Chapter 7
Monoclonal Antibody Production and It’s Applications

- A single type of antibodies having the same antigenic determinant produced by a single hybridoma clone is called monoclonal antibody.
- The hybridoma is made by fusing a lymphocyte (B cell) with a myeloma cell. Presence of a single antigenic determinant is the useful feature of the monoclonal antibodies. Monoclonal antibodies bind with only one type of epitope on the antigens.
- In 1975, Georges Köhler and Cesar Milstein devised a method for preparing monoclonal antibody, which quickly became one of immunology’s key technologies. The significance of the work by Köhler and Milstein was acknowledged when each was awarded a Nobel Prize in 1984.
- The antibodies produced against a single antigen but differing in antigenic determinants are called Polyclonal antibodies. They bind with different determinant sites on the antigen.
- In the early years, animals were immunized against a specific antigen were isolated and cultured in vitro for producing monoclonal antibodies.
- This approach was not successful since culturing normal B-Lymphocytes is difficult, and the synthesis of monoclonal antibody was short lived and very limited.
- It is interesting that immortal monoclonal antibody producing cells do exist in nature. They are found in the patients suffering from a disease called multiple myeloma.
- The hybridoma cells posses the growth and multiplying properties of myeloma cells but secrete antibody of B-Lymphocytes.
- Production of monoclonal antibodies by hybrid cells is referred to as hybridoma technology.

**MONOCLONAL ANTIBODY PRODUCTION BY HYBRIDOMA TECHNOLOGY:**
Hybridoma is a fusion product of a B-lymphocyte and myeloma cell. The production of hybridoma clones for monoclonal antibody production involves different steps.

**Monoclonal antibodies produced in the following steps:**
1) Isolation of B-Lymphocytes
2) Isolation of Myeloma Cells
3) Somatic Cell Fusion
4) Selection of Hybridoma cells
5) Screening of culture for antibody production
6) Propagation of screened colony
7) Isolation of antibodies
8) Commercially Production of Monoclonal Antibodies

1. Isolation of B-Lymphocytes
   B-lymphocytes are isolated from immunized mouse. It involves the following steps:
   i) 2-4 weeks old mice are immunized with the known antigen by sub cutaneous injection.
   ii) A mouse is killed after 72 hours of immunization, especially within 4 days, and its spleen is taken.
   iii) The spleen is minced into small fragments and the fragments are sterilized.
   iv) The fragments are then macerated into individual cells using enzymatic method.
   v) The cell suspension so obtained is immediately suspended in a balanced salt solution.
   vi) The suspension is washed 2 or 3 times with balanced salt solution to get pure plasma cells (spleenocytes).
   vii) Some of the spleenocytes are the antibody producing B-lymphocytes (B-cells). They are grown in fresh medium for cell fusion.

2. Isolation of Myeloma Cells
   - Myeloma cells are fast growing large cells hematopoietic portion of bone marrow.
   - Myeloma is taken from a bone and macerated to get a suspension of myeloma cells.
   - The myeloma cells have the ability to produce a specific antibody in larger amount.
   - HGPRT- mutant myeloma cells are raised by inducing mutations using 8-azaguanine. This will help us to select hybridomas from fused and un-fused cells.

3. Somatic Cell Fusion
   - After preparing B cell and myeloma cell, they are fused with the help of sendai virus or polyethylene glycol (PEG). These fusogens fuse the cells to produce fused B cells-myeloma cells, cybrid and hybrids. Some unfused cells may also present in the medium.
   - The spleenocytes and myeloma cells are mixed together in the ratio of 2:5:1 and treated with PEG.
• The cell mixture is shaken well for 3 minutes.
• The PEG brings the two cells together and induces cell fusion.
• As a result, spleenocyte-1 myeloma hybrids called hybridomas are formed. Sometimes, PVA is used as a fusagen to induce the cell fusion.

4. Selection of Hybrid Cells in HAT Medium:
• HAT medium (hypoxanthine aminopterin thymidine medium) is used for selection of hybrid cells. Nucleotide synthesis is essential for a survival.
• In HAT medium, aminopterin blocks the cellular synthesis of purines and pyrimidine from simple sugars (de novo pathway). But cells can thrive by utilizing Hypoxanthine an thymidine present in the medium by salvage pathway using the hypoxanthine guanir phosphoribosyl transferase (HGPRT) enzyme.

Principle of HAT medium works in the selection of hybrid cells:
• Myeloma cells are HGPRT deficient.
• So these cells cannot survive in HAT medium aminopterin blocks de novo pathway.

**Fig: HAT selection medium principle**

- **B- Cells** are HGPRT+ and can survive in the HAT medium. After some division, B cells undergo normal cell death.
- **Hybrid cells** has HGPRT enzyme from the B cells. So, only hybrid cells can survive in HAT medium.

