

*Medical Biotechnology 2026'*  
**Biological therapies**

**Lecture 3-4<sup>th</sup>**

**Monoclonal antibodies for therapy. I.**

# Biological therapy

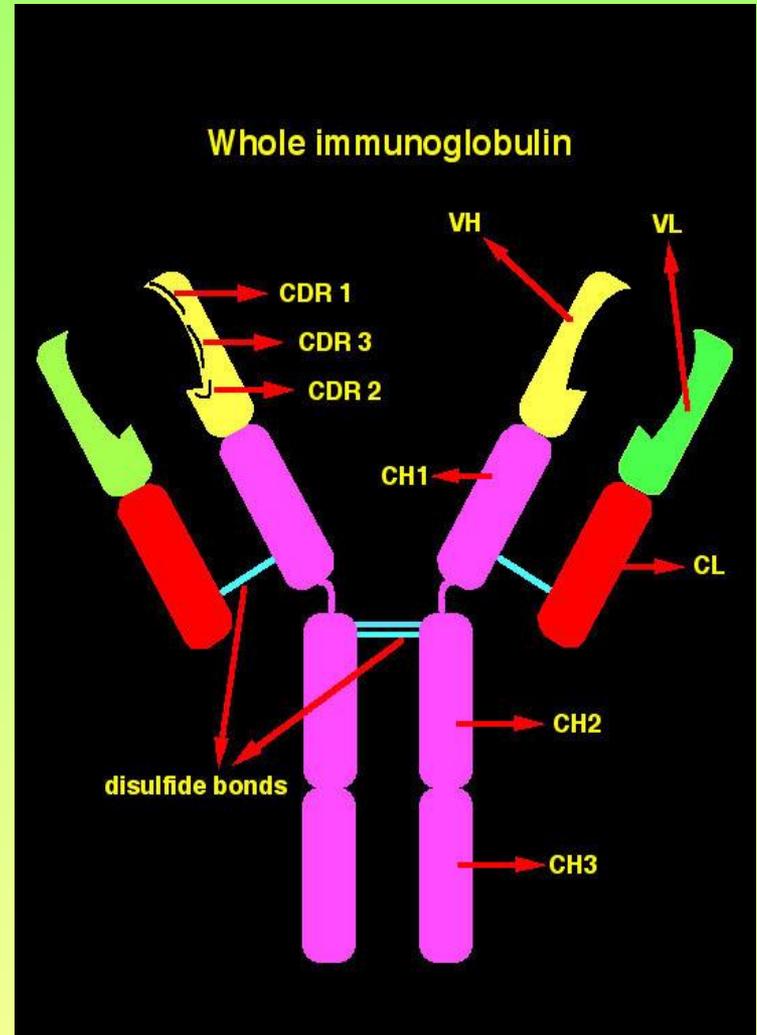
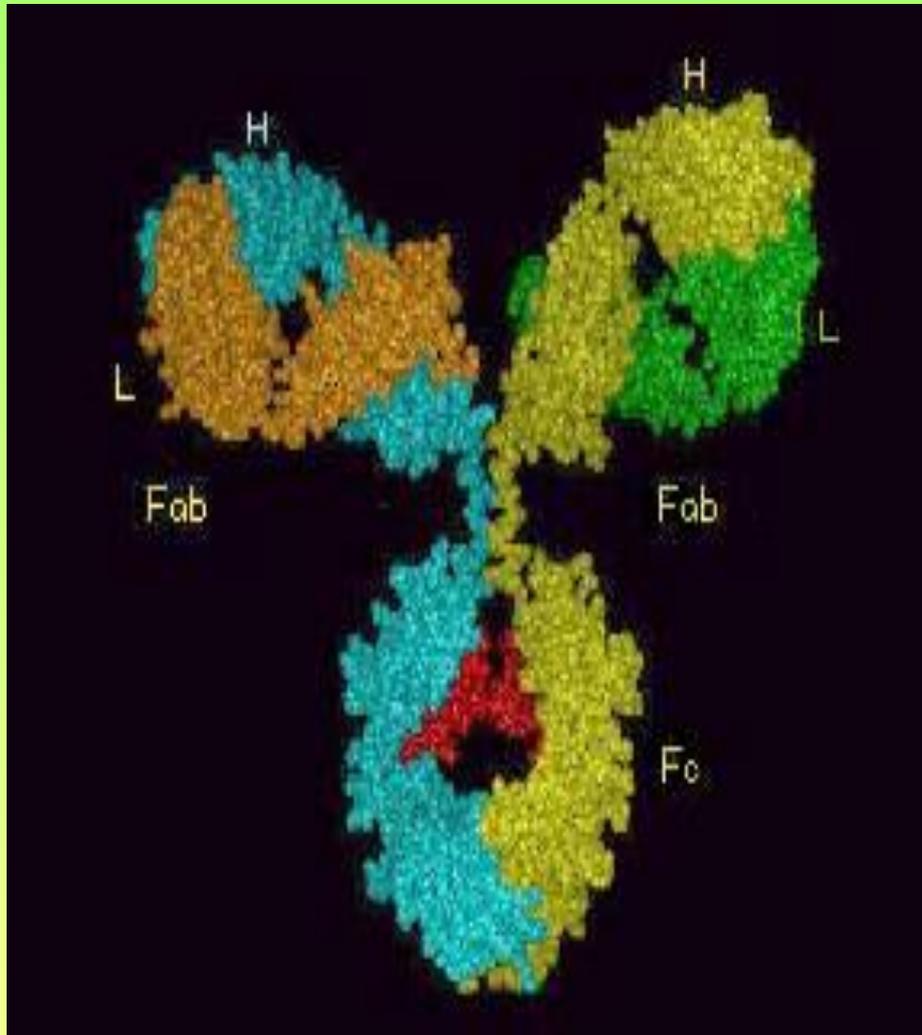
- Biological or biologic therapy is a type of treatment used to stimulate or restore the ability of the body's immune system.
- Forms of biological therapies:
  - Monoclonal antibodies
  - Interferons and colony stimulating factors
  - Vaccines and adoptive cell transfer
  - Tumor vaccines
  - Gene therapy

# Therapeutic monoclonal antibodies

## Antibodies produced by gene technologies:

- Antibodies produced by somatic cell fusion (hybridoma technology )
- Humanized antibodies
- Recombinant monoclonal antibodies

# Immunoglobulin structure



3-3 amino acids determine the idiootype of an immunoglobulin

# Cytokines Dictate the Isotype Production

## Role of cytokines in regulating Ig isotype expression

Cytokines	IgM	IgG3	IgG1	IgG2b	IgG2a	IgE	IgA
IL-4	Inhibits	Inhibits	Induces		Inhibits	Induces	
IL-5							Augments production
IFN- $\gamma$	Inhibits	Induces	Inhibits		Induces	Inhibits	
TGF- $\beta$	Inhibits	Inhibits		Induces			Induces

Figure 9-7 Immunobiology, 6/e. (© Garland Science 2005)

IL-4 leads to IgG and IgE production (TH2)

IFN- $\gamma$  leads to IgG production (TH1)

TNF- $\alpha$  leads to IgG and IgA production (TH1)

They will also inhibit the production of other isotypes

# Main steps of antibody production

- Polyclonal antibodies - antisera
  - immunization
  - antibody purification
- Hybridomas and monoclonal antibodies for therapeutic use
  - antigen design and antibody production
  - humanization
  - large scale fermentation

# Polyclonal antibody production

Polyclonal antibody production involves the repeated immunization of an animal with a desired antigen.

Animals such as rabbits, goats, and sheep are generally used for polyclonal antibody production, as they are relatively easy to handle for immunization and bleeding purposes. Polyclonal antibodies can be produced quickly and relatively cheaply and do not require the same amount of expertise or time as monoclonal antibody production. Polyclonal antiserum contains a heterogeneous population of antibodies.

# Animals for immunization





FIG. 4

The production of an antiserum: bleeding an immunized horse from the jugular vein.

# Factors influencing antibody production

- MHC haplotype of recipient
- Nature of the antigen
- Dose of the antigen
- Compartment of the administration
- Adjuvants
- Kinetics of sequential immunisation

# Characteristics of polyclonal antibodies

- Blood serum (mixture of different antibodies with altered isotype, idiotype and affinity)
- Characterised by avidity (working dilution)
- Standard (during the bench)

# Immunoglobulin purification

Salt precipitation  $(\text{NH}_4)_2\text{SO}_4$  precipitation

Liquid chromatography

Affinity chromatography (Fc end, antigen)



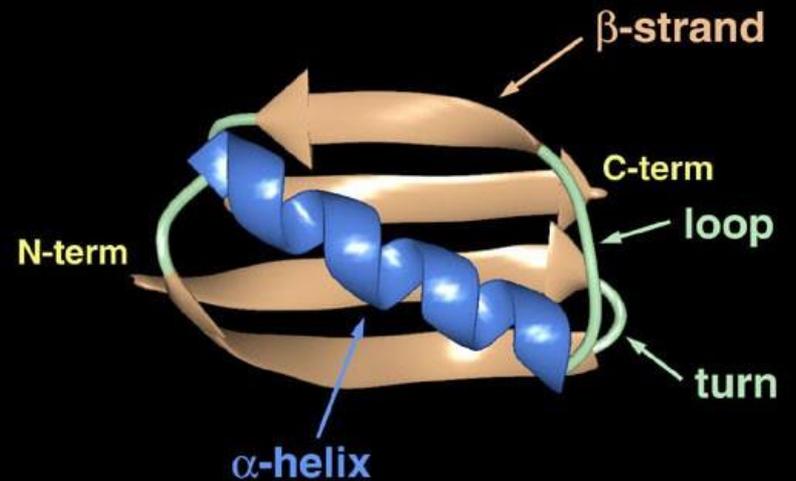
# Affinity purification

## Protein A



## Protein G

### immunoglobulin binding domain of protein G



**„Discovery of monoclonal antibody production was not a simple laboratory technical development, but a new area which overrode the biological and medical sciences and the daily diagnostic and industrial practice.“**

