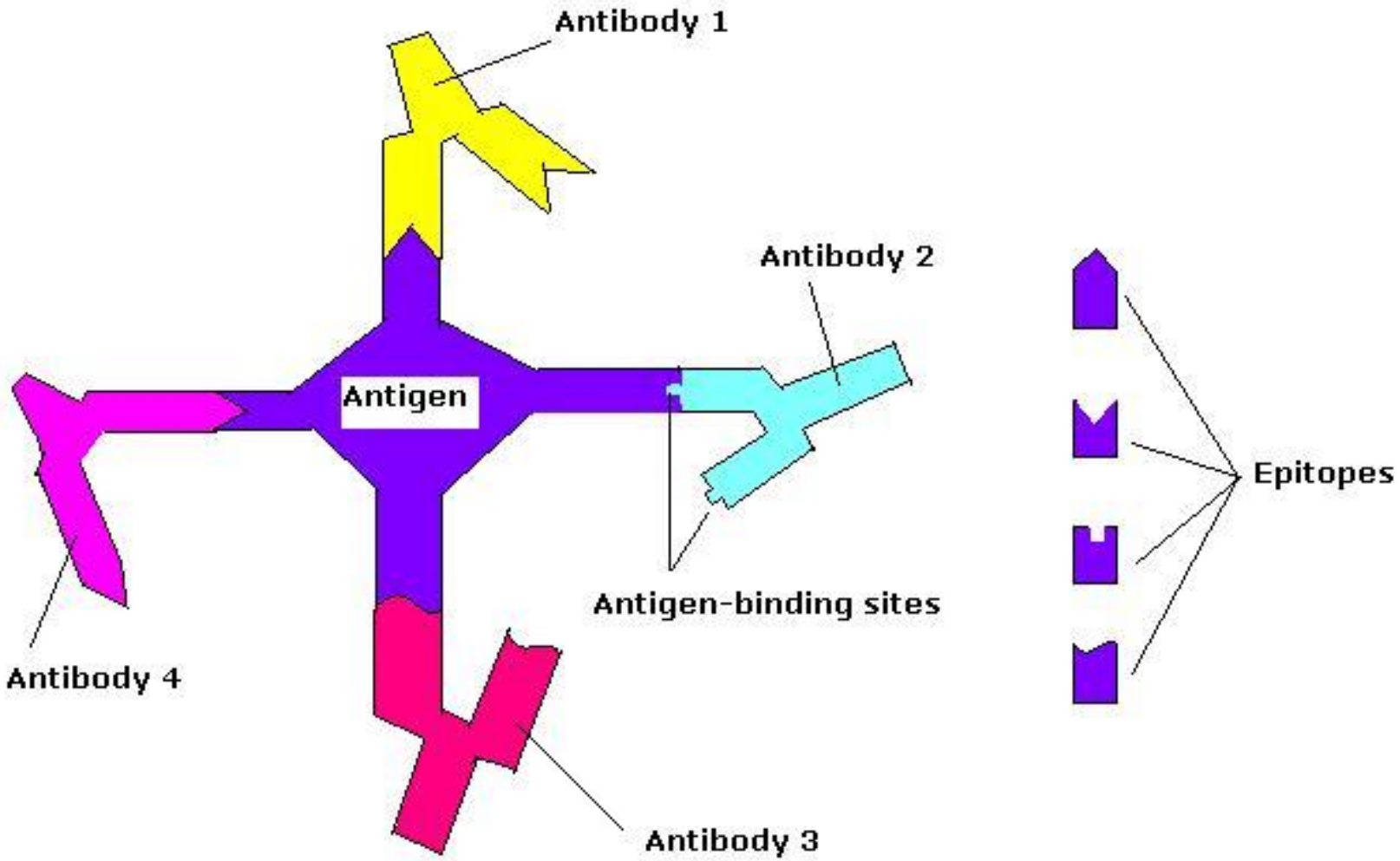
MONOCLONAL ANTIBODIES

Definition

Monoclonal antibodies *(MAbs)* are antibodies obtained from a **single** clone (of B cells) and are directed towards **one *epitope* *(antigenic determinant)*** of an antigen.

Background

- An antigen has many **epitopes.**
- Each antibody secreting cell *(B cells in spleen)* ***recognise only one epitope*** and therefore produce antibody of a **single** type.
- Because it is produced from clone of a single cell, it is called ***monoclonal antibody*****.**


Antibody 1
Antibody 2
Antigen
Antigen-binding sites
Epitopes
Antibody 4
Antibody 3

History

- In 1975, **Kohler and Milstein** first **fused lymphocytes** (B cells and myeloma cells) to produce a cell line which was **both immortal and a producer of monoclonal antibodies.**

- The two scientists were awarded the Nobel Prize for Medicine in 1984 for the development of this **"hybridoma”** (hybrid cell).

Hybridoma

- If number of **descendants of epitope recognizing B cells *(clone)*** can be sufficiently increased, we can get **relevant antibody in large amounts.**

- The problem is that these **antibody secreting B cells can not** be propagated in culture.

