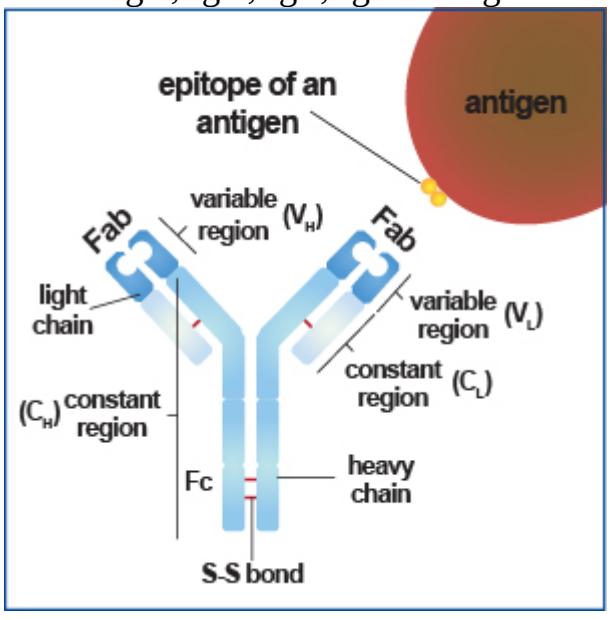
HYBRIDOMA TECHNOLOGY FOR PRODUCTION OF MONOCLONAL ANTIBODIES

An **antibody**, also known as an immunoglobulin (Ig), is a protein that is produced by plasma cells after stimulation by an antigen. Antibodies are the functional basis of humoral immunity. Antibodies occur in the blood, in gastric and mucus secretions, and in breast milk.

Antibodies have a basic structure reminding of the letter Y. The so called heavy chains and light chains, named after their weight, make up the antibody. Heavy chains and light chains are divided into a constant region and variable region where the variable region is what determines the specificity of the antibody and the constant regions determines the class of the antibody. The arms of the antibody are called Fab regions (fragment antigen binding), having a heavy chain and a light chain connected by disulphide bonds.

The stem of the antibody is called Fc region (fragment crystallisable), which is made up of heavy chains. Fc regions are not involved in the specificity of the antibodies, but in effector functions such as binding to cell receptors and class determination of the antibody. The two heavy chains in the Fc region are connected with a disulphide bond that also makes the antibody flexible to increase the chance of good binding to antigens.

There are several sorts of antibodies that differ among species. The different classes of mammalian immunoglobulins are divided into classes named IgA, IgD, IgE, IgG and IgM where IgG is most abundant.



The **high specificity of antibodies** makes them an excellent tool for detecting and quantifying a broad array of targets, from drugs to serum proteins to microorganisms.

If one takes serum from mammalians, the serum will contain lots of different antibodies and the mix is said to contain polyclonal antibodies.
Hybridomas are cells that have been engineered to produce large amounts of monoclonal antibodies.
Monoclonal antibodies can be produced in specialized cells through a technique now popularly known as hybridoma technology.

Monoclonal Antibodies versus Polyclonal Antibodies

Polyclonal antibodies are produced from different lines of B-cells while monoclonal antibodies on the other hand comes from the same line of B-cells. Monoclonal antibodies are clones from one B cell line and thus specific to the same epitope of an antigen**.**

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.