# N° of research articles in the NCBI PubMed

at 02/12/2026

*„monoclonal antibody“*  
415.576

*„therapeutic monoclonal antibody“*  
217.297

• 1976	169	18
• 1977	186	31
• 1978	204	23
• 1979	331	45
• 1980	705	95
• 1985	6.839	245
• 1995	9.698	1.628
• 2005	8.429	4.080
• 2013	11.356	7.048
• 2014	12.728	8.798

**2024**

More than 2000  
therapeutic  
monoclonal  
antibodies are  
under clinical trial

# Preliminaries

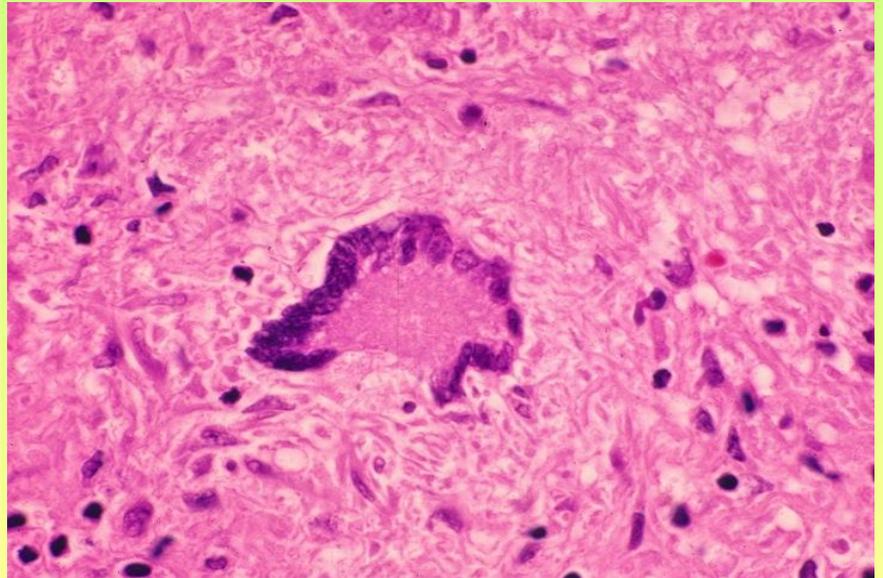
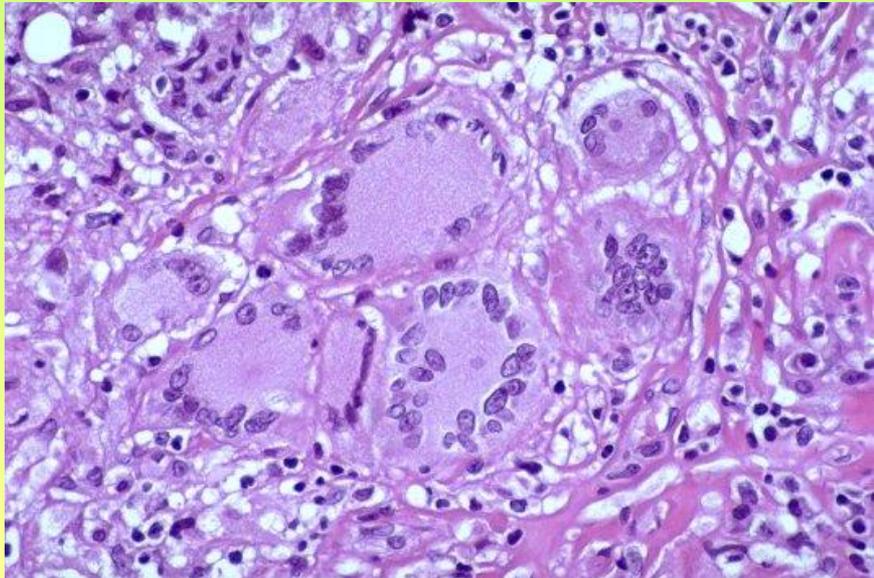
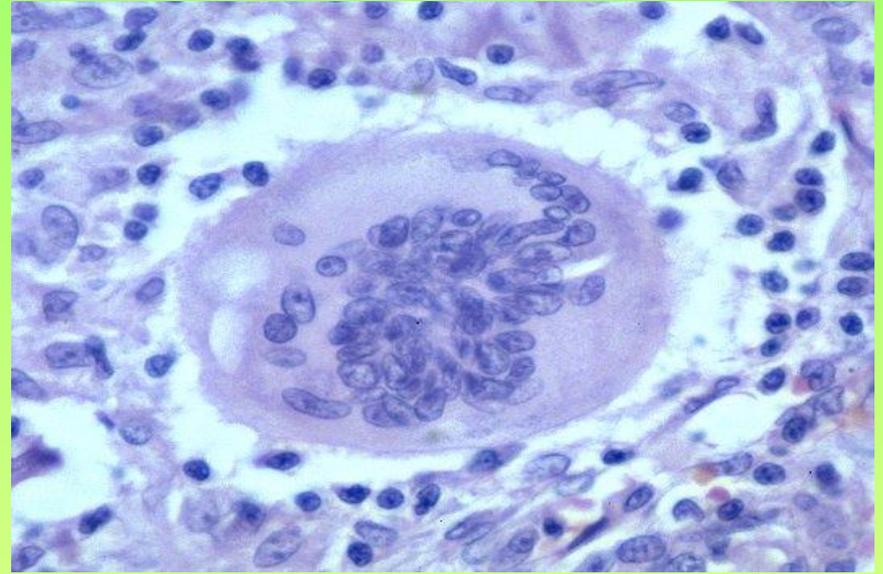
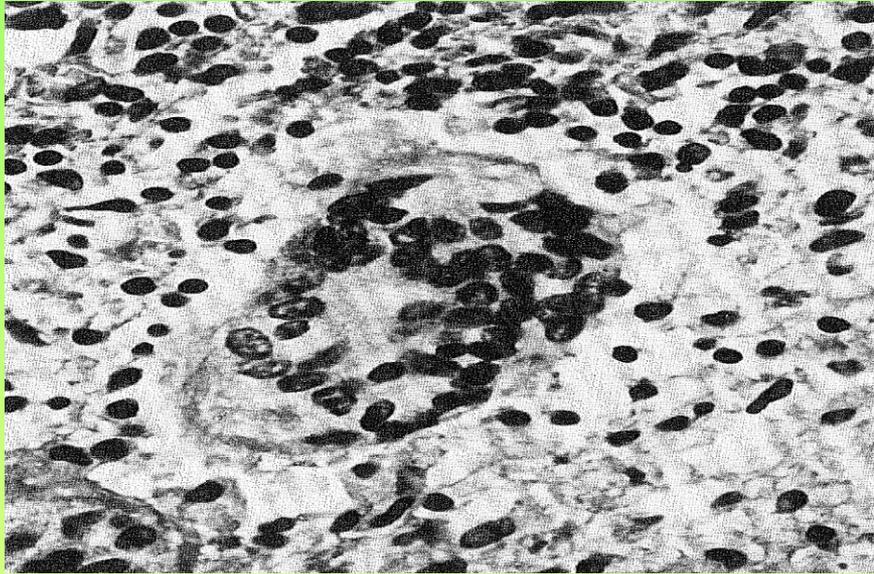
**Johannes Müller (1801-1858):** firstly described the fusion of somatic cells