- The reason is that as they **mature, they die** as any normal cell does**.**

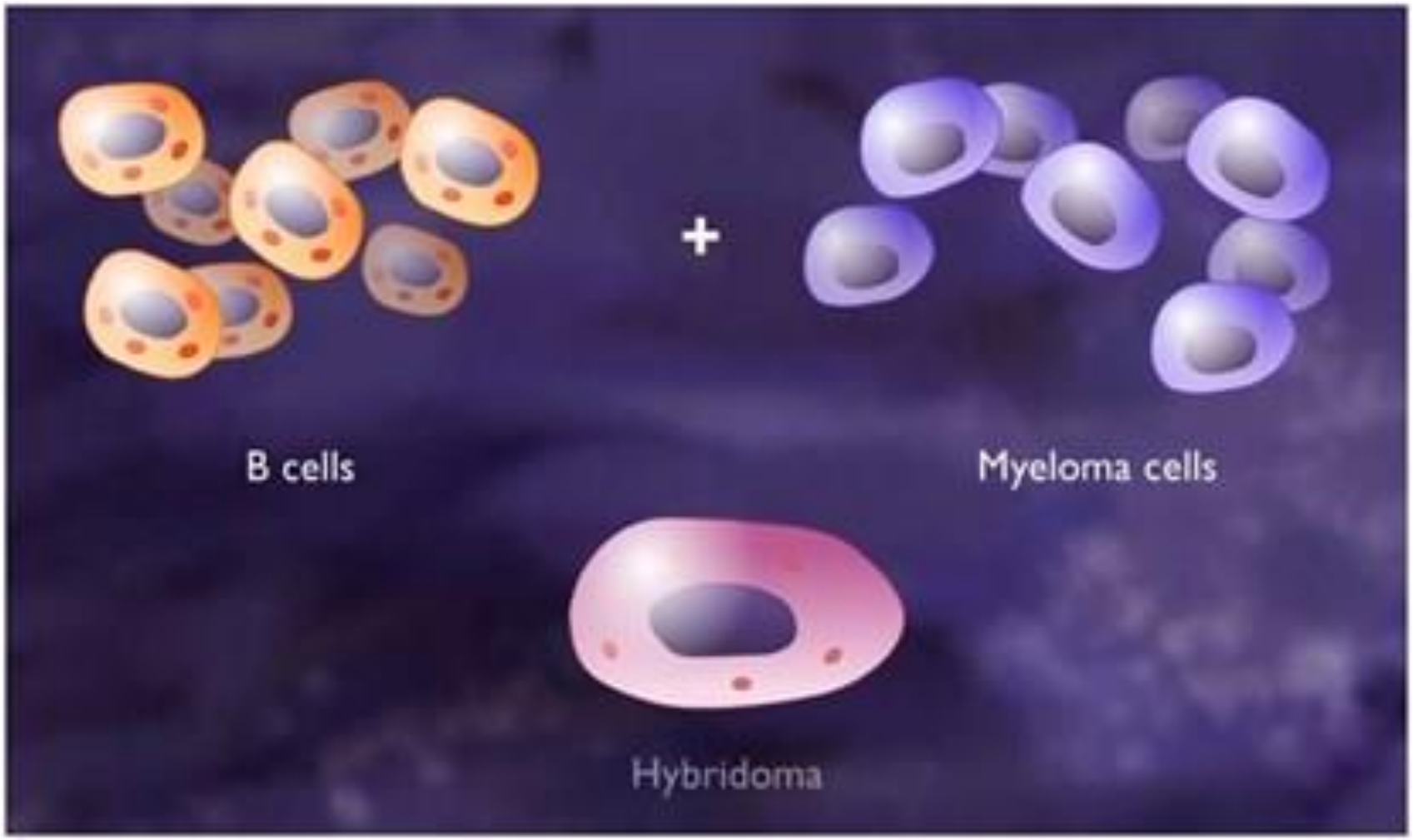
- So they are **mortal.**

- To overcome this problem, these B cells are fused with an **immortal** cell, i-e., **myeloma** cell.

- The resultant cell [because it is a **hybrid** of two cells] is called ***hybridoma***.

- It can grow **indefinitely** and keep producing the **Mab** as its culture grows.

Myeloma is blood cancer arising from plasma cells (antibody producing B cells).


B cells
+
Myeloma cells
Hybridoma

- Fusion of myeloma cells and B cells is done by different chemicals, most notably ***PEG (polyethylene glycol)*** or by using **Sendai** virus.

But there is a problem:

- **Not every** B cell fuses with myeloma cells.
- In fact, **only 1 in 100,000 cells** form a ***hybridoma***.
- To resolve this problem we have to do **drug selection**.

Drug selection (HAT selection)

- When we mix B cells and myeloma cells, there are three types of cells in the mixture, i-e, **B cells, myeloma cells and hybridomas.**
- When this mixture is put in culture medium, **unfused B cells automatically die** out because they are **mortal.**
- Myeloma cells grow as efficiently as hybridoma cells, and in fact, they **overgrow** hybridoma cells grossly because they are **also immortal** and in **much greater numbers**.
- So it is necessary to **eliminate** the myeloma cells to have **pure hybridoma cells.**
- This is done by **drug selection**.

