People's Democratic Republic of Algeria

Ministry of Higher Education and Scientific Research

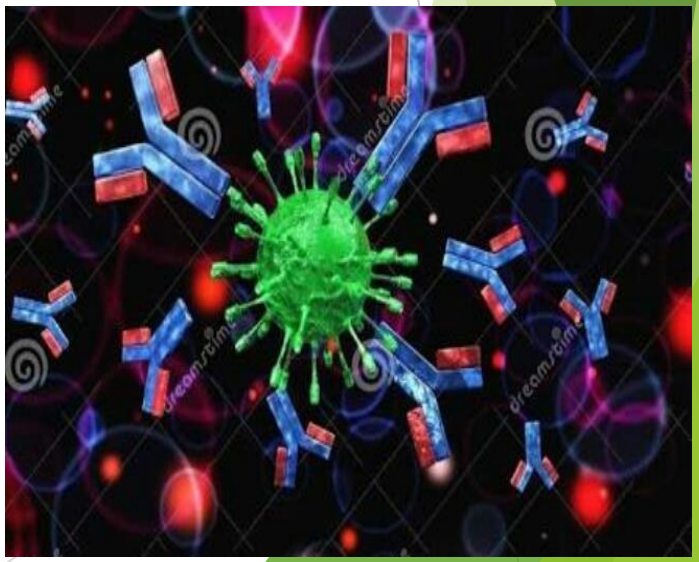
Mila University Center.

Faculty of Natural and Life Sciences *3 rd*

year Biochemistry

Module*:*Immunology

Monoclonal antibodies



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1 INTRODUCTION

- In recent years, new pharmacological agents based on the use of monoclonal antibodies have appeared in oncology .These agents rely on the fact that tumor cells express antigenic targets at higher levels than normal cells.
- There are two types of monoclonal antibodies that can be either unconjugated or conjugated so-called "armed" to a cytotoxic agent that will be delivered directly to the tumor cells.
- Other approaches are also being considered, such as the use of specific monoclonal antibodies, for example bis-peaceful antibodies, antibodies with intracellular action or recombinant antibody fragments

2.- Definition .

- Monoclonal antibodies are antibodies produced naturally by the same line of activated B lymphocytes or plasma cells, recognizing the same episode of an antigen .
- In order to be able to be used as therapy ,they are produced thanks to a cell resulting from the fusion between a B lymphocyte and a cancer cell (myeloma)