**Virchow (1821-1902) and Langerhans (1847-1888):** published cell fusions in pathologic tissues

**Ringertz (1876):** described the giant cell formations caused by somatic cell fusions in pathologic conditions

**Lewis (1927)** spontaneous cell fusions in *in vitro* cultured tumor cells

# Multinucleated „giant cells” occurred *in vivo*



**Barski, Sorieul and Confert** (1960, 1961) produced the first *in vitro* somatic cell fusions

**Okada** (1972): developed the technique of UV inactivated Sendai virus for somatic cell hybridization

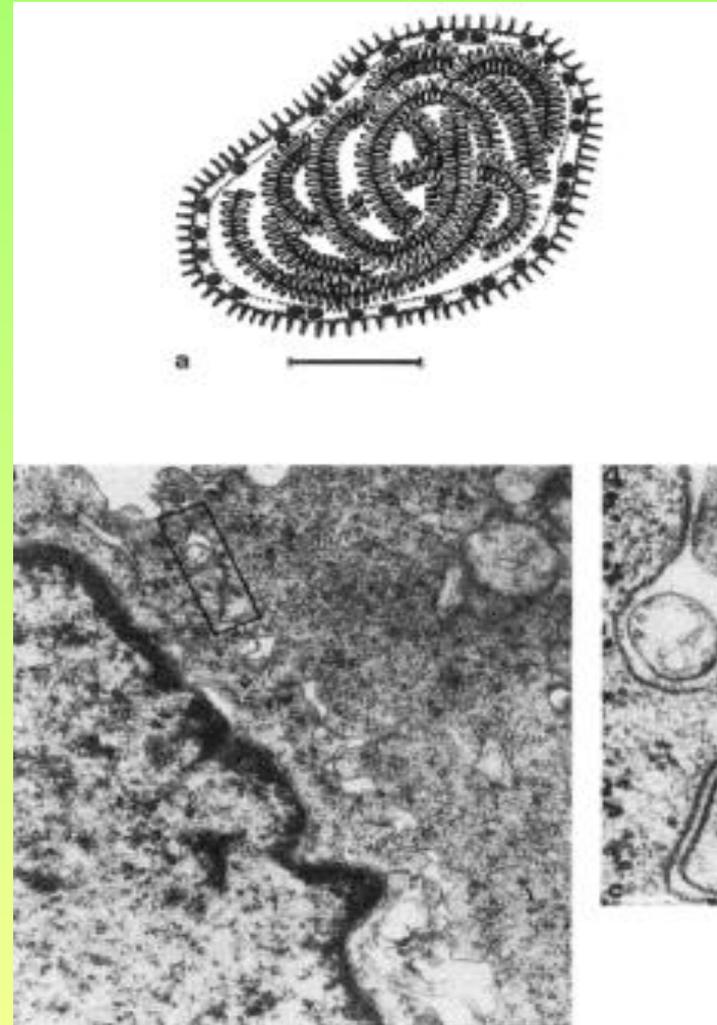
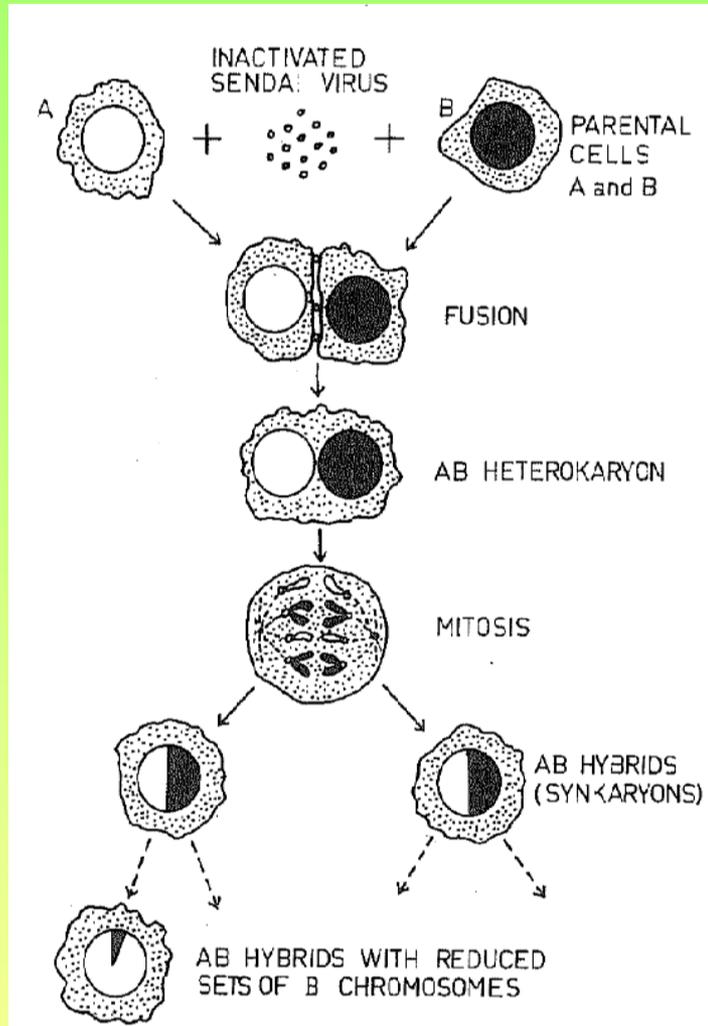
**Littlefield** (1964, 1966): selection of cell hybrids by the use of enzyme (HGPRT, TK) deficient mutant cell lines

**Harris and Watkins** (1965, 1969), and **Okada and Murayama**: first interspecies hybrids

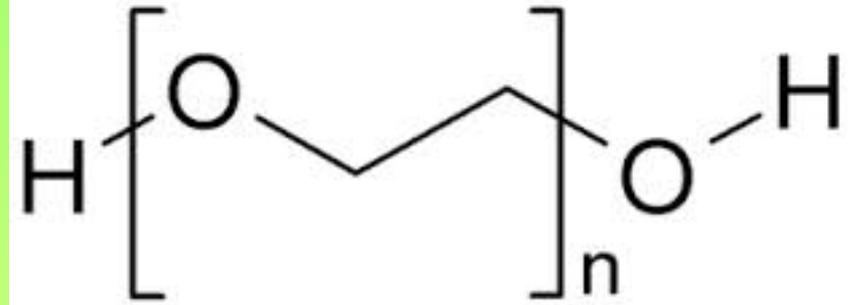
**Harris and Klein** (1969): hybrids of normal and tumor cells

**Köhler and Milstein** (1975): somatic cell fusion for mouse light chains research

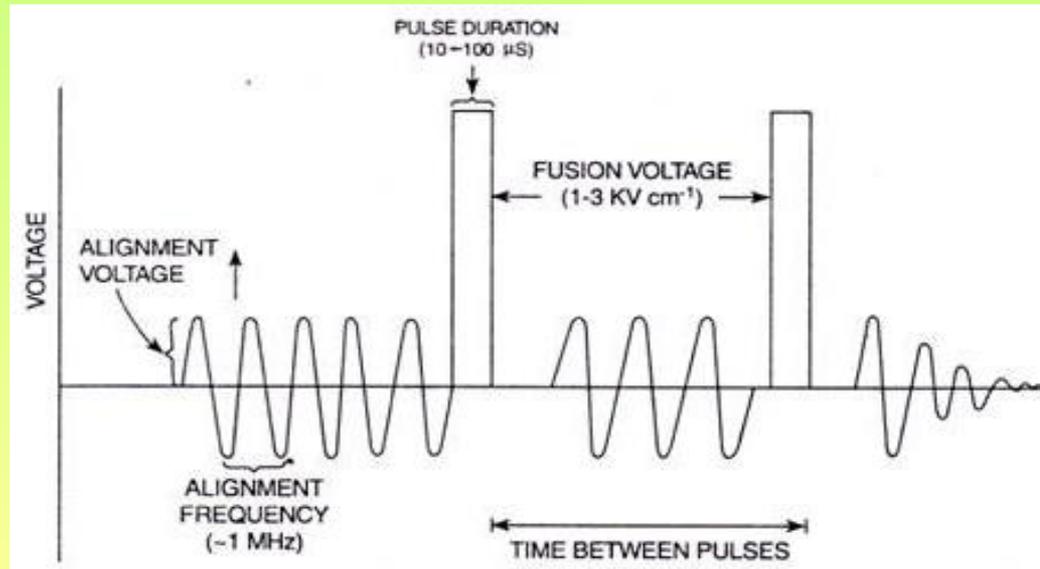
# Sendai virus induced somatic cell hybrids