- The Myeloma cells used for this purpose usually have a **mutation** in one of the enzymes of the **salvage pathway** of purine nucleotide biosynthesis.

- Selection with **8-azaguanine** (for mutation of myeloma cells) often yields a myeloma cell line harboring a **mutated hypoxanthine - guanine phosphoribosyl transferase (HPRT)** gene so that it **can not use the salvage pathway**.

- Due to this mutation, it can use only **de novo synthesis**.

De novo synthesis *refers to the synthesis of complex molecules from simple molecules such as sugars or amino acids, as opposed to recycling after partial degradation.*
A ***salvage pathway*** *is a pathway in which nucleotides (purine and pyrimidine) are synthesized from intermediates in the degradative pathway for nucleotides.*

- Addition of any compound which **blocks the de novo nucleotide synthesis pathway** will **force cells** to **use salvage pathway.**

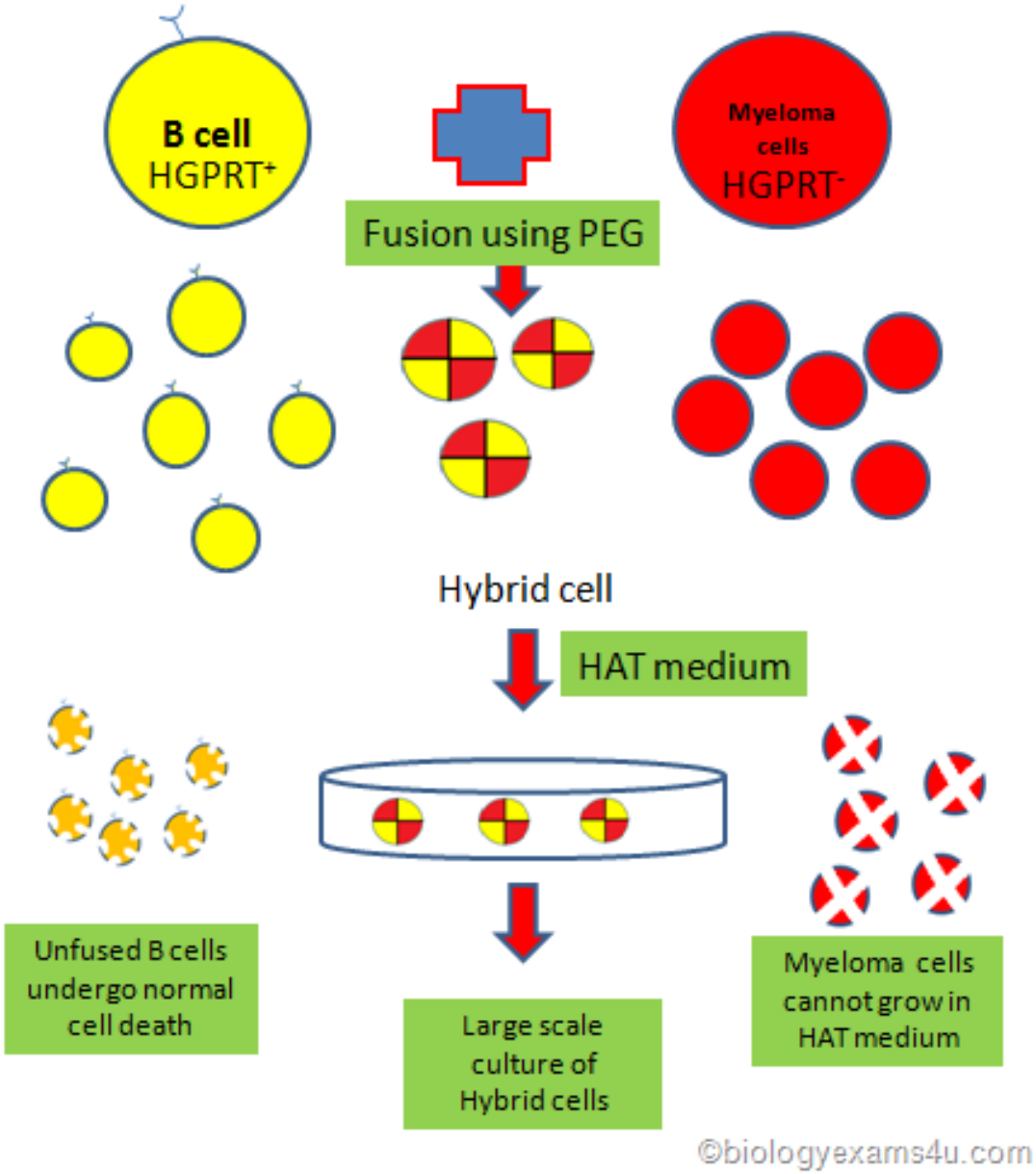
- Cells containing a **non-functional HPRT gene (myeloma cells) will die** in these conditions as they **cannot use either de novo synthesis or salvage pathway.**

- Only the **hybridomas** which have a **functional HPRT gene** (provided by antibody producing **B cells**) will be **able to survive** because **they can use salvage pathway.**

- Selection is commonly done with **aminopterin, methatrexate**, or **azaserine** (for **blockage of de novo synthesis**).