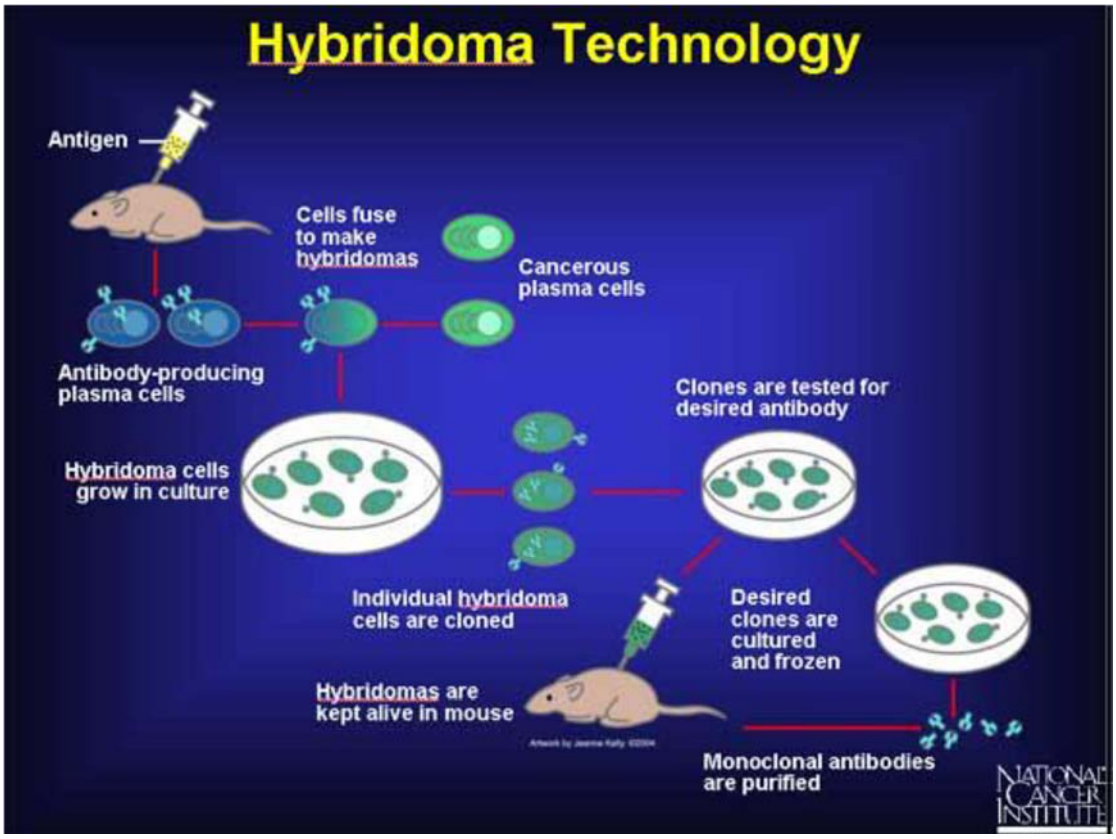
METHADOLOGY

A hybridoma, is produced by the injection of a specific antigen into a mouse, procuring the antigen-specific plasma cells (antibody-producing cell) from the mouse's spleen and the subsequent fusion of this cell with a cancerous immune cell called a myeloma cell.
The hybrid cell, which is thus produced, can be cloned to produce many identical daughter clones. These daughter clones then secrete the immune cell product. Since these antibodies come from only one type of cell (the hybridoma cell) they are called monoclonal antibodies. The advantage of this process is that it can combine the qualities of the two different types of cells; the ability to grow continually, and to produce large amounts of pure antibody.
HAT medium (Hypoxanthine Aminopetrin Thymidine) is used for preparation of monoclonal antibodies.
Laboratory animals (eg. mice) are first exposed to an antigen to which we are interested in isolating an antibody against. Once splenocytes are isolated from the mammal, the B cells are fused with immortalized myeloma cells .
Fused cells are incubated in the HAT (Hypoxanthine Aminopetrin Thymidine) medium.

Practical steps for production

1. Immunize animal
2. Isolate spleen cells (containing antibody-producing B cell)
3. Fuse spleen cells with myeloma cell

4. Allow unfused B cell to die
5. Add aminopterin to culture and kill unfused myeloma cells
6. Clone remaining cells (place 1 cell/well and allow each cell to grow into a clones of cell)
7. Screen supernatant of each clone for presence of desired antibody
8. Grow chosen clone of cells in tissue culture indefinitely
9. Harvest antibody from the culture.



Applications of monoclonal antibodies

1.**Diagnostic Applications**

a. Biochemical analysis

• 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, thyroid stimulating hormone) and several **other tissue and cell products** (blood group antigens, blood clotting factors, interferon's, interleukins, tumor markers).

Eg. Pregnancy by detecting the urinary levels of human chorionic gonadotropin.

Hormonal disorders analysis of thyroxine.

Cancers estimation of plasma carcinoembryonic antigen in colorectal cancer, and prostate specific antigen for prostate cancer

b. 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.

• Myocardial infarction and atherosclorosis .

2. Therapeutic applications

Direct use of MAbs as therapeutic agents

• **In destroying disease-causing organisms:** MAbs promote efficient opsonization of pathogenic organisms (by coating with antibody) and enhance phagocytosis.

• **In the immunosuppression of organ transplantation:** In the normal medical practice, immunosuppressive drugs such as cyclosporin and prednisone are administered to overcome the rejection of organ transplantation. In recent years, MAbs specific to T-lymphocyte surface antigens are being used for this purpose

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 immune-affinity method.

• There are certain advantages of using MAbs for protein purification. These include the specificity of the MAb to bind to the desired protein, very efficient elution from the chromatographic column and high degree of purification.

Advantages of using Monoclonal Antibodies:

• In some cases, 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 damaged cells needing treatment.
• They treat a wide range of diseases.