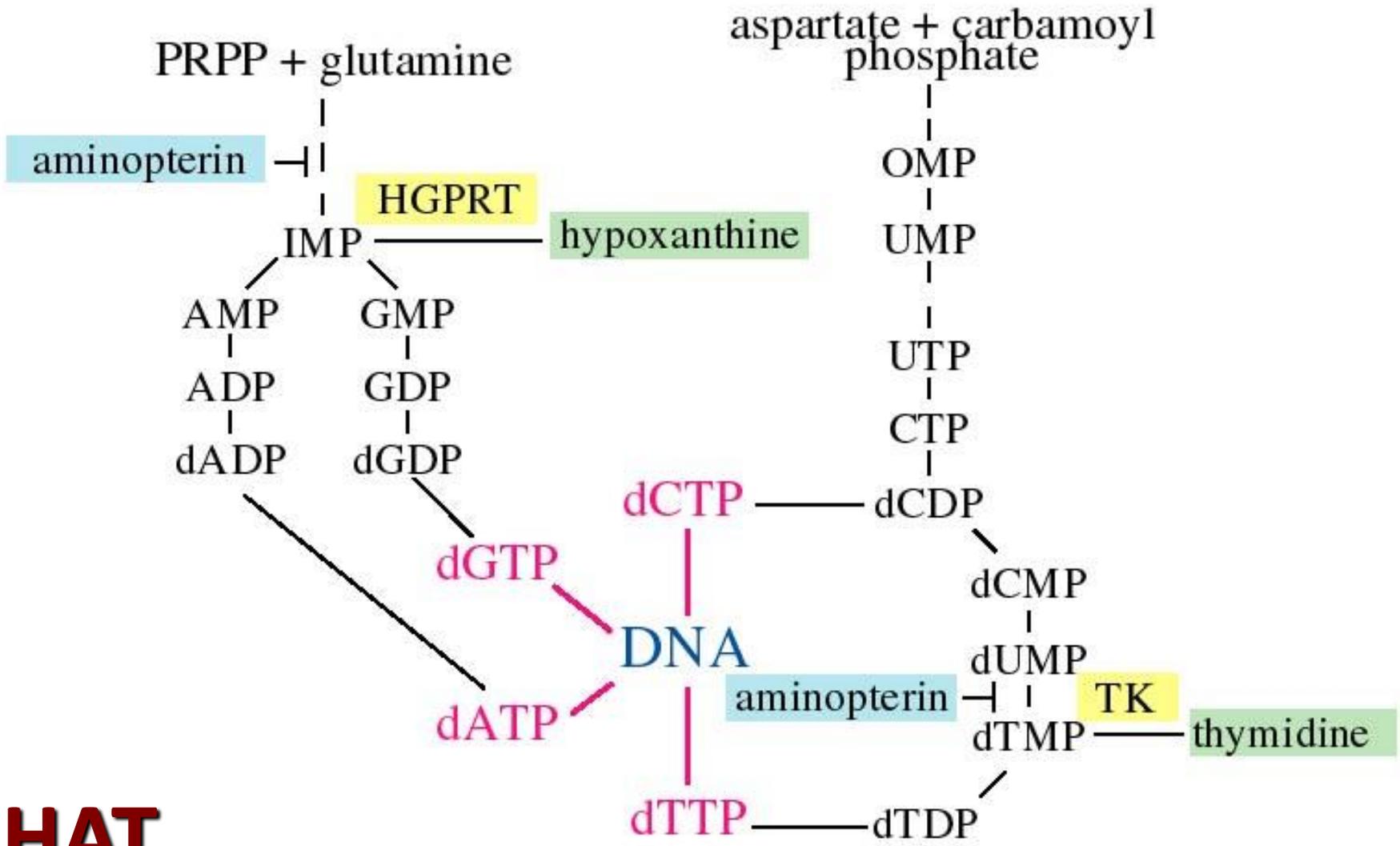


# Polyethylene glycol



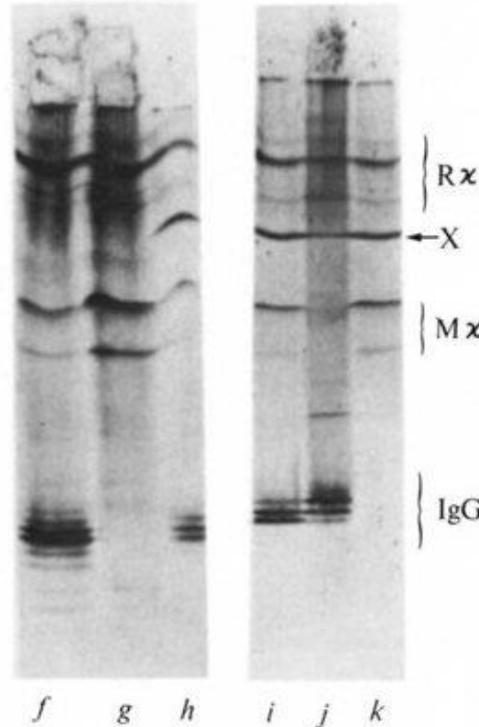
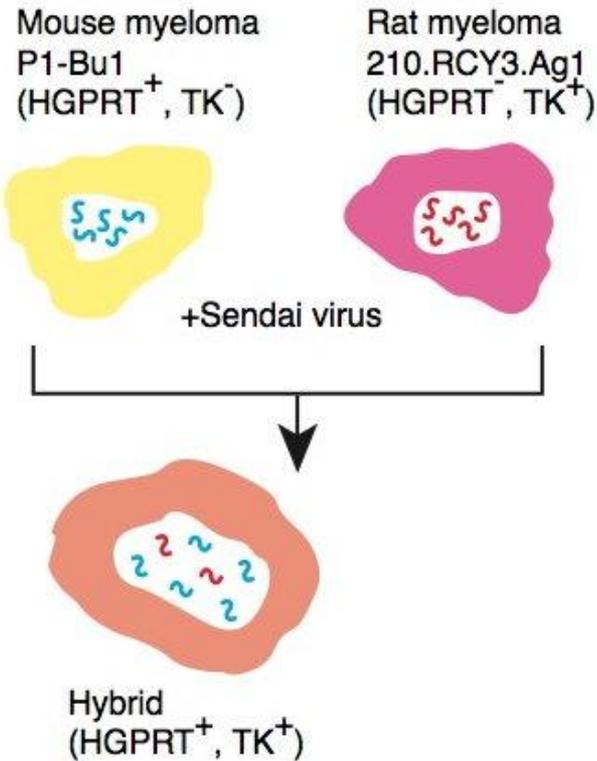
## Electrofusion





**HAT**

# Fusion of two Ig-producing myeloma cell lines



- f* P1-Bu1 + 210.RCY3.Ag1
- g* P1-Bu2 + 210.RCY3.Ag1
- h* hybrid clone 21
- i* hybrid clone 16
- j* hybrid clone 21
- k* hybrid clone 19

RGH Cotton and C Milstein  
 Nature 244: 42 (1973)

# **Nature 256, 495 - 497 (07 August 1975); Continuous cultures of fused cells secreting antibody of predefined specificity**

**G. KÖHLER & C. MILSTEIN**

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

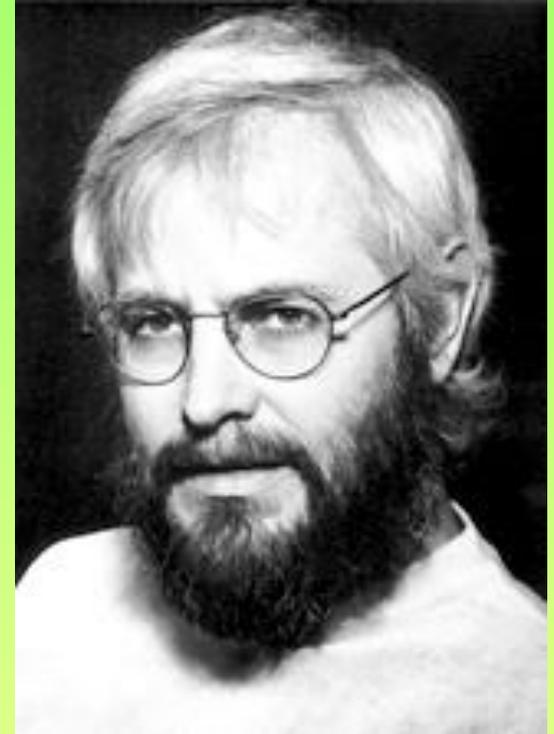
**THE manufacture of predefined specific antibodies by means of permanent tissue culture cell lines is of general interest. There are at present a considerable number of permanent cultures of myeloma cells<sup>1,2</sup> and screening procedures have been used to reveal antibody activity in some of them. This, however, is not a satisfactory source of monoclonal antibodies of predefined specificity. We describe here the derivation of a number of tissue culture cell lines which secrete anti-sheep red blood cell (SRBC) antibodies. The cell lines are made by fusion of a mouse myeloma and mouse spleen cells from an immunised donor. To understand the expression and interactions of the Ig chains from the parental lines, fusion experiments between two known mouse myeloma lines were carried out.**

## **References**

1. Potter, M., *Physiol. Rev.*, **52**, 631–719 (1972).
2. Horibata, K., and Harris, A. W., *Expl Cell Res.*, **60**, 61–70 (1970).
3. Milstein, C., and Munro, A. J., in *Defence and Recognition* (edit. by Porter, R. R.), 199–228 (MTP Int. Rev. Sci., Butterworth, London, 1973).
4. Cotton, R. G. H., and Milstein, C., *Nature*, **244**, 42–43 (1973).
5. Schwaber, J., and Cohen, E. P., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2203–2207 (1974).
6. Littlefield, J. W., *Science*, **145**, 709 (1964).
7. Svasti, J., and Milstein, C., *Biochem. J.*, **128**, 427–444 (1972).
8. Milstein, C., Adetugbo, K., Cowan, N. J., and Secher, D. S., *Progress in Immunology*, II, **1** (edit. by Brent, L., and Holborow, J.), 157–168 (North-Holland, Amsterdam, 1974).
9. Harris, H., and Watkins, J. F., *Nature*, **205**, 640–646 (1965).
10. Awdeh, A. L., Williamson, A. R., and Askonas, B. A., *Nature*, **219**, 66–67 (1968).
11. Milstein, C., Brownlee, G. G., Cartwright, E. M., Jarvis, J. M., and Proudfoot, N. J., *Nature*, **252**, 354–359 (1974).
12. Frangione, B., and Milstein, C., *Nature*, **244**, 597–599 (1969).
13. Jerne, N. K., and Nordin, A. A., *Science*, **140**, 405 (1963).
14. Cotton, R. G. H., Secher, D. S., and Milstein, C., *Eur. J. Immun.*, **3**, 135–140 (1973).