- **Hypoxanthine** and **thymidine** are added to encourage use of salvage pathway.

- The medium used is called **HAT** (hypoxanthine-aminopterin-thymidine) medium.


B cell
HGPRT+
Myeloma cells
HGPRT-
Fusion using PEG
Hybrid cell
HAT medium
Unfused B cells undergo normal cell death
Large scale culture of Hybrid cells
Myeloma cells cannot grow in HAT medium
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Outline for production of monoclonal antibodies

If antigen is a toxin, it is treated with **0.1% formalin to neutralize toxicity**.

Mice are immunized as follows:

1st dose:	App. 20 μg with adjuvant i.p.
2nd dose:	App. 10 μg without adjuvant i.v. after 5 weeks
3rd dose:	App. 10 μg without adjuvant i.v. after 4 days

(Adjuvant is usually **Freund's incomplete adjuvant**)

- Two weeks after the third dose, animal is sacrificed and **spleen cells are removed**.

- Spleen cells and myeloma cells are mixed at a **ratio of 5:1** and **fused with 50% PEG** with stirring.

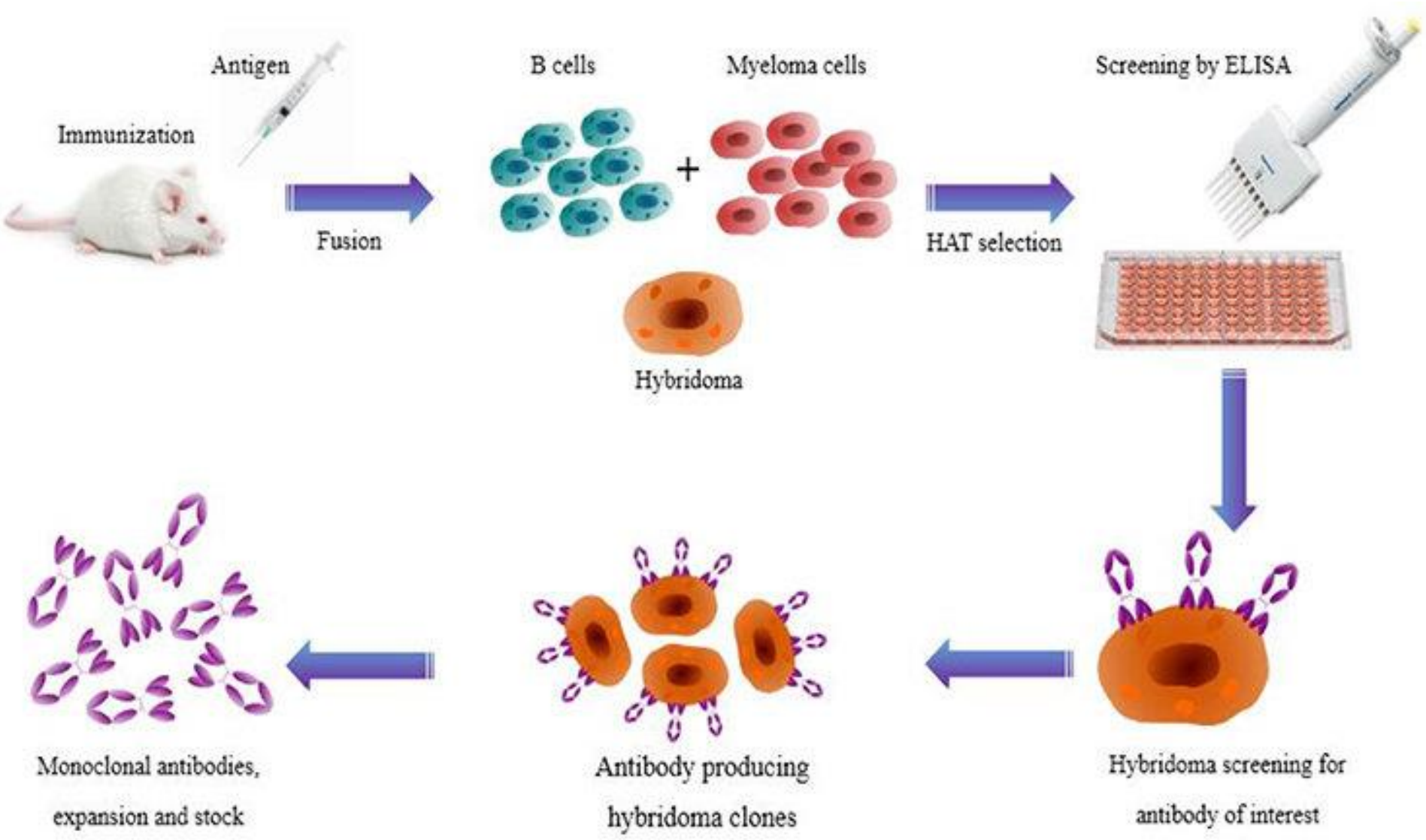
- Cells are grown in **Dulbecco's Modified Eagle's Medium** containing **HAT selection drugs** at 37°C.