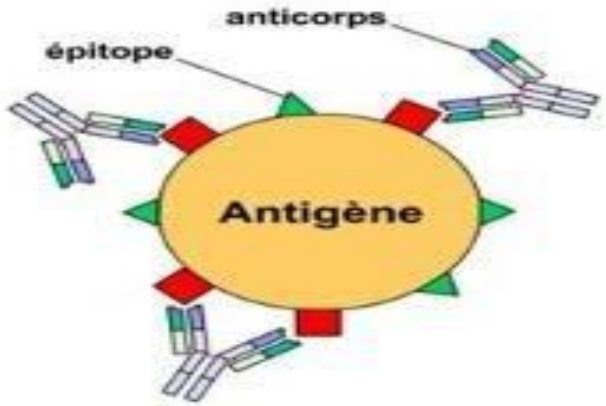
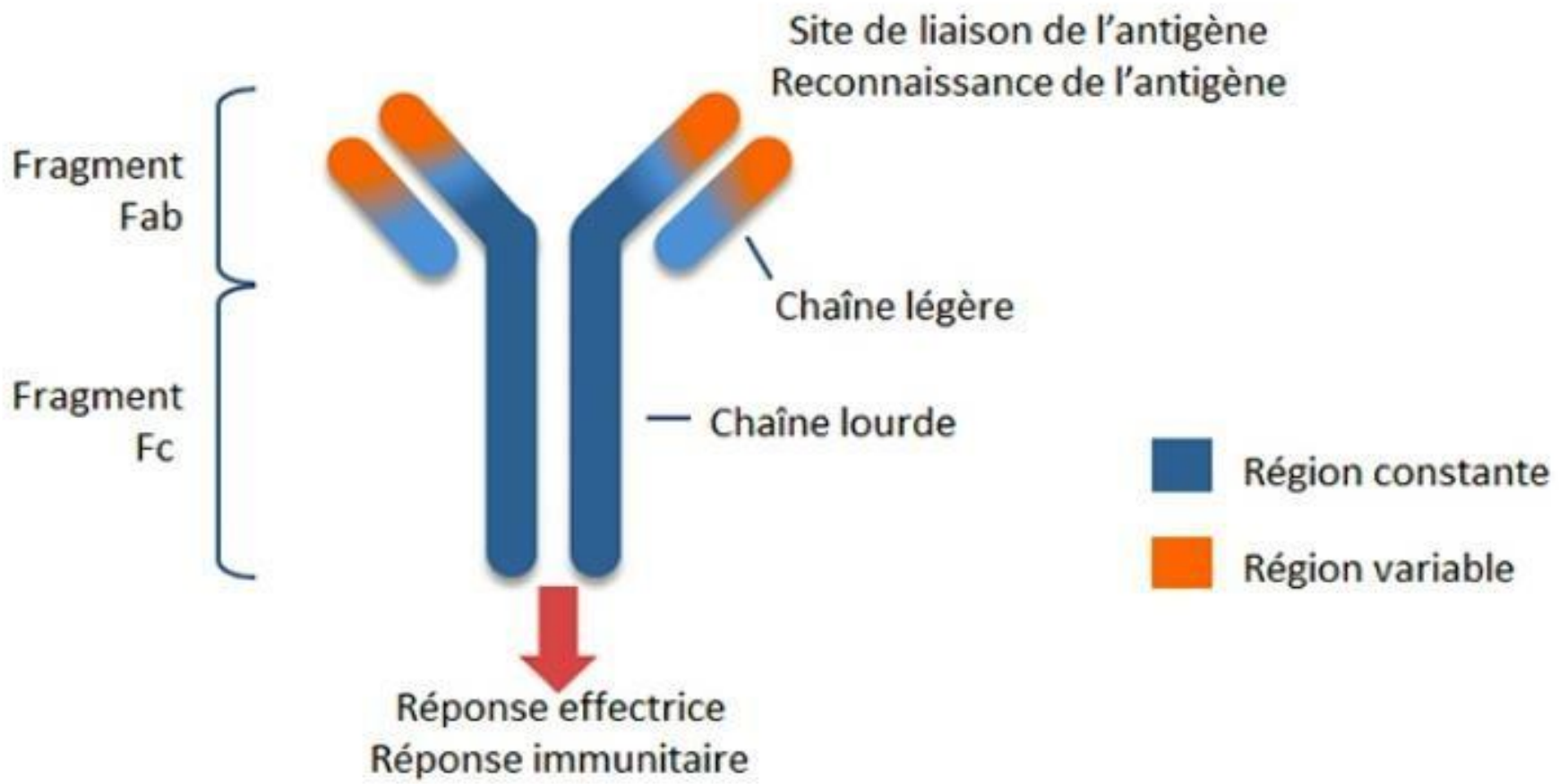


Fig 1 : Des anticorps monoclonaus couplés a un Antigène

3 /Structure and nomenclature:



- Monoclonal antibodies that are used therapeutically end with a

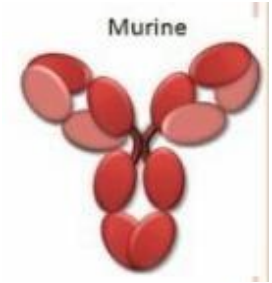
  mAb (Monoclonal Antibody) suffix.According to their nature, 4 generations are determined

4/The different types of monoclonal antibodies:

●*murine antibodies:*

The[first] used from **1975,**they bear the suffix MOMAB

They were produced by the hybridoma technique which consists in forming hybrids between the B lymphocytes of mice immunized with a given antigen and murine myeloma cells.



