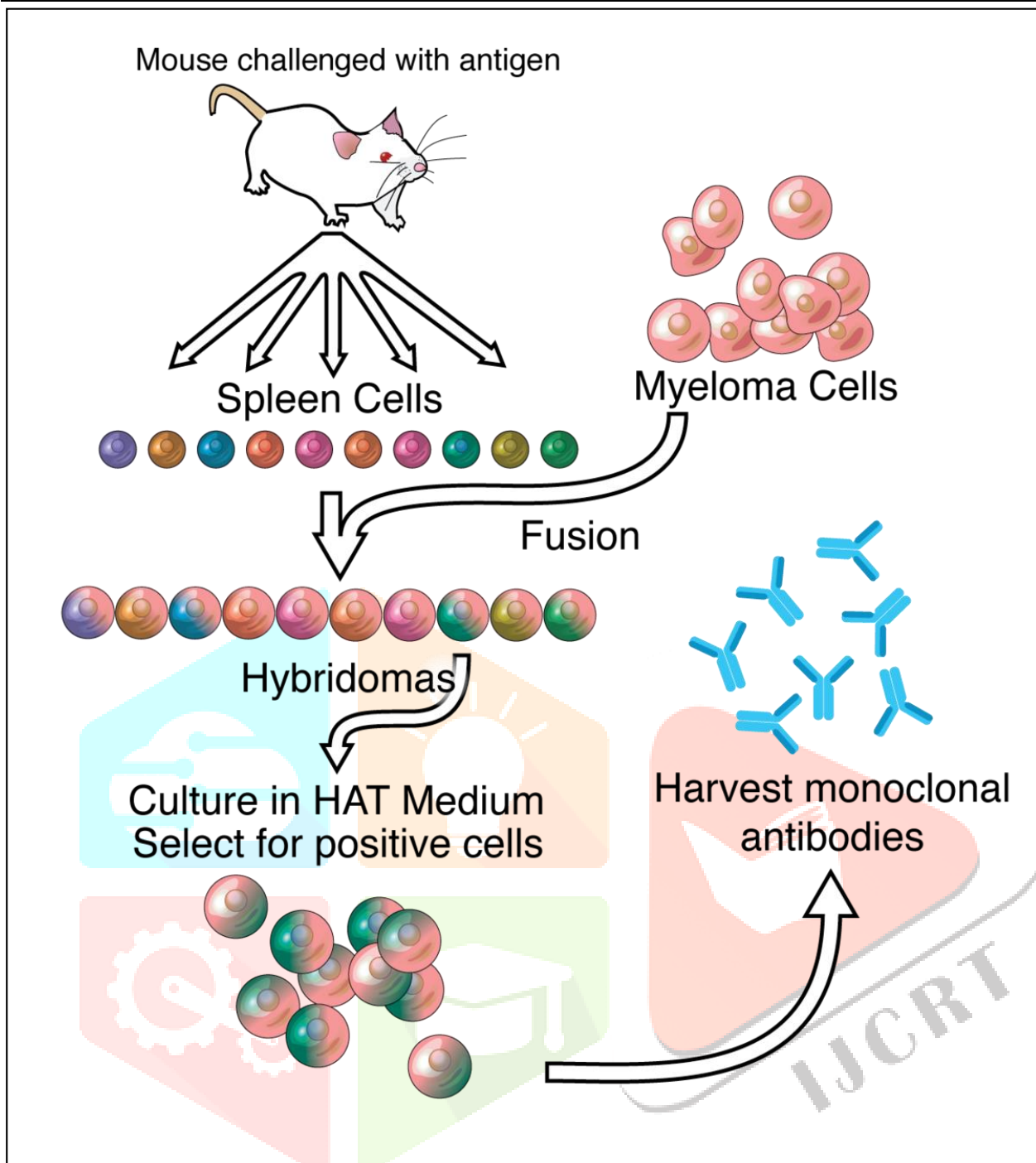
The production of monoclonal antibody can be done in vitro and in vivo.

(a) In vitro production of antibodies:

Take a well established tissue culture plate which is activated for 24 hours. Then take a flask which contains suitable culture medium. The cell density of flask is maintained between 10^5 cells/ml. A perfect culture medium is used to produce $100\mu\text{g/ml}$ of antibody. By this method a pure version of antibody is produced.

(b) In vivo Production

For producing monoclonal antibodies in vivo, mice are primed by intraperitoneal injection with $10^5 - 10^7$ hybridoma cells. The rate of growth of the resulting ascites tumor is in general very variable and can be from less than two or more than five weeks. The ascites fluid can be collected from an anaesthetized mouse. It is possible to obtain 10 ml of ascites fluid or more from a mouse by regular tapping. Ascites fluid will be contaminated with mouse immunoglobulins to a small extent and if a very pure antibody is required this may prove inconvenient. ⁽¹⁰⁾



Hybridoma Technology

Applications of monoclonal antibodies:

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 by using the Western blot and immuno dot blot tests. In immuno histochemistry, monoclonal antibodies can be used to detect antigens in fixed tissue sections, and similarly, immuno fluorescence can be used to detect a substance in either frozen tissue section or live cells. (11)

Analytical and chemical uses:

Antibodies can also be used to purify their target compounds from mixtures by using the method of immuno precipitation.

Therapeutic uses:

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 signaling pathways. (12)

Cancer treatment

One of the most possible treatments for cancer involves monoclonal antibodies that bind only to cancer cell-specific antigens and induce an immune response against the target cancer cell. Such monoclonal antibodies can be modified for delivery of a toxin, radioisotope, cytokine or other active conjugate that can bind with surface easily in order to target antigen. Some of the FDA approved monoclonal antibodies for treatment of cancer are: (13)

Monoclonal antibodies approved by the FDA for cancer include:

- Alemtuzumab
- Bevacizumab
- Cetuximab
- Gemtuzumab ozogamicin
- Ipilimumab
- Ofatumumab
- Panitumumab
- Pembrolizumab
- Ranibizumab
- Rituximab
- Trastuzumab

Autoimmune diseases:

Monoclonal antibodies which are used for the treatment of autoimmune disorders are: infliximab and adalimumab. These monoclonal antibodies are used for the treatment of rheumatoid arthritis, Crohn's disease, ulcerative colitis, spondylitis etc.(14)

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