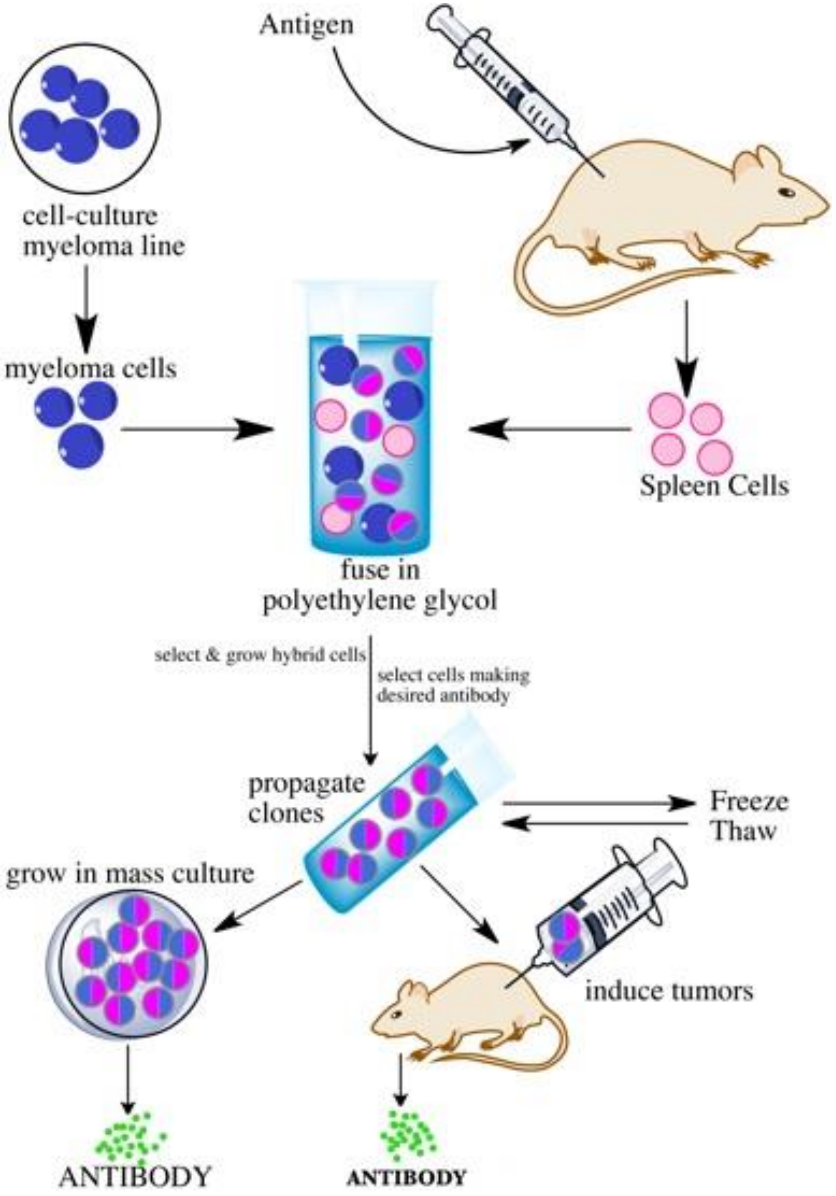
- After 2-3 days, cell number is counted and **dilutions are made** for distribution in multiple well plates.

- The dilutions are such that **each well contains less than one cell**, theoretically.

- Cells are grown at **37°C**.
- Everyday the plates are observed **microscopically**.
- Wells that show **clusters or clones of cells** are selected and their **supernatant** is checked for MAbs by **ELISA**.
- The cells from wells which give **positive** ELISA results are washed and injected into **BALB/C mice** at a concentration of **10^7 cells per mouse**.
- These mice are injected two weeks earlier with **pristane** to suppress immune system.

- This is done to allow the **clones to grow freely and produce MAbs *in vivo*.**
- After **10-14 days**, the mice develop **ascitic fluid** which is rich in MAbs.
- It is **purified** and used.


Antigen
Immunization
Fusion
B cells
Myeloma cells
Hybridoma
HAT selection
Screening by ELISA
Hybridoma screening for antibody of interest
Antibody producing hybridoma clones
Monoclonal antibodies, expansion and stock


Antigen
cell-culture
myeloma line
myeloma cells
Spleen Cells
fuse in
polyethylene glycol
select & grow hybrid cells
select cells making
desired antibody
propagate
clones
Freeze
Thaw
grow in mass culture
induce tumors
ANTIBODY
ANTIBODY

Factors Affecting MAb Production

- The **purity of the antigen**
- the **mouse strain** which is used
- the **age** and **sex** of the mouse
- the **tolerance of the individual mouse** may affect the **outcome of Ab-producing procedures.**

- Because **so many variables** are present, one general way to maximize the animal's immune response is to use **adjuvants**.