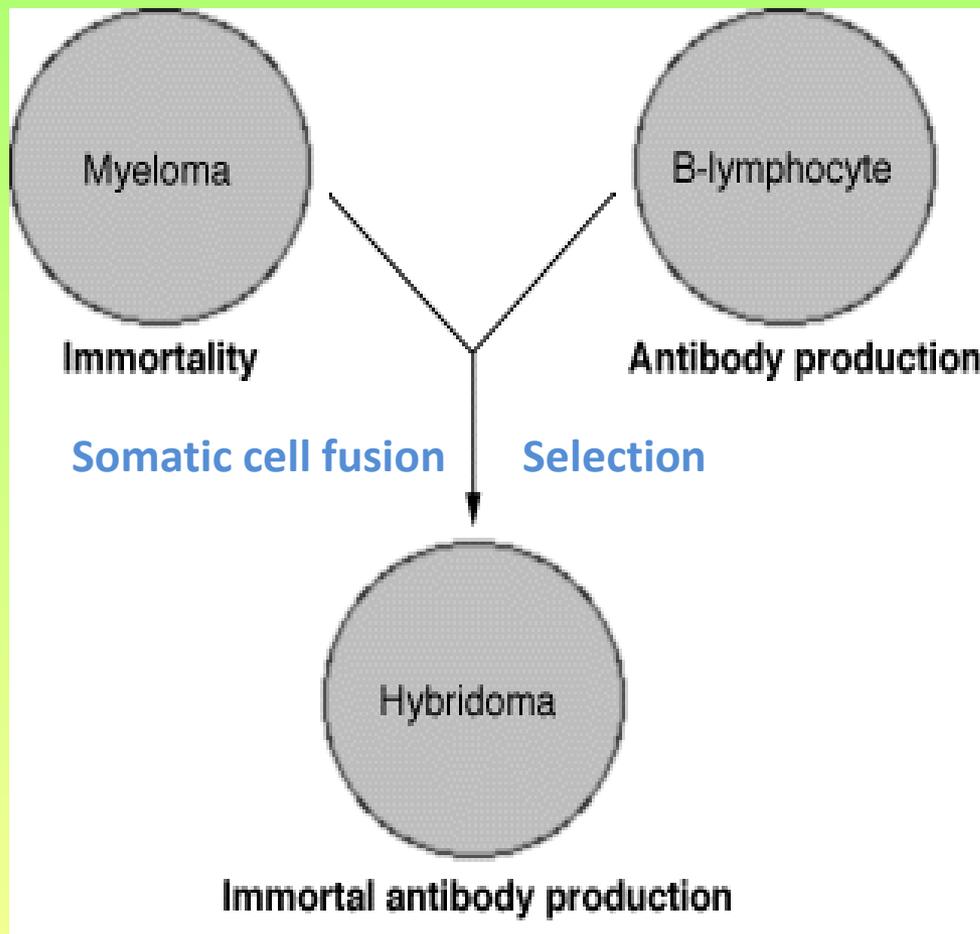
**César Milstein**



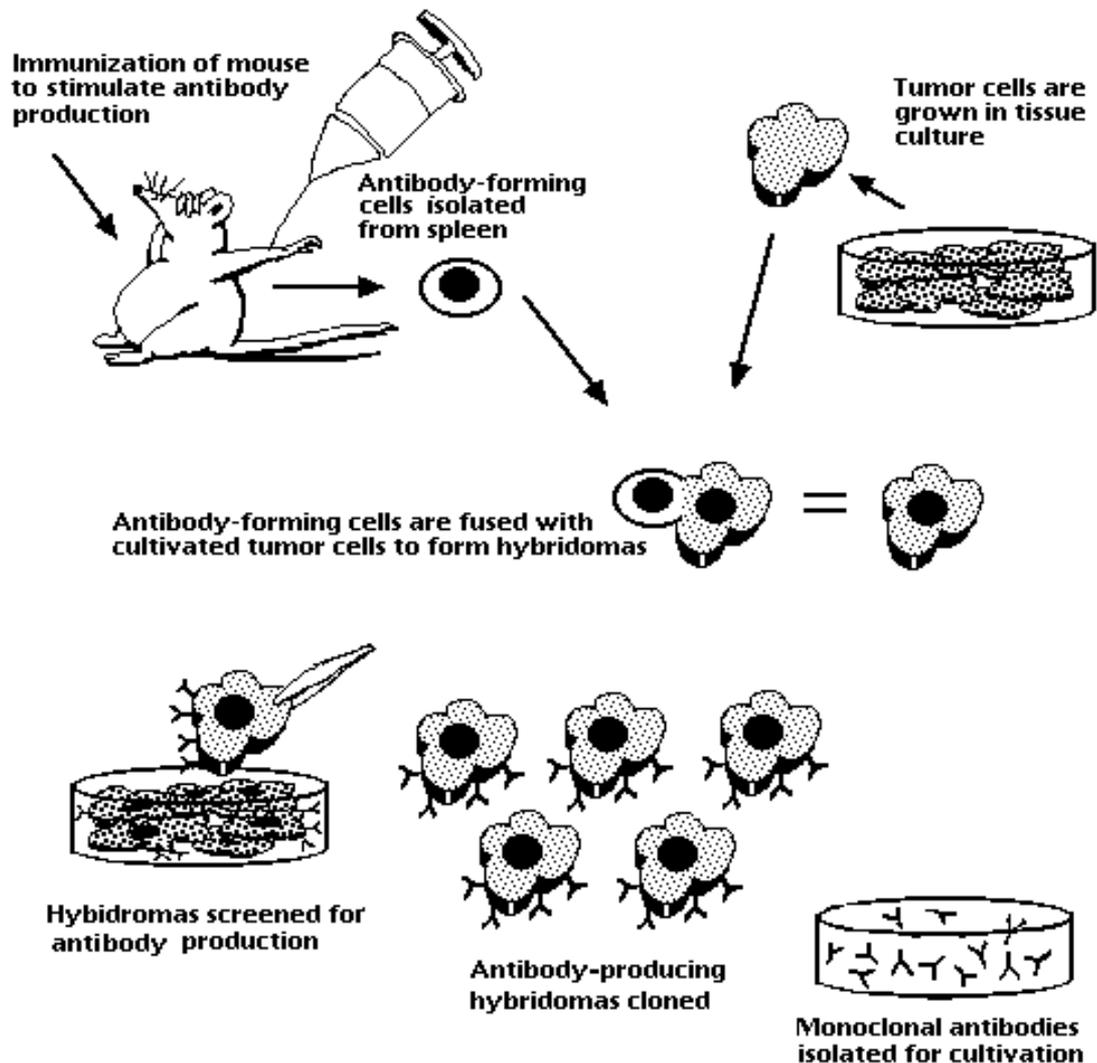
**Georg Köhler**

**Nobel prize, 1984:** *"for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies"*

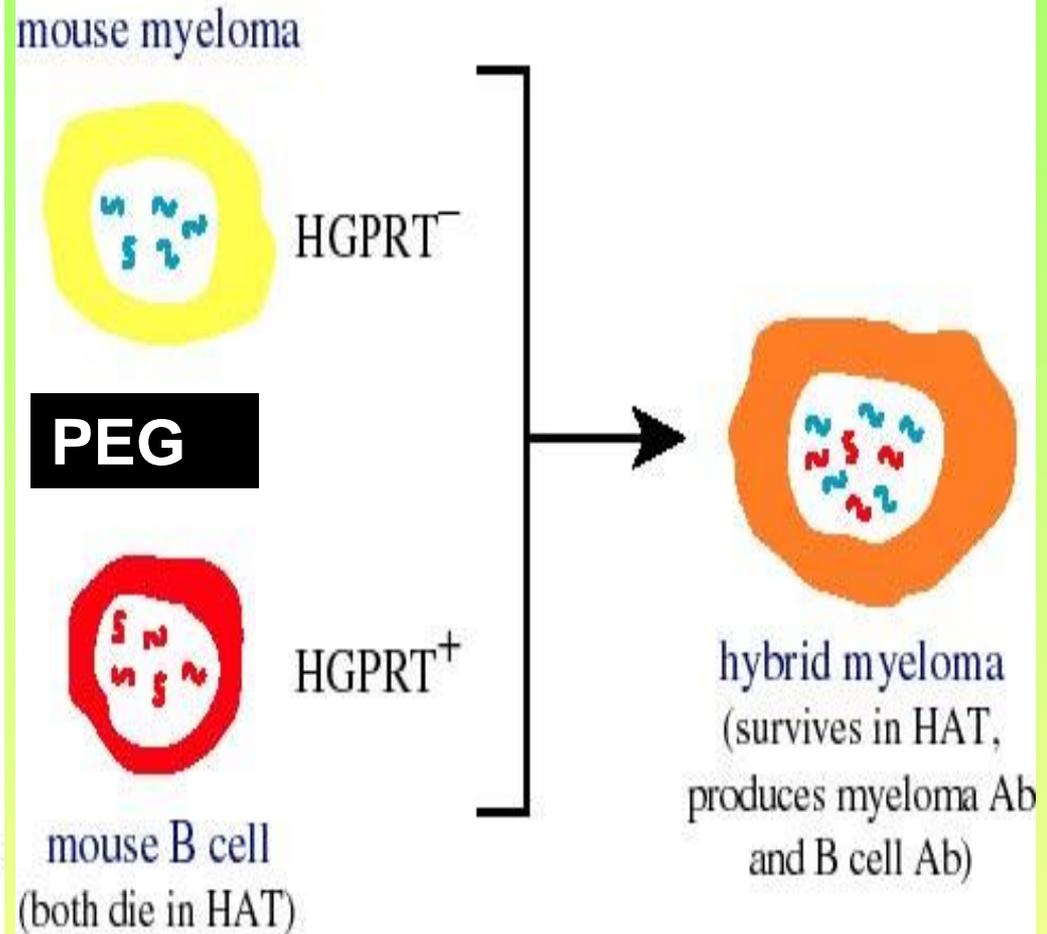
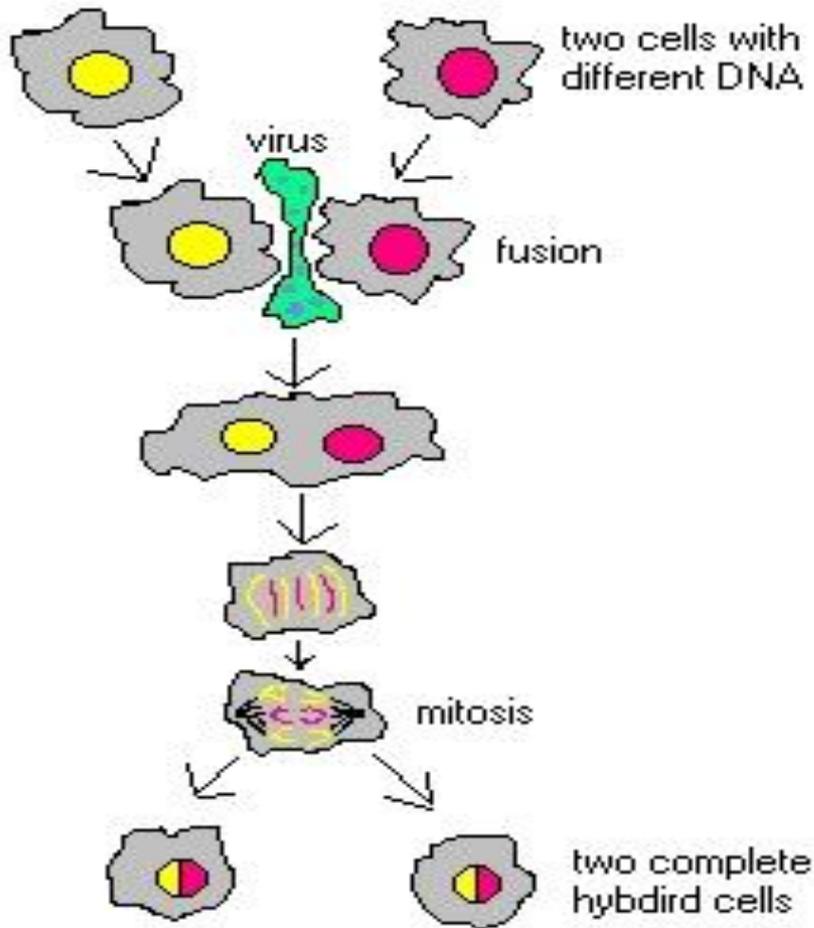
# Hybridoma



