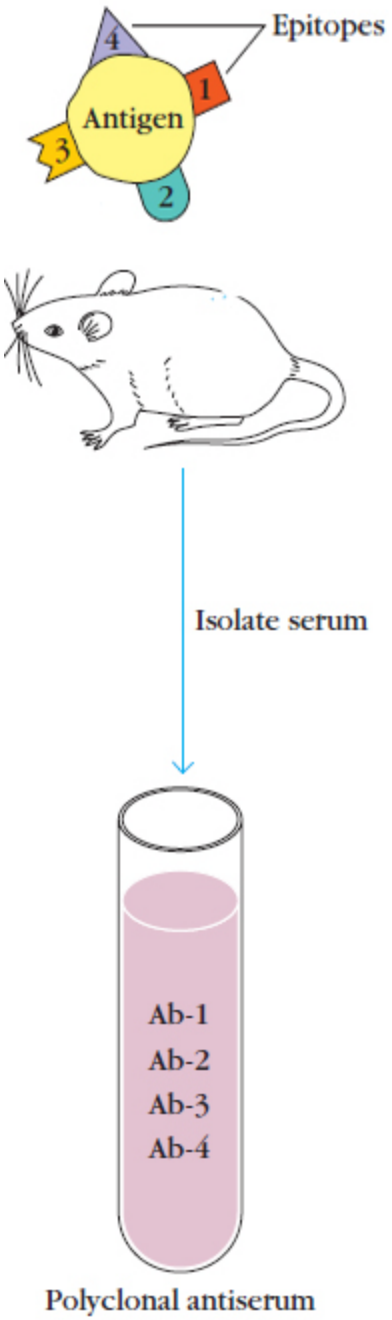
INVERTIS UNIVERSITY BAREILLY

LECTURE 7

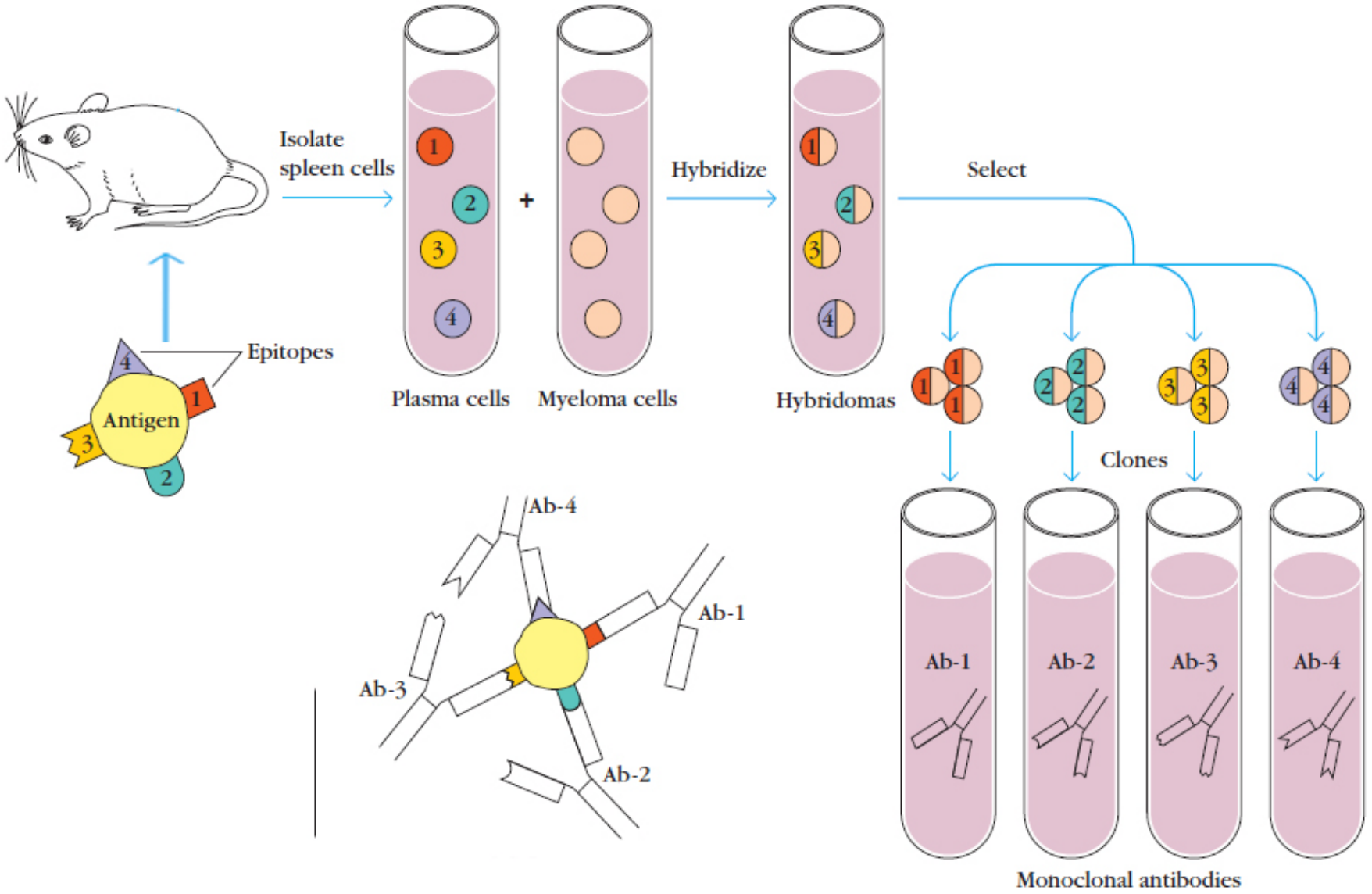
PRODUCTION AND APPLICATION OF MONOCLONAL ANTIBODY