- Adjuvants **hold the antigen at the site of immunization** and **release it over a long period of time**.

- The major hazards of MABs are **contamination with retroviral particles** from mouse myeloma cells.

In Vitro Production:

- The expansion of hybridomas in animals has **ethical implications.**

- In addition, although *in vivo* production is **relatively inexpensive** and **yield is very high (1-10 mg/ml),** ascites fluid of mice may yield **commercially unsuitable antibody.**

- So ***in vitro* production of MAbs** has been developed.

Two popular alternatives are:

1. **Bulk tissue culture:** It is the process or technique of making body tissue grow in a culture medium outside the organism.

2. **Expression of cloned antibody genes** in high producing eukaryotes.

   It is accomplished through **recombinant DNA technology.**

In both cases, the yield runs from **10-60 µg/ml** which is **500 -1000 times less** than mouse ascitic fluid ((1-10 mg/ml) **.**

Medical applications

- The idea of using antibodies as **therapeutic agents** is appealing.

- It seems probable that **specific antibodies** could be used to attack toxins, bacteria, viruses and even cancer cells.

  In fact, in case of **cancer treatment**, monoclonal antibodies are already playing a significant role.

- With hybridoma technology, a **continuous supply** of pure mono-specific antibody can be maintained.

- However, the problems of **cross-reactivity** [with similar epitopes somewhere else] leading to an immune response and anaphylaxis may arise.

- The recipient might still produce **antibodies to a monoclonal antibody**, which carry **mouse (murine) determinants** [from mouse myeloma cells].

- To overcome this problem **chimeric** monoclonal antibodies have been developed.

In biology, **chimeric** means made from parts of two different animals.

Chimeric antibodies

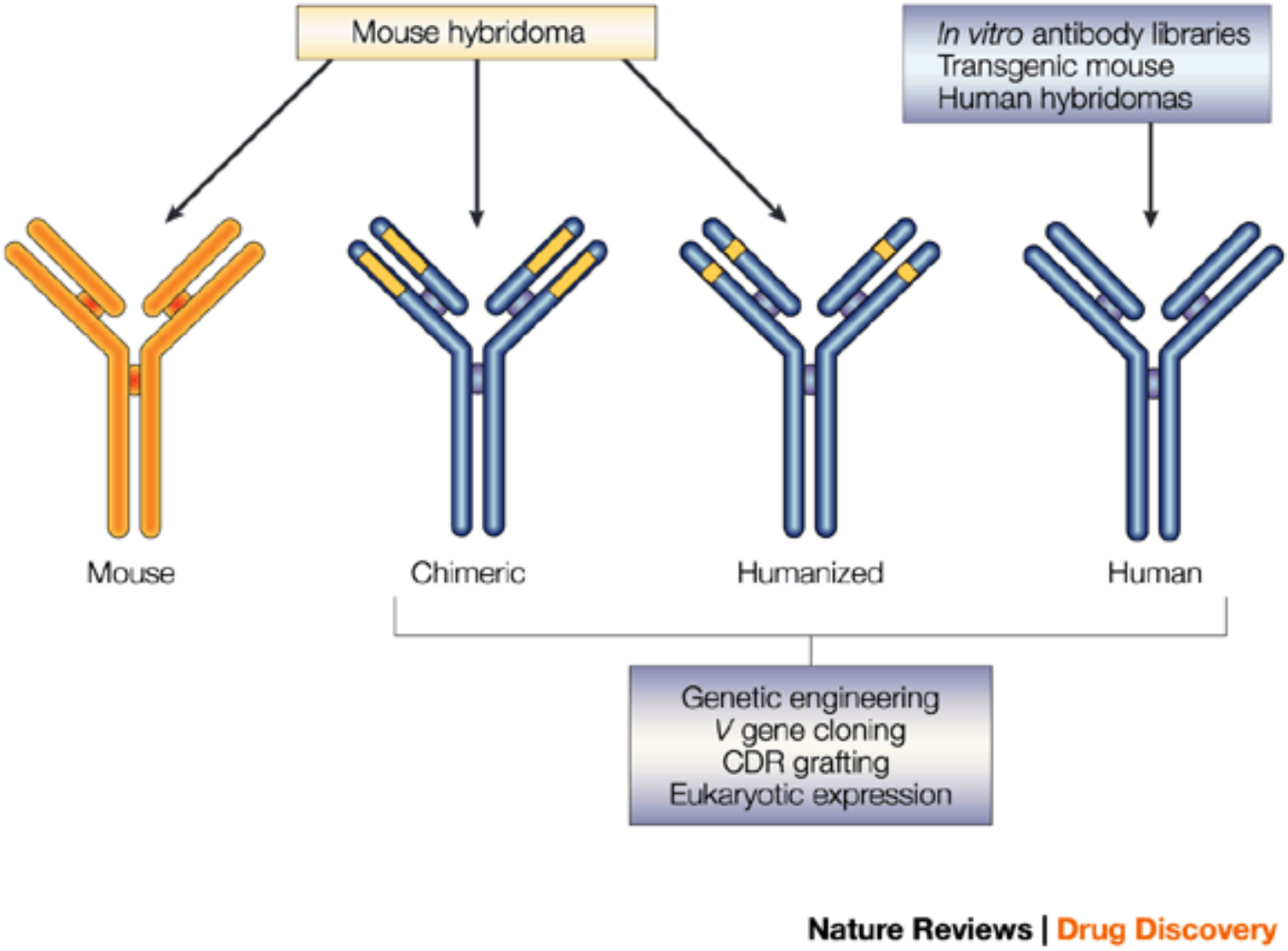
- The molecular nature of antibody function has made it possible to convert mouse MAb into one that has **some human segments but still retain its original antigen-binding specificity** [those parts that leads to unwanted reactions are changed].