# Monoclonal Antibody Production



# Somatic cell hybridization and selection

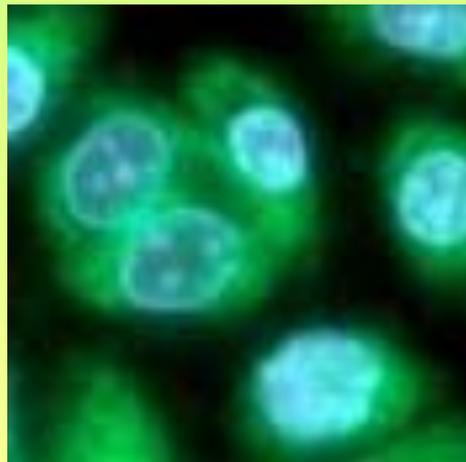


# **Main steps of monoclonal antibody production**

- **Antigen design**
- **Immunisation**
- **Hybridoma production**
- **Selection**
- **Cloning**
- **Mass production**
- **Application for practical use**

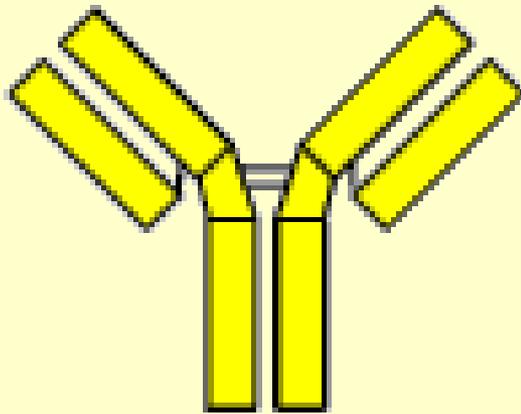
# Main characteristics of monoclonal antibodies

- Genetically engineered antibodies
- Uniform immunoglobulin molecules specific in a single epitope
- Characterized by chemical affinity
- Standard during the life time of hybridoma cell line

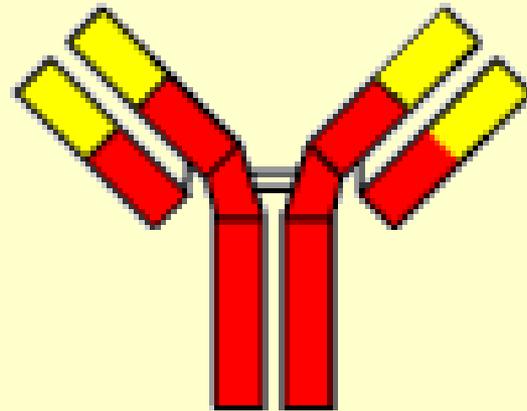


# **Recombinant monoclonal antibodies for therapeutic use**

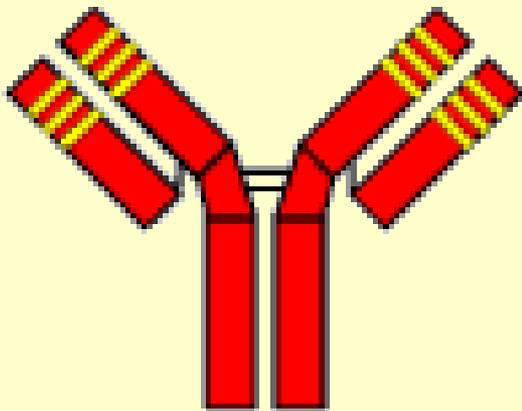
- Chimeric mabs
- Humanized mabs
- Human mabs
- Ig like constructions