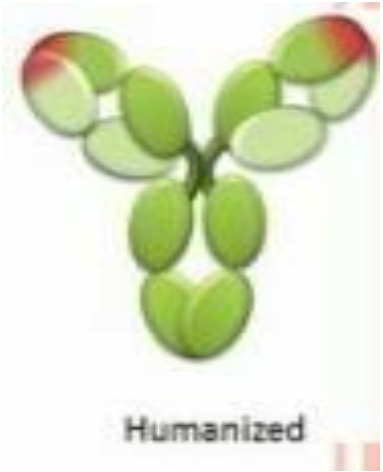
●*Chimeric Human /Mouse Antibodies (1984):*

•Consisting of murine variable (VH) and (VL) regions and human H and L constant regions. Such a genetic construct makes it possible to produce a hybrid CA whose human constant part is not or only slightly immunogenic in humans. Their name includes the syllable XIMAB

•*Antibodies, Humanized*

They exhibit a greater degree of humanization of variable regions with a substitution of murine ACm hypervariable regions for human VH and VL domain hypervariable regions

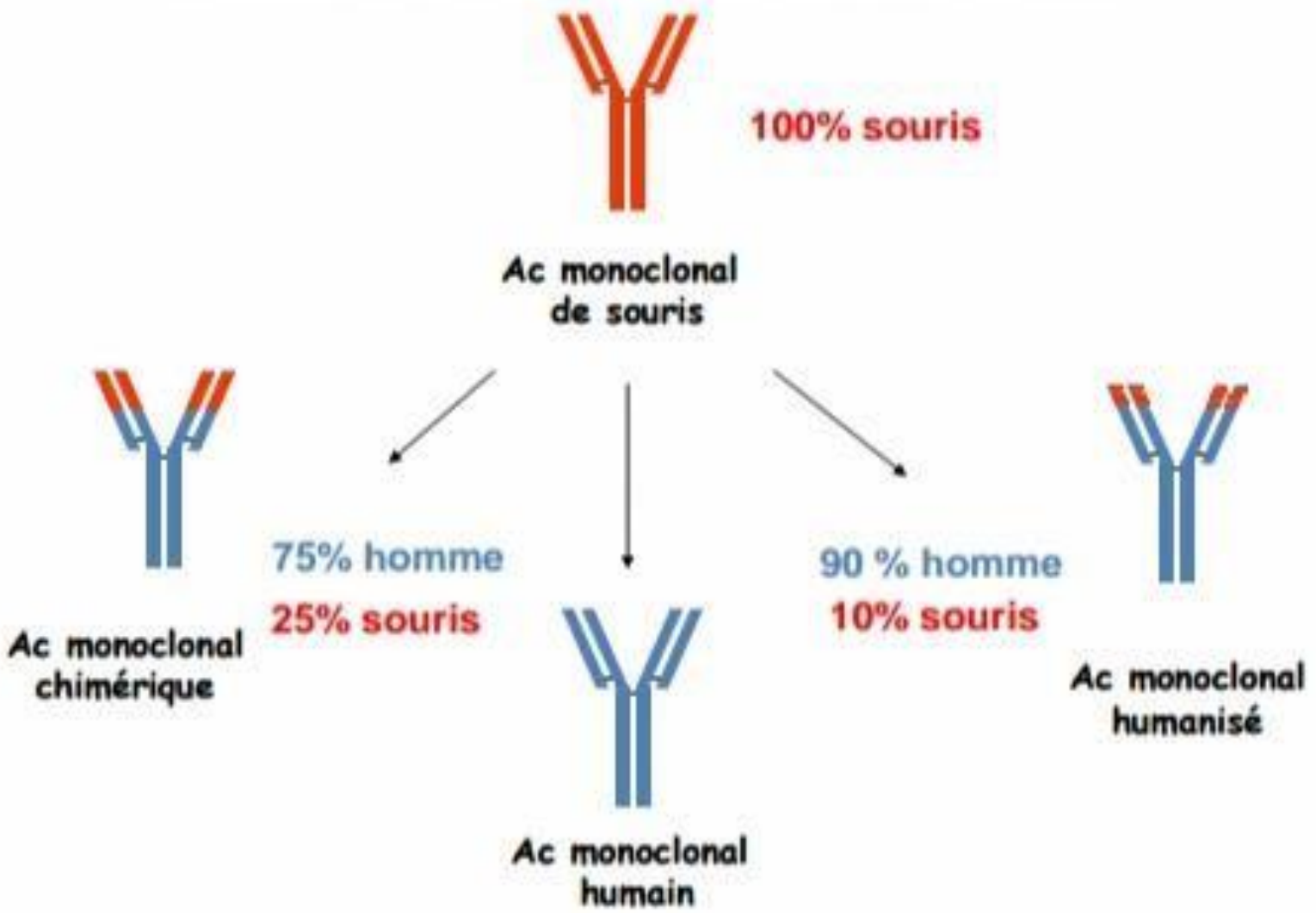
They are identified by the syllable ZUMAB.