MONOCLONAL ANTIBODIES:

- Most Ags shows multiple epitopes & therefore induce proliferation & differentiation of a variety of B-cell clones, each derived from B cell that recognizes a particular epitope.
- Thus, the resulting serum antibodies are heterogeneous (mixture of antibodies), each specific for one epitope.
- Such a **polyclonal antibody** response facilitates the phagocytosis, and complement-mediated lysis of antigen.
- It has clear advantages for the organism in vivo.
- Unfortunately, in experiments the Ab heterogeneity, reduces the efficiency of an antiserum for various uses.
- Thus, for research and diagnostic purposes, **monoclonal antibodies** (derived from a single clone and thus specific for a single epitope) are preferred.
- But, direct biochemical purification of a monoclonal Ab from a polyclonal Ab mixture is not feasible.



- In 1975, Georges Kohler and Cesar Milstein given a method for preparing monoclonal antibody and each of them was awarded a Nobel Prize.
- By fusing a normal activated, antibody-producing B cell with a myeloma cell (a cancerous plasma cell), they were able to generate a hybrid cell, called a **hybridoma.**
- This **hybridoma** shows the property of both fused cells i.e. immortal growth properties of myeloma cell and antibody secretion property of the B cell.
- The resulting clones of hybridoma cells, which secrete large quantities of monoclonal antibody, can be cultured indefinitely (enormously).


Isolate spleen cells
Plasma cells
Myeloma cells
Hybridize
Hybridomas
Select
Clones
Epitopes
Antigen
Ab-1
Ab-2
Ab-3
Ab-4
Monoclonal antibodies

HYBRIDOMA TECHNOLOGY:

- In this technology, cells of interest (plasma cells) hybridized with cancer cells. The aim of this technology is to provide immortality to the cells of interest.
- When cells of interest fused with cancer cells with the help of fusogens like sendaivirus or polyethylene glycol (PEG):
- First cytoplasmic membrane of two cells are fused to form heterokaryon i.e. cells with two different nucleuses.
- Then the two nucleuses combined to form cybrid or hybrid cells.
- **Hybrid cells** contain combination of both cell nuclear materials but maintaining species usual chromosomal number by loss of extra chromosome.
- **Cybrid cells** genetic material of either of the cell maintained completely and the other one is lost completely.
- Of the three possibility i.e. unfused cells, hybridoma cells and cybrid cells, hybridoma cells selected by using HAT (Hypoxanthine Aminopterin Thymidine) medium.

HAT MEDIUM SELECTION:

- HAT selection depends on the fact that mammalian cells can synthesize nucleotides by two different pathways: the de novo and the salvage pathways.
- The de novo pathway: in which a methyl or formyl group is transferred from an activated from of tetrahydrofolate to a folic acid analog (this is blocked by aminopterin of HAT medium).
- Thus, when de novo pathway is blocked, cells utilize salvage pathway (bypasses the aminopterin block) for synthesizing nucleotides.
- The enzymes catalyzing the salvage pathway include hypoxanthine-guanine phosphorribosyl transferase (HGPRT) and thymidine kinase (TK) and a mutation in either of these two enzymes blocks the salvage pathway.
- HAT medium contains aminopterin to block the de novo pathway and hypoxanthine and thymidine to allow growth via the salvage pathway.
- When two types of cells, one with mutation in TK & other with mutation in HGPRT are fused, only hybrid cells will contain necessary enzymes for growth on HAT medium via the salvage pathway. Thus only hybrid cells will grow in HAT medium.