The selection of spleenocyte-myeloma hybrids is mentioned below:

i) After cell fusion, the cell suspension is treated with 1 fresh medium lacking blood serum at a rate of 1 ml/minute.

ii) The diluted suspension is centrifuged to remove the fusagen along with the liquid medium.
iii) The cell suspension is then diluted slowly with a serum free HAT medium containing hypoxanthine, aminopterin and thymidine.

iv) The diluted cell suspension is distributed into the wells of a multiwell plate and the plate is incubated at 25-29°C for 2-3 weeks in an incubator.

v) Later, the wells of the multiwell plate are visualized for cell clumps.
   - The HGPRT mutant myeloma cells fail to synthesize purines from hypoxanthine. Aminopterin blocks the metabolism of purines. So myeloma cells and myeloma-myeloma hybrids do not grow in the HAT medium.
   - Un-fused spleenocytes and spleenocyte-spleenocyte hybrids never grow in the medium.
   - Spleenocytes contribute functionally active HGPRT enzyme to the spleenocyte-myeloma hybrids. Hence hybridomas synthesise purines from hypoxanthine and grow into cell clumps.

vi) The cell clumps are allowed to grow till each clone attains 500 or more cells by providing enough medium. Each of these cell lines is called Hybridoma or Clone.

vii) After hybridoma cells are selected, they are cultured in microtiter wells in such a way that each well consists of single hybridoma cells. So that, mono clone is produced i.e. Group of cells derived from single hybridoma cells.

5. Screening cells for antibody production:
   - Even though hybridoma cells survived in the selection medium and they also form clones, there ability to form antibody is not tested yet.
   - This property is tested using ELISA or RIA tests. Those clones which have the ability to produce antibody of our interest are allowed for next step of monoclonal antibody production.

6. Propagation of screened clones:
   - It is nothing but culturing or allowing selected clone cells to grow by in-vitro or in-vivo conditions respectively.
   - In-vitro technique clone cells are cultured in tissue culture flask with suitable medium. The production rate is found to be 10 – 100 mcg/ml.
   - In-vivo method, selected clones are injected into the peritoneal cavity of histocompatible mice and allowed to multiply and produce antibody. The rate of antibody production is 25 mg/ml.
Fig: Monoclonal Antibody Production by Hybridoma Technology

1. Primed B-cells (HGPRT⁺, Ig⁺) and Myeloma cells (HGPRT⁻, Ig⁻, immortal)
2. Polyethylene glycol
3. Heterokaryons
4. HAT selection (only B-cell/myeloma hybridoma grows)
5. Hybridoma (containing genes for HGPRT⁺, Ig⁺, immortal growth)
6. Assay for desired antibody in culture supernatant
7. Reclone Ab³ hybridomas
8. Expand positive clones
9. Culture
10. Ascites fluid
11. Monoclonal antibodies
12. Monoclonal antibodies

Mr. Jaydeep B Pawar
7. Isolation of antibodies:
   • In either of above methods, samples are collected and from these monoclonal antibodies are separated by affinity chromatography and they are used for different studies.

8. Commercially Production of Monoclonal Antibodies:
Monoclonal antibodies have been produced commercially in the following ways:

I) In Vivo Method
   • The hybridoma cell line that makes a desired monoclonal antibody is injected into the body of mice through a-muscular injection.
   • The mice are grown in the laboratory for 3 or 4 weeks.
   • Their blood is taken and the monoclonal antibodies are isolated from it.
   • By this method a mouse can produce about 50 mg of MCA.

II) Suspended Cell Culture in Fermenters
   • A hybridoma clone is cultured in a large fermenter using chemically defined complex medium having all minerals, vitamins and co-factors.
   • Airlift Fermenters are more useful for this purpose.
   • In this method, one litre of culture broth can yield 100 mg of MCA in 2 weeks.

III) Immobilized Cell Reactors
   • Cells of a hybridoma clone are immobilized in a hollow fiber reactor using polyacrylamide gel.
   • By this method, a few grams of MCA can be produced within 2 weeks

TYPES OF MONOCLONAL ANTIBODIES:
Following are the different types of monoclonal antibodies:
   1) Immunotoxins
   2) Chimeric Immunotoxins
   3) Humanized antibodies
   4) CDR grafted antibodies
   5) Heterconjugate antibodies

APPLICATIONS OF MONOCLONAL ANTIBODIES:
   • Monoclonal antibodies are proving to be very useful as diagnostic, imaging, and therapeutic reagents in clinical medicine. Initially, monoclonal antibodies were used primarily as in vitro diagnostic reagents.
Among the many monoclonal antibody diagnostic reagents now available are products for
detecting pregnancy, diagnosing numerous pathogenic microorganisms, measuring the blood
levels of various drugs, matching histocompatibility antigens, and detecting antigens shed by
certain tumors.

Radiolabeled monoclonal antibodies can also be used in vivo for detecting or locating tumor
antigens, permitting earlier diagnosis of some primary or metastatic tumors in patients.