• *Fully human antibodies* (from 1994):

(or fully human recombinant monoclonal antibodies) Developed using human GI gene expression technology in phages. These antibodies have the suffix MUMAB

LES DIFFÉRENTES FORMES DES AC MONOCLONAUX:



5/ Production of monoclonal antibodies:

- The production of these antibodies in-vitro is very difficult because of the low plasma cell lifespan
- In vivo, the production of these antibodies can be obtained by injecting the animal with a given antigen and then extracting them from the blood. This method is very expensive and very few antibodies are obtained.
- the development of the hybridoma technique by CESAR MILSTEN and GEORGES KHOLER in 1975 made it possible to obtain a large quantity of antibodies at low cost and thus allow them to be used in many applications.

6/ The hybridoma technique:

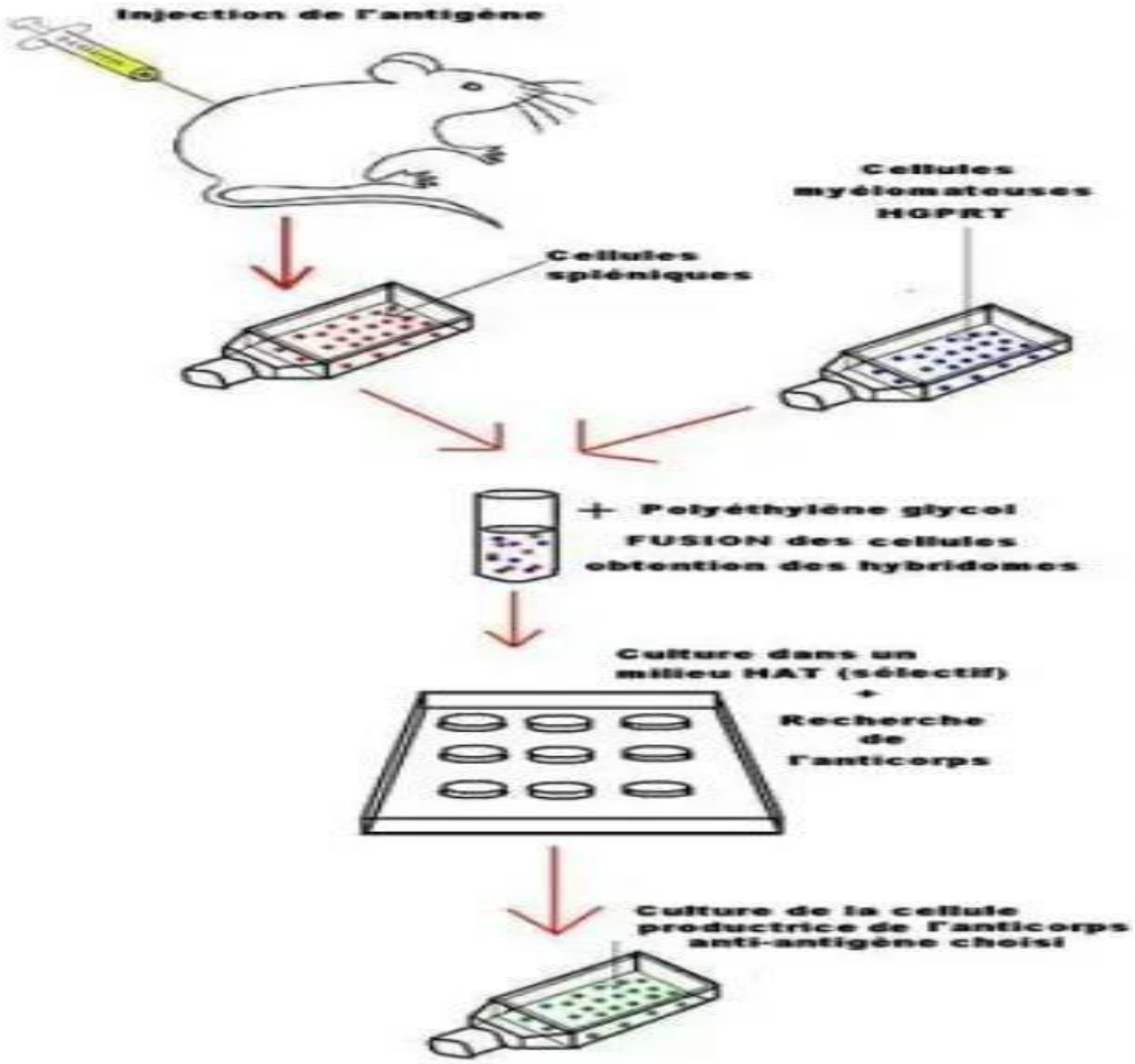
- This technique consists of injecting the antigen of interest into a mouse and then taking, after a few weeks, the cells from the spleen. Among these cells are plasma cells that secrete antibodies specifically directed against the chosen antigen. These plasma cells are fused with tumor cells called myeloma cells (Immortal cells) through the addition of **polyethylene glycol (PEG)** which induces membrane fusion and thus makes it possible to obtain hybridomas that have the ability to multiply faster than normal antibody-producing cells of the body and to develop specific antibodies indefinitely. The cells are then distributed in these multi-well plates in such a way that it