- This hybrid antibody is called ***chimeric*** antibody, or a ***humanized*** antibody.

- In **chimeric** antibodies, **67%** of the antibody has been replaced by human antibody.

- In **humanized** antibodies the human portion is increased to **90%-95%.**

- **Human** antibodies by definition are **100%** human, although **near human antibodies** can also be generated in a genetically modified mouse.


Mouse hybridoma
In vitro antibody libraries
Transgenic mouse
Human hybridomas
Mouse
Chimeric
Humanized
Human
Genetic engineering
V gene cloning
CDR grafting
Eukaryotic expression
Nature Reviews | Drug Discovery

Diagnostic Uses

Monoclonal antibodies are currently utilized in many **diagnostic procedures** including:

-- measuring **protein** and **drug levels** in serum

-- typing **tissue** and **blood**

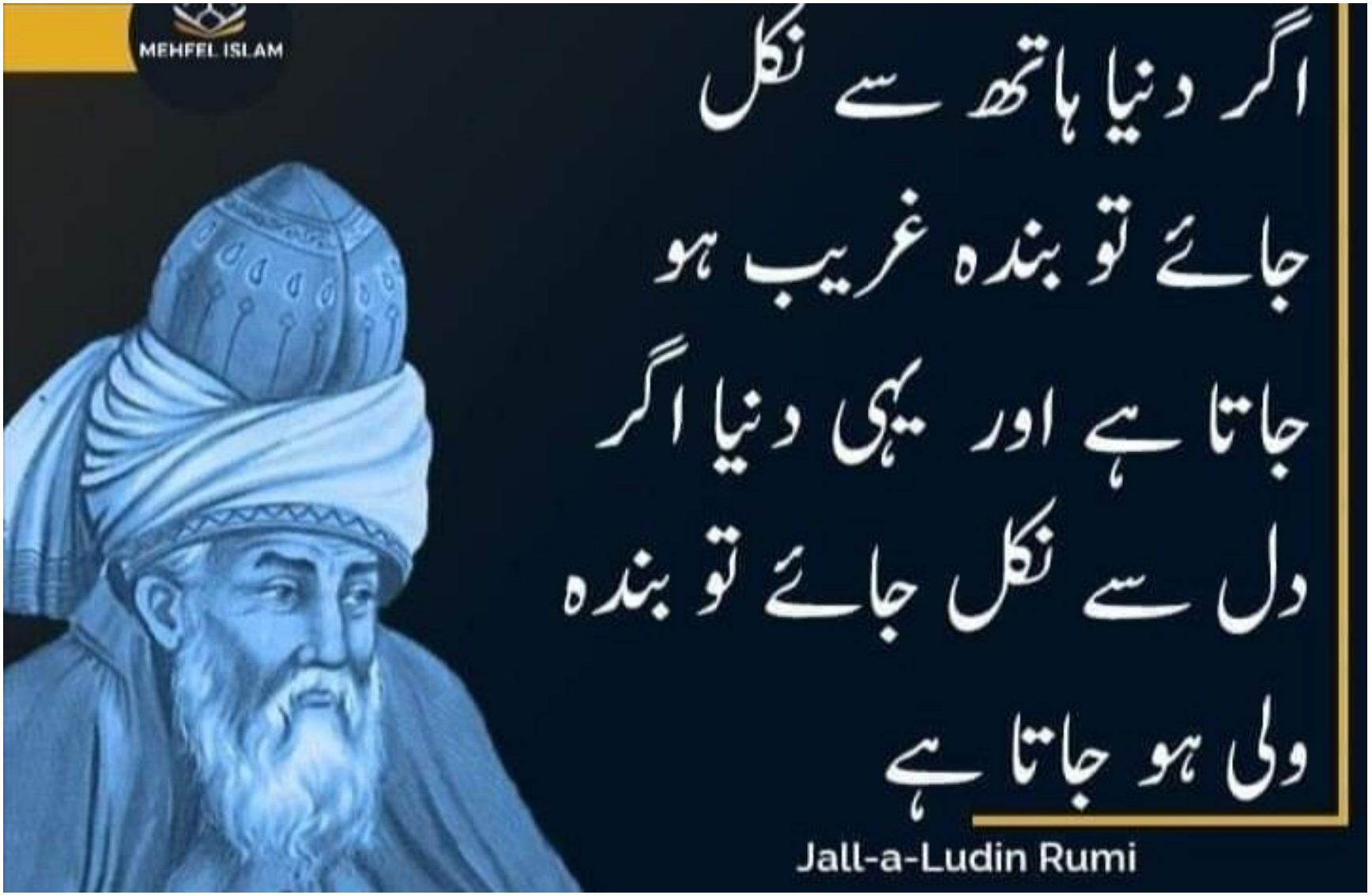
-- identifying **infectious agents**

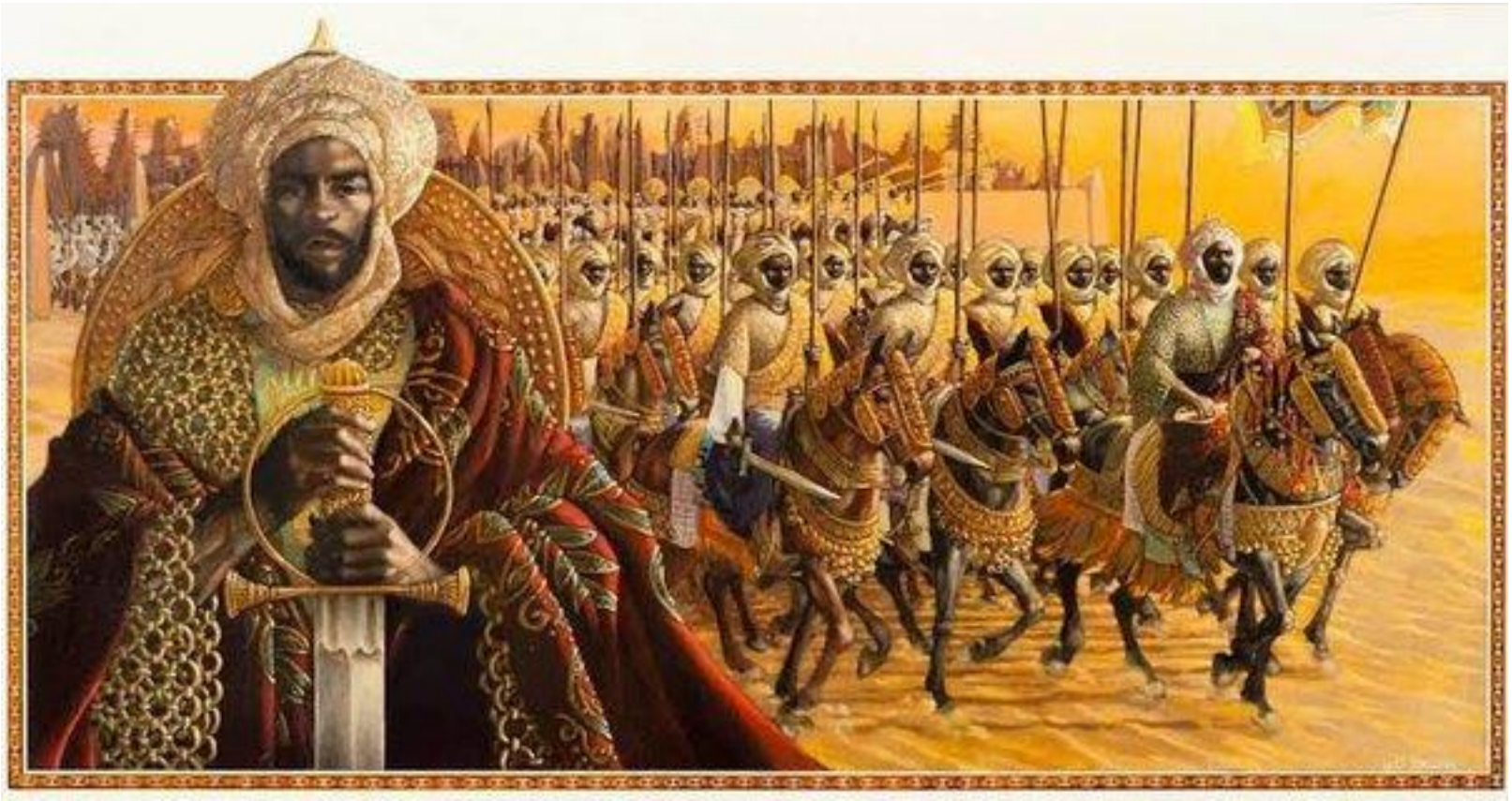
-- identifying **clusters of differentiation** for the classification and follow-up therapy of leukemias and lymphomas

-- identifying **tumor antigens** and **auto-antibodies**

-- identifying the **specific cells** involved in the immune response

-- identifying and quantifying **hormones**


MEHFEL ISLAM
اگر دنیا ہاتھ سے نکل
جائے تو بندہ غریب ہو
جاتا ہے اور یہی دنیا اگر
دل سے نکل جائے تو بندہ
ولی ہو جاتا ہے
Jall-a-Ludin Rumi



Richest man in history

Mansa Musa Emperor of Mali 1312 –1337 AD

Net worth: US $ 400 billion

3 times richer than Jeff Bezos, the richest man in world today


Thank You!