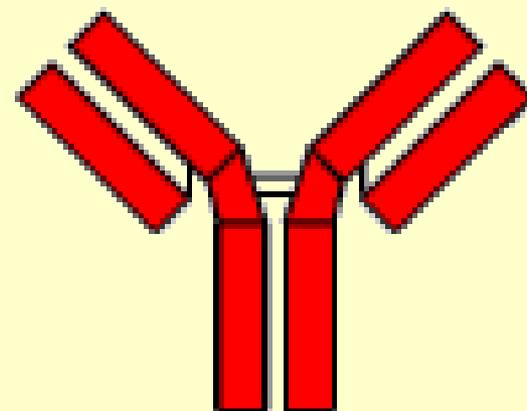
**Murine**



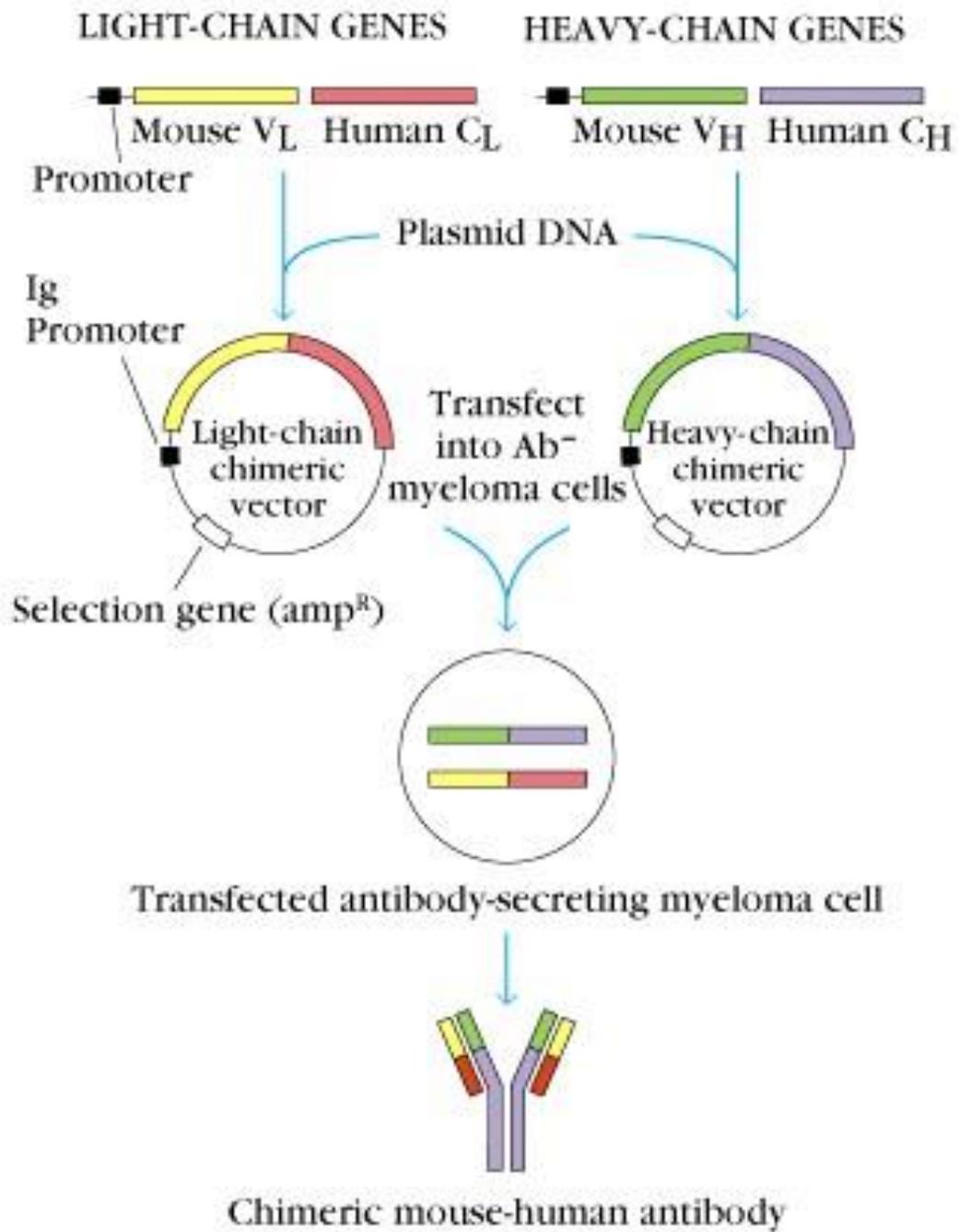
**Chimaeric**



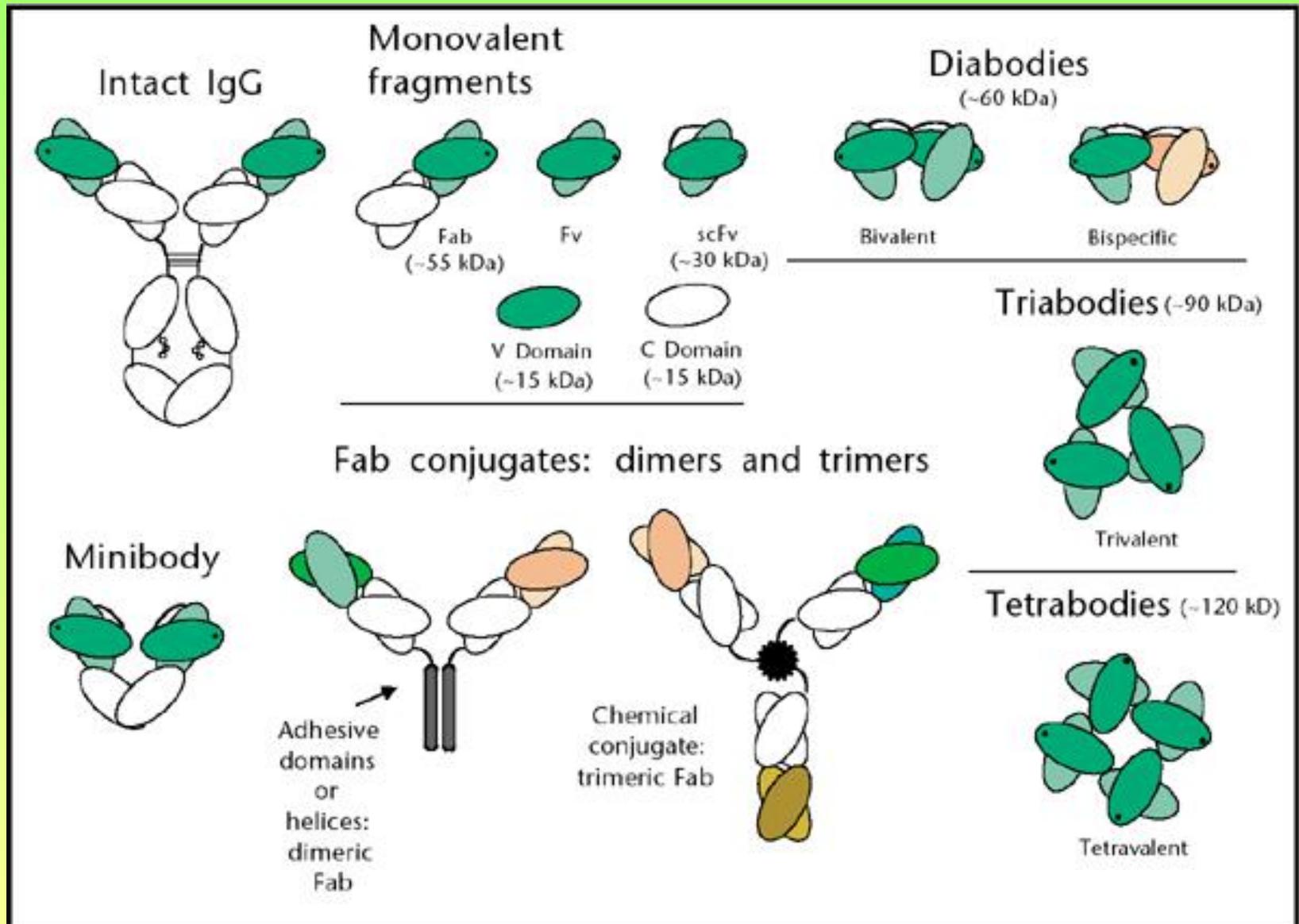
**Humanised**



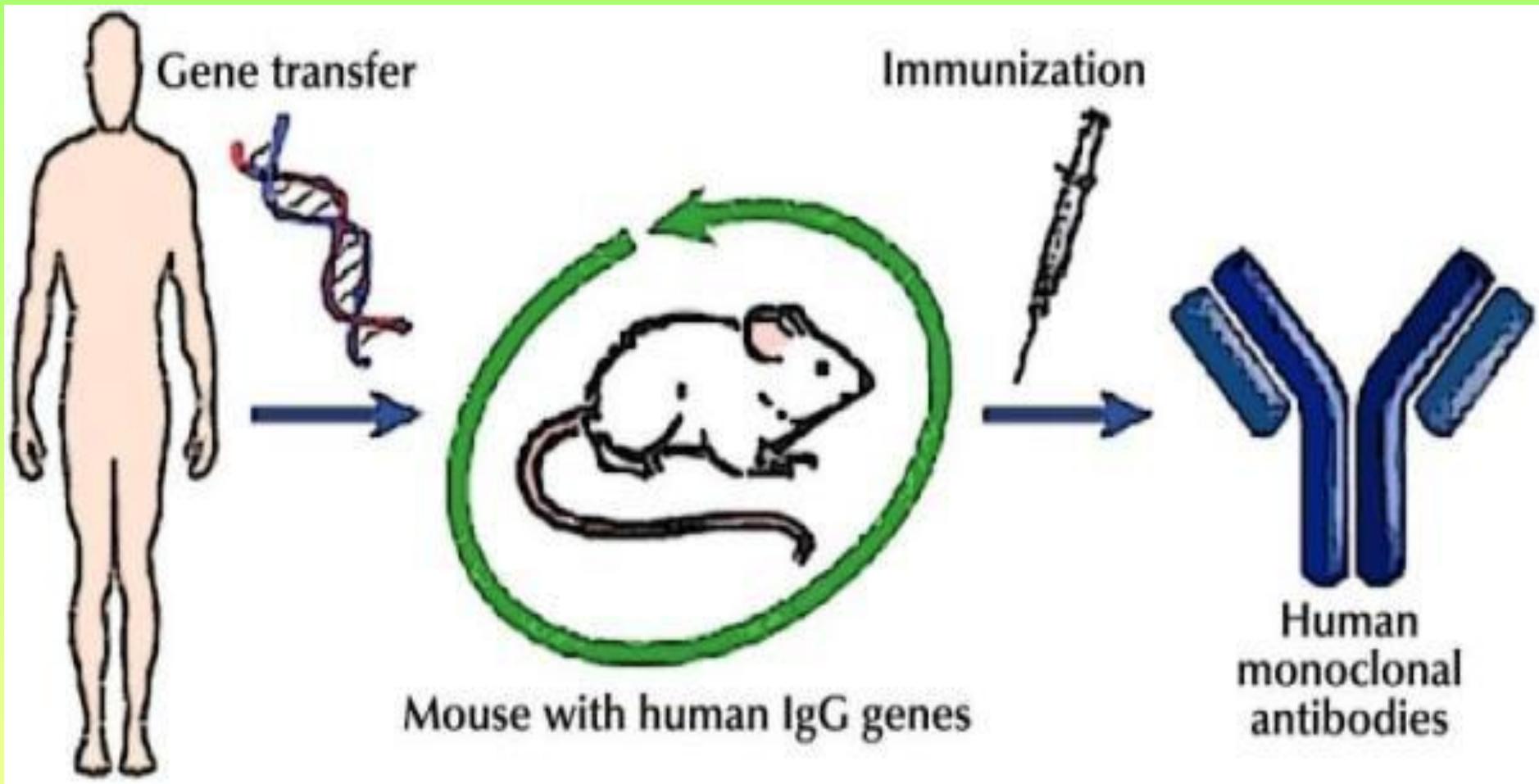
**Human**



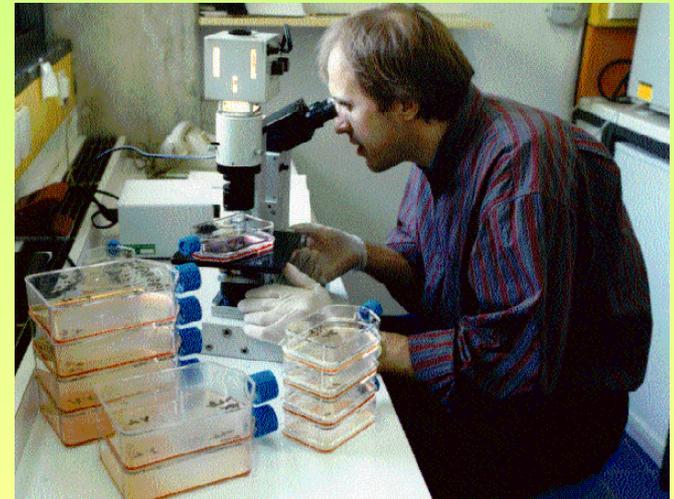
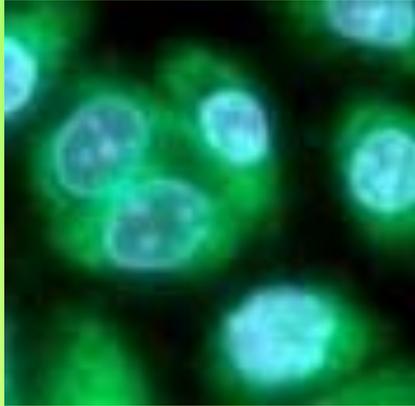
# Ig like constructions