For example, monoclonal antibody to breast-cancer cells is labeled with Iodine-131 and
introduced into the blood to detect the spread of a tumor to regional lymph nodes.

This monoclonal imaging technique can reveal breast-cancer metastases that would be
undetected by other, less sensitive scanning techniques.

Mainly monoclonal antibodies applied in two fields:

**I. Diagnostic Field:**

Since 1979, monoclonal antibodies have been put in the clinical diagnosis of certain severe
diseases and also for the following purposes:

a) Leucocyte Identification
b) Lymphocyte subset determination
c) HLA typing
d) Viral detection and sub typing
e) Parasitic determination
f) Polypeptide hormone detection
g) Detection of cancer with tumor marker determination
h) Detection of cardiac myosin in cardiac injury
i) Pregnancy detection

In the above mentioned conditions, the basic principle is production of antibodies against antigen
and identification or quantification of antigen and antibody complex.

**II. Therapeutic Field:**

There are four different headings available in this field namely,

a) Anti-tumor therapy:

In anti-tumor therapy, antibodies against tumor antigen produced and they are converted
into either Immunotoxins, or chimeric Immunotoxins or Heterconjugate antibodies. When
these antibodies utilized, they damage tumor cells and tumor growth controlled. These
anti-tumor antibodies are called as “magic bullets”.

b) **Immunosuppression:**
During transplantation between partially incompatible individuals, host versus graft rejections are suppressed using monoclonal antibodies against TCR, BCR, Co-receptor complex and cytokines etc., Hypersensitivity reactions are also treated with blocking monoclonal antibodies.

c) **Fertility control:**
By producing antibodies against HCG or trophoblast, fertility controlled.

d) **Drug toxicity reversal:**
Toxicity produced by drugs is treated using monoclonal antibodies against drugs, so that the functions of drugs are blocked and effect reversed.

### III. **Research Field:**
Monoclonal antibodies have been used as research tools in the fields of medicine, immunology, biochemistry, genetic engineering, carcinobiology and so on. Some of their research applications are given below:

a) In genetic engineering, monoclonal antibody is used to screen recombinants.

b) In immunological studies, MCAs are used to identify various cell types involving in immune responses and to detect interactions among them.

c) Monoclonal antibodies are used to determine the structure of cell membranes.

d) They are employed in serological classification of closely related bacteria, viruses etc.

e) They are used in radioimmuno assay (RIA), ELISA and immune fluorescence assay in research to identify and detect; some target products.

f) MCAs are used to detect the exact position of enzymes in the cells by using immune cytochemical methods.

g) MCAs are being used to detect and classify enzymes.

h) Monoclonal antibodies are used in affinity columns to pick up proteins from the solution. They help to isolate and purify proteins and enzymes from solutions.

i) The rejection of transplanted organs, especially kidney transplants, is associated with OKT-3 antigen present on the surface of T-cells. Monoclonal antibody against OKT-3 antigen is made and injected into the patient to suppress the transplant rejection. The MCA named *Orthoclone OKT-3* is mainly used for this purpose.
Below are examples of clinically important monoclonal antibodies.

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<tr>
<th>Category</th>
<th>Type</th>
<th>Application</th>
<th>Mode</th>
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<tbody>
<tr>
<td>Anti-Inflammatory</td>
<td>Infliximab</td>
<td>• Rheumatoid Arthritis&lt;br&gt;• Crohn's Disease&lt;br&gt;• Ulcerative Colitis&lt;br&gt;• Ankylosing Spondylitis</td>
<td>Chimeric</td>
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<td></td>
<td>Adalimumab</td>
<td>• Rheumatoid Arthritis&lt;br&gt;• Crohn's Disease&lt;br&gt;• Ulcerative Colitis&lt;br&gt;• Ankylosing Spondylitis</td>
<td>Human</td>
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<td>Basiliximab</td>
<td>• Acute Rejection Of Kidney Transplants</td>
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<td>Daclizumab</td>
<td>• Acute Rejection Of Kidney Transplants</td>
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<td>Omalizumab</td>
<td>• Moderate-To-Severe Allergic Asthma</td>
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<td>Gemtuzumab</td>
<td>• Relapsed Acute Myeloid Leukemia</td>
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<td>Alemtuzumab</td>
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<td>Rituximab</td>
<td>• Non-Hodgkin's Lymphoma&lt;br&gt;• Rheumatoid Arthritis</td>
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<td>Trastuzumab</td>
<td>• Breast Cancer With Her2/Neu Overexpression</td>
<td>Humanized</td>
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<td>Nimotuzumab</td>
<td>• Approved In Squamous Cell Carcinomas, Glioma&lt;br&gt;• Clinical Trials For Other Indications Underway</td>
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<td>Bevacizumab &amp; Ranibizumab</td>
<td>• Anti-Angiogenic Cancer Therapy</td>
<td>Humanized</td>
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<td>Anti-Cancer</td>
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<td>Bavituximab</td>
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<td>• Rsv Infections In Children</td>
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