MONOCLONAL ANTIBODY PRODUCTION BY HYBRIDOMA TECHNOLGY:

- Monoclonal antibodies produced in the following steps:
    1. Preparation of Cells
    2. Fusion of cells
    3. Selection of Hybridoma cells
    4. Culturing of selected hybridoma cells
    5. Screening of culture for antibody production
    6. Propagation of screened colony
    7. Isolation of antibodies

1. Preparation of Cells:

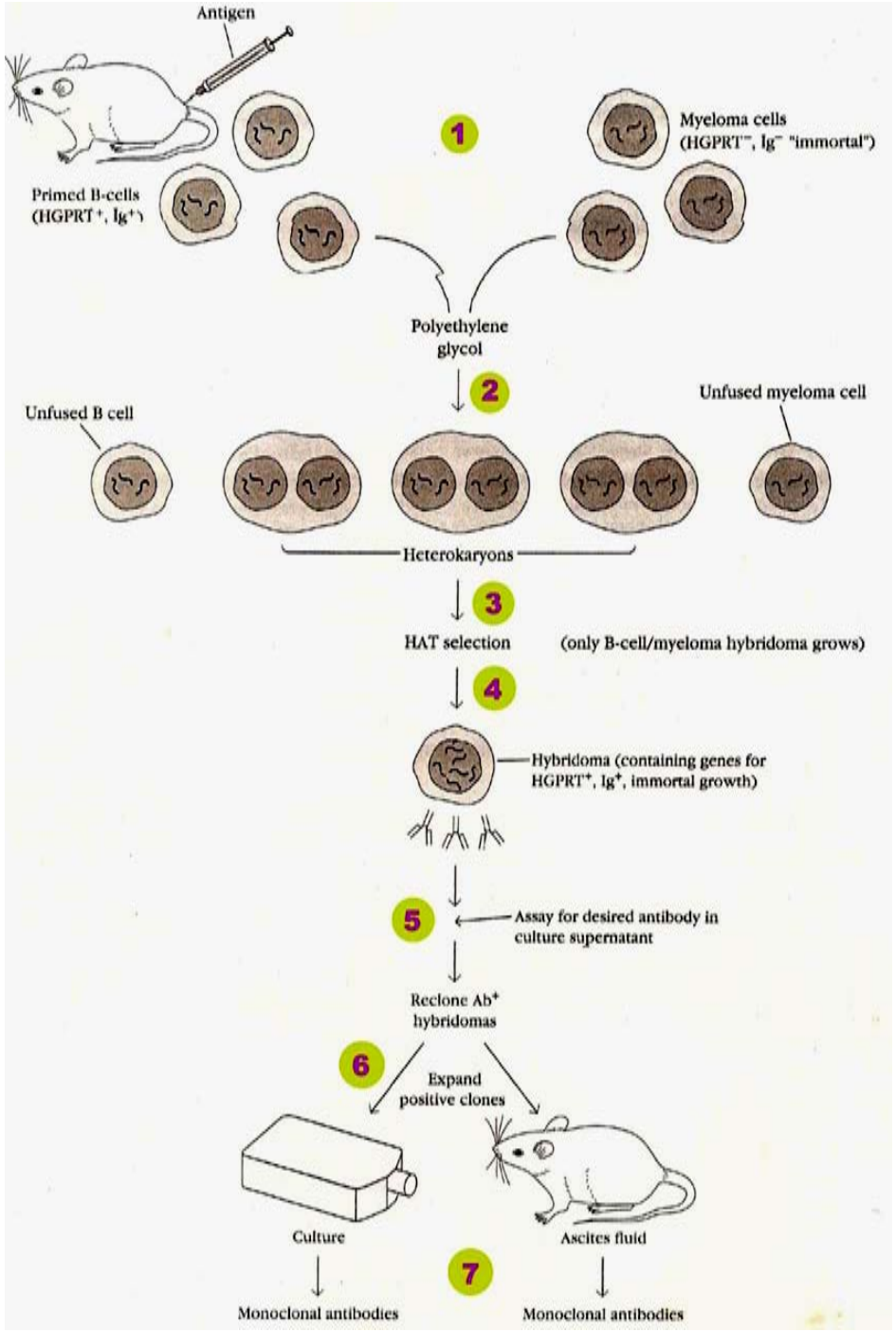
- B cell and myeloma cells are used for monoclonal antibody production.
- Genetic machinery of B cells should be modified as Ig^{+}, $HGPRT^{---}$ and TK^{+}.
- This is achieved through site directed mutagenesis process.
- *These B cells are actually isolated from mice to which antigen of our interest is injected and it also posses the ability of producing antibody against antigen of our interest.*
- Genetic machinery of myeloma cells are modified as Ig^{---}, $HGPRT^{+}$ and TK^{---}.
- Myeloma cells are used to provide immortality property.