Disadvantages of using Monoclonal Antibodies:
• Time consuming project – between 6 -9 months.
• Very expensive and needs considerable effort to produce them.
• 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 against an antigen

Monoclonal Antibodies in Cancer Therapy

Cancer is non-normal and uncontrolled cell division that often spread to other tissues or locations within the body. It has long been treated with surgery, radiotherapy and chemotherapy and surgery being most effective to treat cancer. But since surgery is invasive, radio- and chemotherapy are often chosen but needs several treatments since the therapy cannot kill all tumor cells with one treatment.

Cancer tissue often expresses specific antigens or growth factors, which makes it possible to target cancer tissue with antibodies. Antibodies are, as mentioned, very specific and this specificity make it possible to create "magic bullets", a term coined by Nobel prize winner Paul Ehrlich in the early 1900s, meaning that drugs can be targeted for specific receptors (Strebhardt and Ullrich, 2008). In this case antibodies only bind to the intended molecule. To target the antigens, a thorough screening of both tumor and normal tissue expression is performed, and also what biological role the antigen has when the tumor is growing.

There are several mechanisms that mAbs can initiate tumor killing:

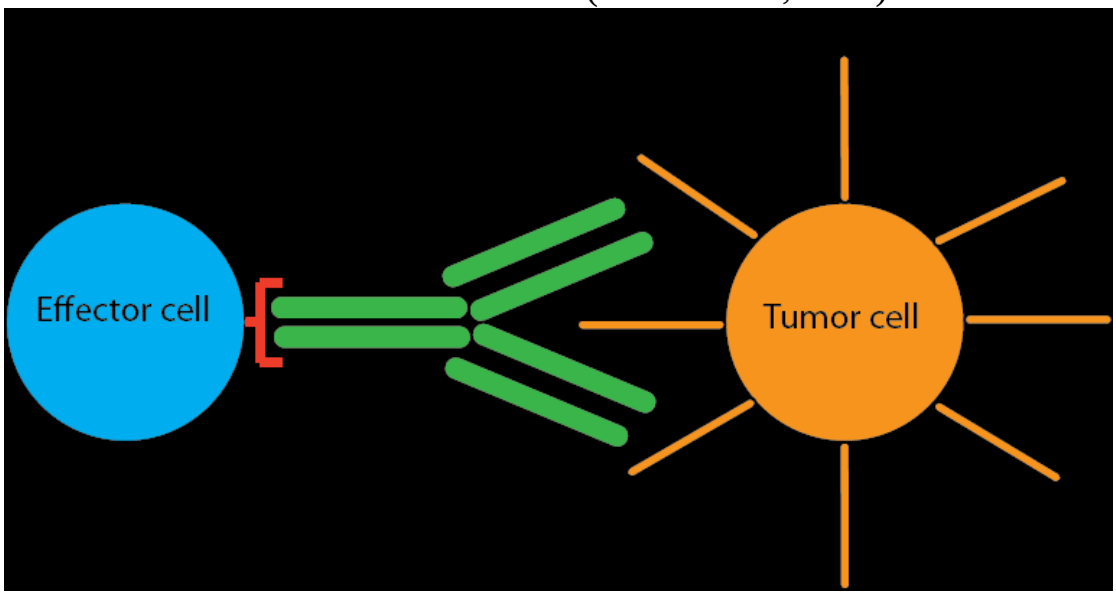
Direct tumor cell killing

Direct tumor cell killing can be initiated when antibodies bind to cell surface receptors with several outcomes that kill the cells. Induction of apoptosis, programmed cell death can be initiated when mAbs bind to certain surface receptor and mimic the binding of a ligand that occurs naturally.

Immune-mediated tumor cell killing

The most used mechanism for carrying out tumor cell killing is antibody-dependent cell-mediated cytotoxicity (ADCC), where the antibodies are recruiting different cytotoxic cells from the immune system, and is used in many clinically approved drugs (Scott et al. 2012). The Fc part of the antibody bind to a Fc gamma receptor (FcgR) at any kind of effector cell in the body and creates a complex that can bind to a cancer cell and initiate ADCC. When the Fab fragment of the antibody

bind to a tumor cell, and the Fc fragment is bound to the FcgR, the mechanism to kill the tumor cell starts (Mellor et al., 2013).



Vascular and stromal ablation

Cancer tumors have stromal cells and a rich vascular net just like other tissues. Therapies targeted towards these two important parts of a tumor can therefore be utilized to kill the tumor. Therapies inducing vascular and stromal ablation can do so by inhibit stromal cells, deliver toxins to stromal cells or vasculature and have antagonists bind to vasculature receptors.