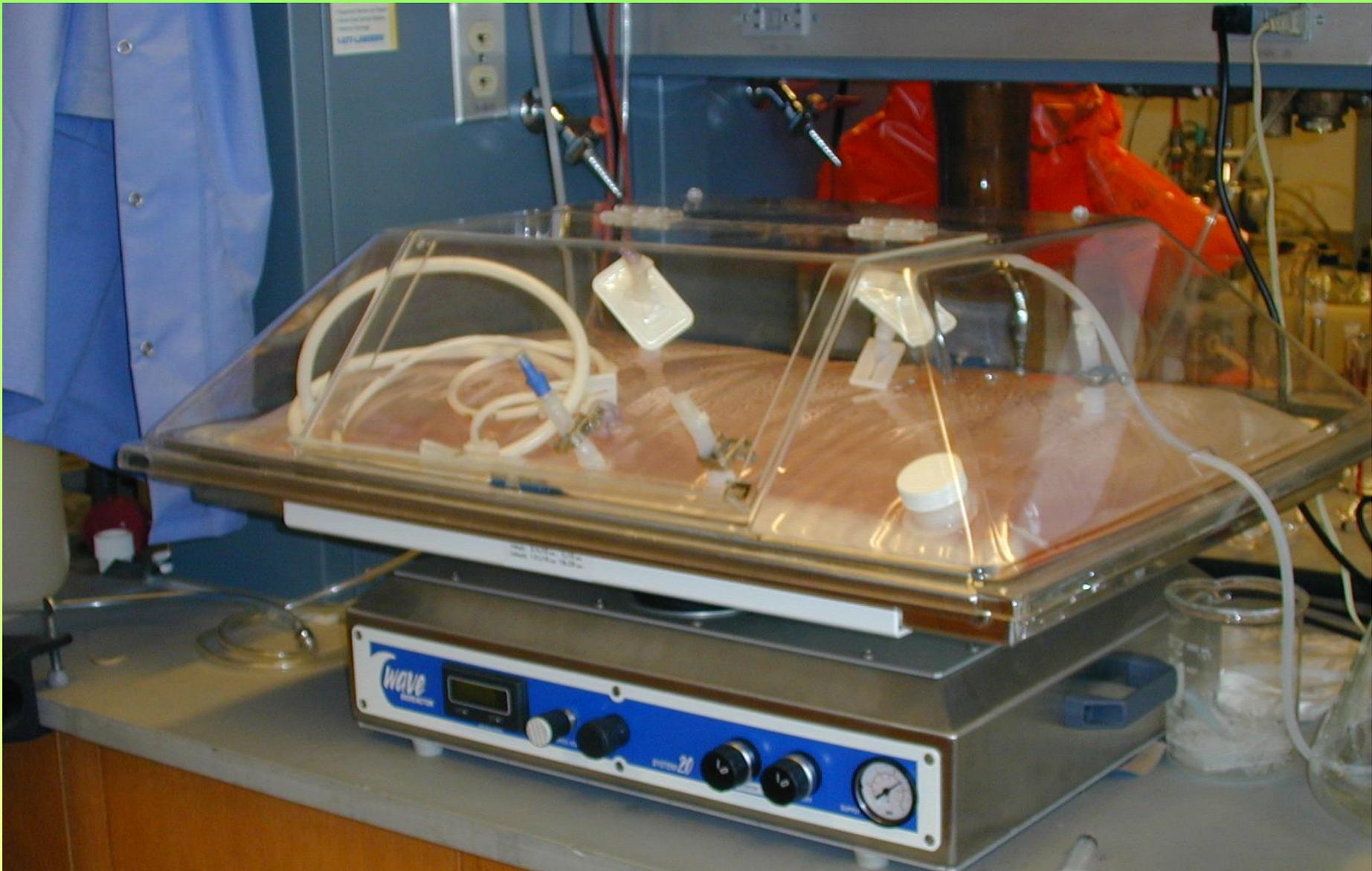
# Human monoclonal antibody construction



# Hybridoma culturing







**Cell fermentation in laboratory scale**

# Cell fermentation in industrial scale

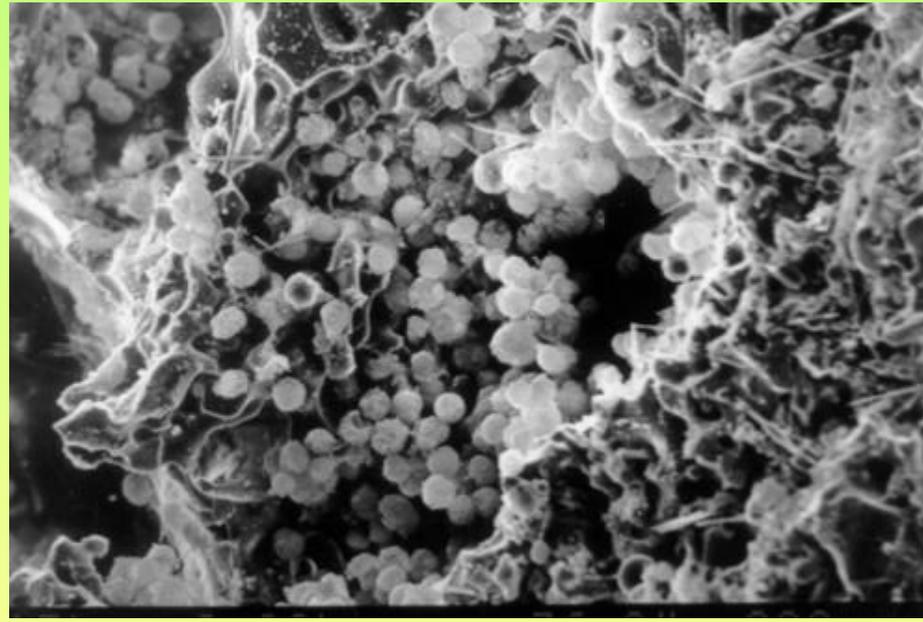








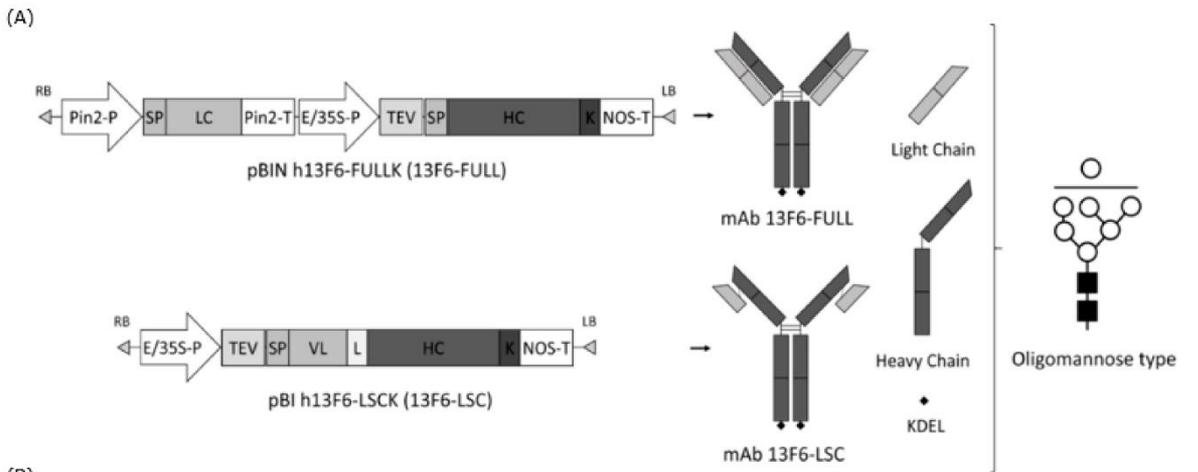




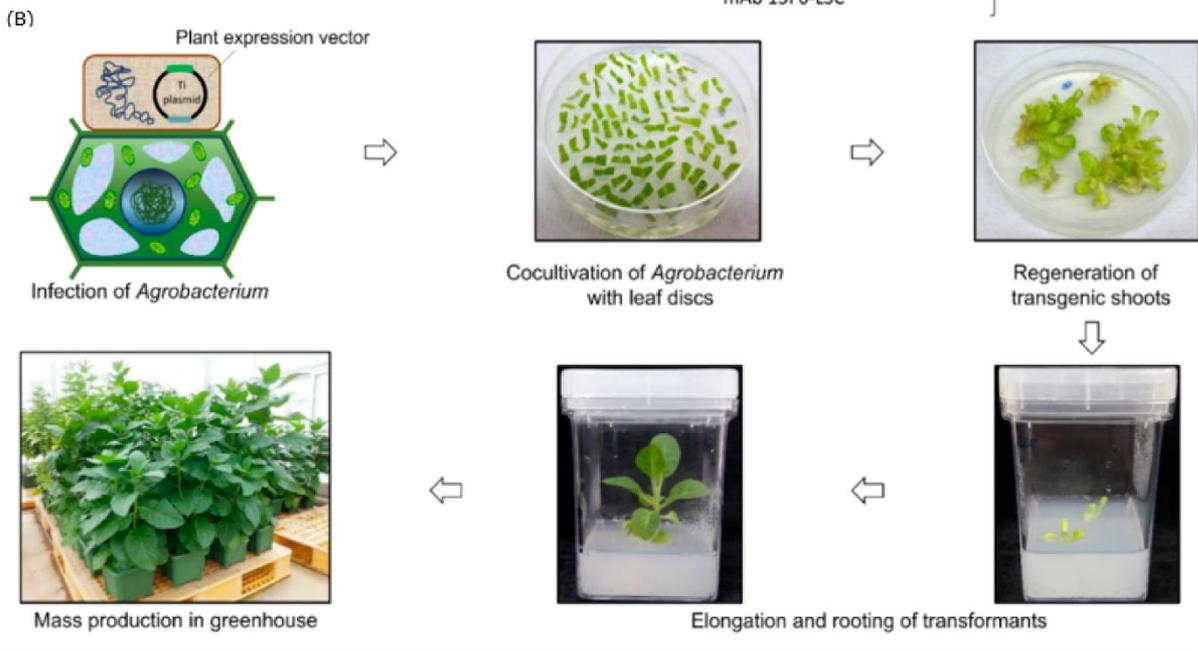








Schematic representation of the plant expression cassette and Agrobacterium-mediated plant transformation for the production of transgenic plants expressing antibodies.



Greenhouse facilities are able to produce transgenic plants for the expression of monoclonal antibodies in plants.