2. Fusion of Cells:

- After preparing B cell and myeloma cell, they are fused with the help of sendaivirus 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

3. Selection of Hybrid cells:

- After fusing the cells with the help of fusogens they are placed in a culture medium with selection medium of HAT.
- Aminopterin inhibits de novo nucleotide biosynthesis.
- When de novo synthesis inhibited cells can use salvage pathway to produce nucleotides for that they require HGPRT and TK.
- Since one of this enzyme is absent in B cells, myeloma cells and cybrids, they are unable to survive in the selection medium.
- Only hybrid cells which are having functional HGPRT and TK are found to be capable of growing in selection medium.



4. **Culturing of selected hybridoma:** After hybridoma selection, they are cultured in MT 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:** Only hybridoma cells survived in HAT medium and they also form clones, but there ability to form Ab is not tested.

- This property is tested using ELISA or RIA and cell clones which have the ability to produce Ab of our interest are allowed for monoclonal Ab production.

6. **Propagation of screened clones:** In this selected clone cells are allowed to grow in *invitro* or *invivo* conditions.

- In *invitro* technique clone cells are cultured in tissue culture flask and production rate is found to be 10–100 ug/ml.
- In *invivo* method, selected clones are injected into the peritoneal cavity of histocompatible mice and allowed to multiply & produce antibody and the rate of antibody production is 25 mg/ml.

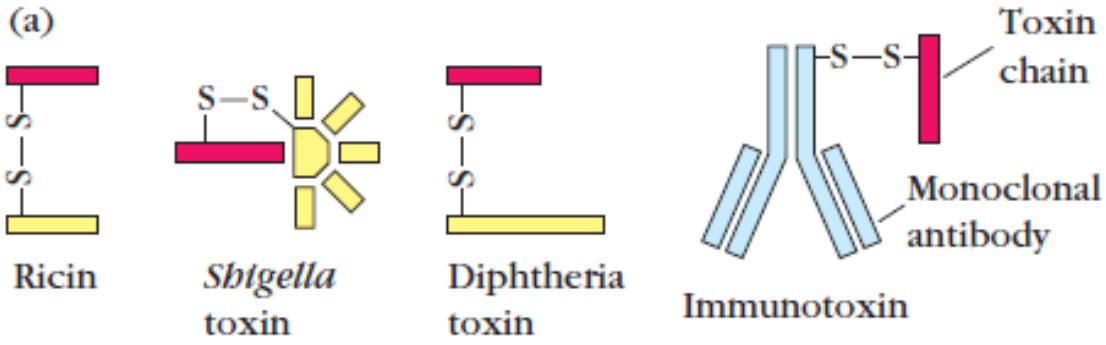
7. **Isolation of antibodies:** In either of above methods, samples are collected and monoclonal Abs are separated by affinity chromatography.

APPLICATIONS OF MONOCLONAL ANTIBODIES:

- Monoclonal antibodies are proving to be very useful as diagnostic and therapeutic reagents in clinical medicine.
- Initially, monoclonal antibodies were used as in vitro diagnostic methods.
- Currently monoclonal antibody diagnostic reagents are available as products, such as, pregnancy detection kit, numerous pathogenic microorganisms diagnosing kit, measuring the blood levels of various drugs, and detecting antigens causative of certain tumors.
- Radiolabeled monoclonal antibodies can also be used in vivo for detecting or locating tumor antigens.
- ***Example:*** Monoclonal antibody to breast-cancer cells is labeled with iodine-131 and introduced into blood to detect tumor spread to regional lymph nodes. This monoclonal imaging technique can reveal breast-cancer diagnosis that would be undetected by other, less sensitive scanning techniques.