  there is only one cell per well

- In order to remove unfused plasma cells and myeloma cells, HAT **(hypoxanthine aminopterin thymidine) selective culture medium will be used.** Unfused plasma cells die rapidly and used myeloma cells with a non-functional gene for an enzyme involved in nucleotide synthesis **hypoxanthine –guanine –phosphoribosyl -transferase (HGRPT)** are unable to survive in HAT **medium**.

Only hybrid cells multiply. After about ten days, each well is tested for the presence of antibodies directed against the antigen used to immunize the mouse. The producer cells are transplanted. In this way, a few producer cell clones are isolated that can be stored in

liquid nitrogen.


Injection de l'antigène
Cellules myélomateuses HGPRT
Cellules spléniques
+ Polyéthylène glycol
FUSION des cellules
obtention des hybridomes
Culture dans un milieu HAT (sélectif)
+
Recherche de l'anticorps
Culture de la cellule productrice de l'anticorps anti-antigène choisi

8/ Use:

- Monoclonal antibodies used as drugs all have a nomenclature ending with "mab", acronym for "monoclonal antibody" such as Rituximab (treatment of rheumatoid arthritis).
- They are used in pregnancy tests, in many areas of research in biology and by many techniques (flow cytometry, western blots.), in immunohematology tests.
- Monoclonal antibodies, as monotherapy or in combination with chemotherapy, have become part of the standard treatment of many forms of cancer and pathologies.

- **Immunosuppressive therapy:** (Autoimmune and inflammatory diseases, transplant rejection).
- **Cancer treatment** (removal of cancer cells).
- **Cardiology** (anti-thrombotic treatment).
- **Infectiology** (antiviral prophylaxis).
- **Allergology** (asthma treatment).
- Many hopes raised in the treatment of many serious pathologies, for which the conventional therapies have shown their limitations. (targeted therapies)