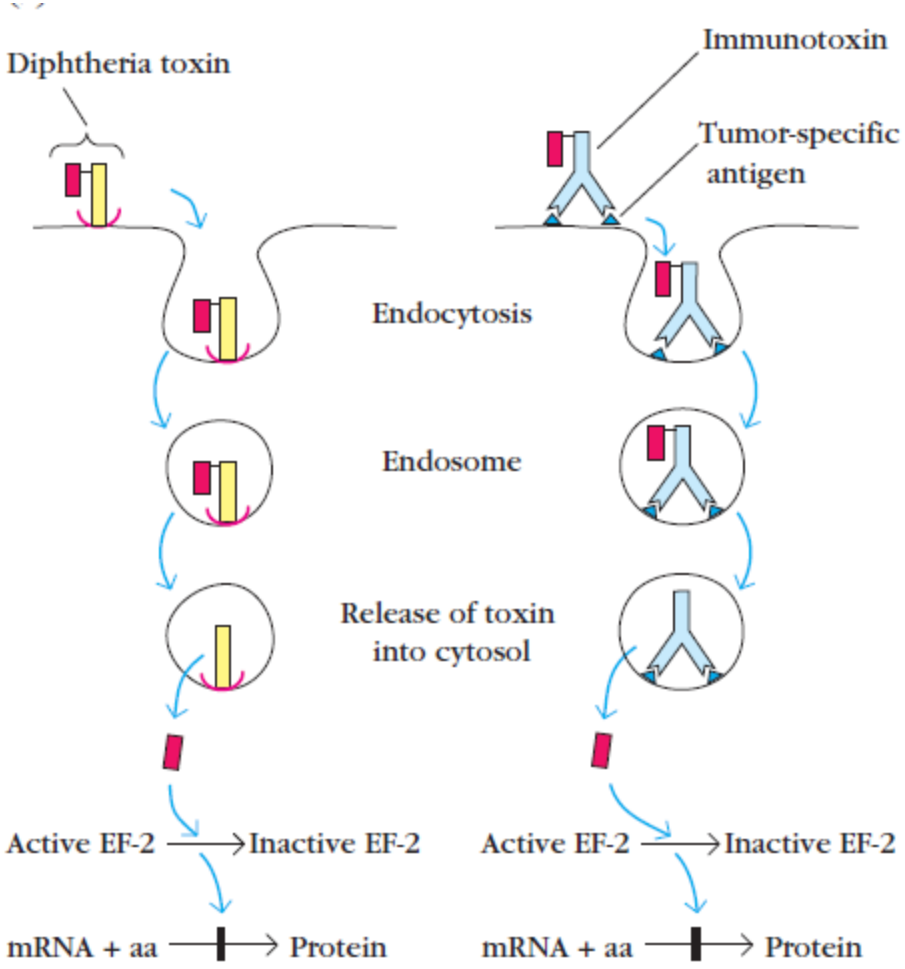
IMMUNOTOXINS:

- Immunotoxins are composed of tumor-specific monoclonal antibodies coupled to lethal toxins, are valuable therapeutic reagents.
- The toxins used in preparing immunotoxins include ricin, *Shigella* toxin, and diphtheria toxin, all of which are able to inhibit protein synthesis.
- These toxins are so potent that a single molecule has been shown to kill a cell.
- Toxins consists of two types of polypeptide components:
    1. An inhibitory (toxin) chain
    2. One or more binding chains which interact with cell surface receptors and thus the toxin can get into cells and without this chain toxin is harmless.
- An immunotoxin is prepared by replacing the binding chain with monoclonal antibody that is specific for a particular tumor cell.



- Theoretically, attached monoclonal antibody will deliver toxin chain to tumor cells, where it cause death of tumor cell by inhibiting its protein synthesis.
- The initial clinical use of immunotoxins in patients with leukemia, lymphoma, and some other types of cancer had done, and currently research to develop and demonstrate their safety and effectiveness is under process.

- ***Diphtheria toxin binds to a cell-membrane receptor (left) and a diphtheria-immunotoxin binds to a tumor-associated antigen (right).***
- ***In both case, toxin is internalized in an endosome. The toxin chain is then released into the cytoplasm, where it inhibits protein synthesis by catalyzing inactivation of elongation factor 2 (EF-2).***



Therapeutic Field: following important applications are available in this:

a. **Anti-tumor therapy:** In anti-tumor therapy, antibodies against tumor antigen produced and they are converted into either Immunotoxins, or chimeric Immunotoxins. These Abs can damage tumor cells and controlled tumor growth.
b. **Immunosuppression:** During the transplantation between partially incompatible individuals, host versus graft rejections are suppressed using monoclonal antibodies against TCR, BCR, Co-receptor complex and cytokines etc.
c. **Fertility control:** By producing antibodies against HCG or trophoblast, fertility can be 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.

References:

- Steven JS, Wayne G, Karoline BP, James A. Current Trends in Monoclonal Antibody Development and Manufacturing. Springer New York Dordrecht Heidelberg London. DOI 10.1007/978-0-387-76643-0.
- Peter JD, Seamus JM, Dennis RB, Ivan MR. Roitt's Essential Immunology. A John Wiley & Sons, Ltd., Publication. 12Edn.
- Judith AO, Jenni P, Sharon AS. Kuby Immunology. W. H. Freeman and Company, New York. 7Edn.

THANKYOU