Medical Biotechnology 2023'

Biological therapies

Lecture 5-6th

Monoclonal antibodies for therapy. I.

Therapeutic monoclonal antibodies

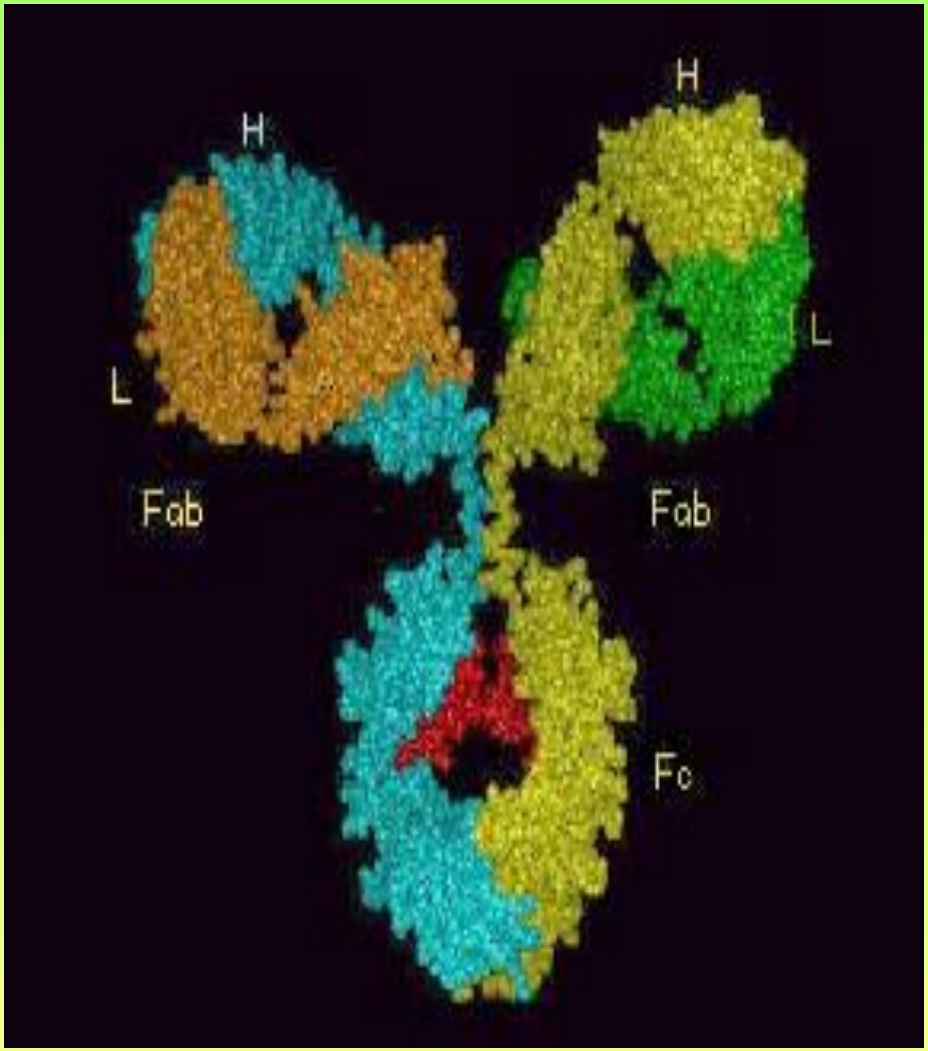
Antibodies produced by gene technologies:

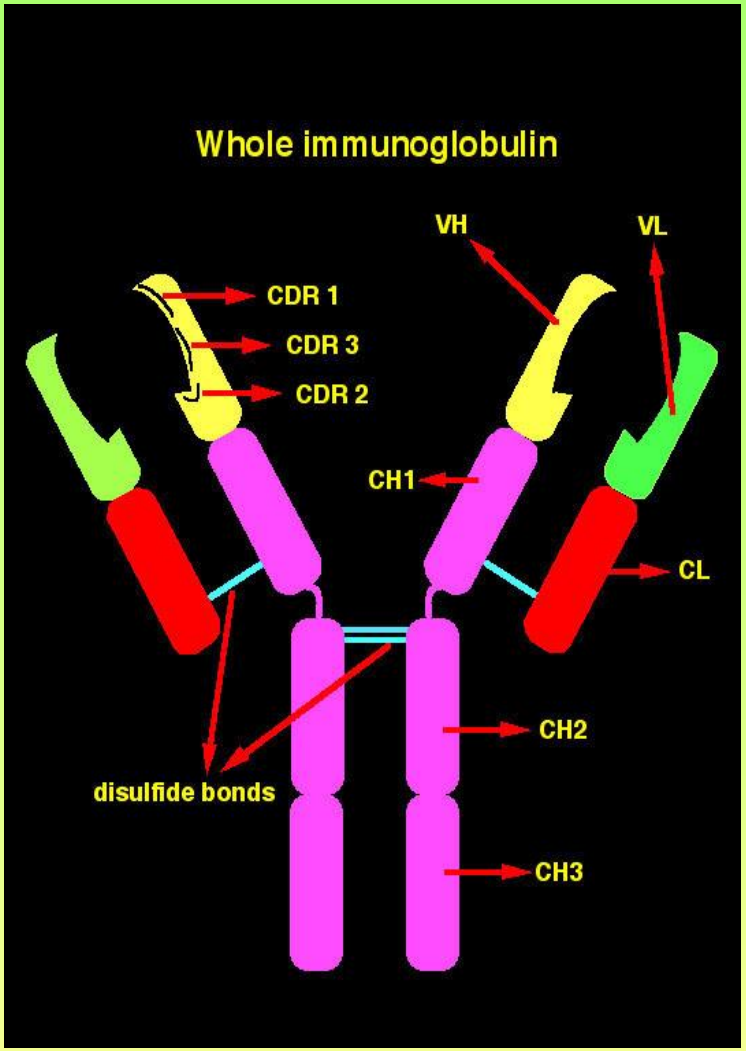
- Antibodies produced by somatic cell fusion (hybridoma technology)

- Humanized antibodies

- Recombinant monoclonal antibodies

Immunoglobulin structure

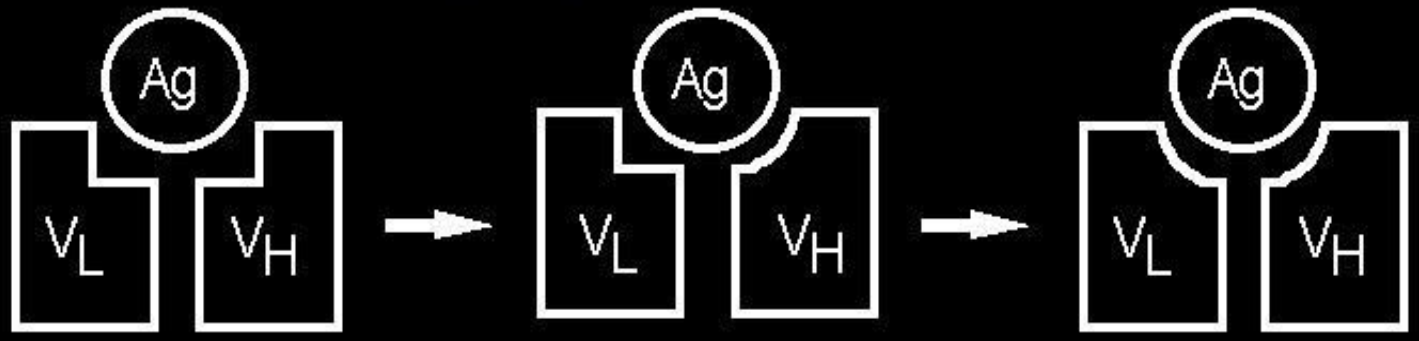


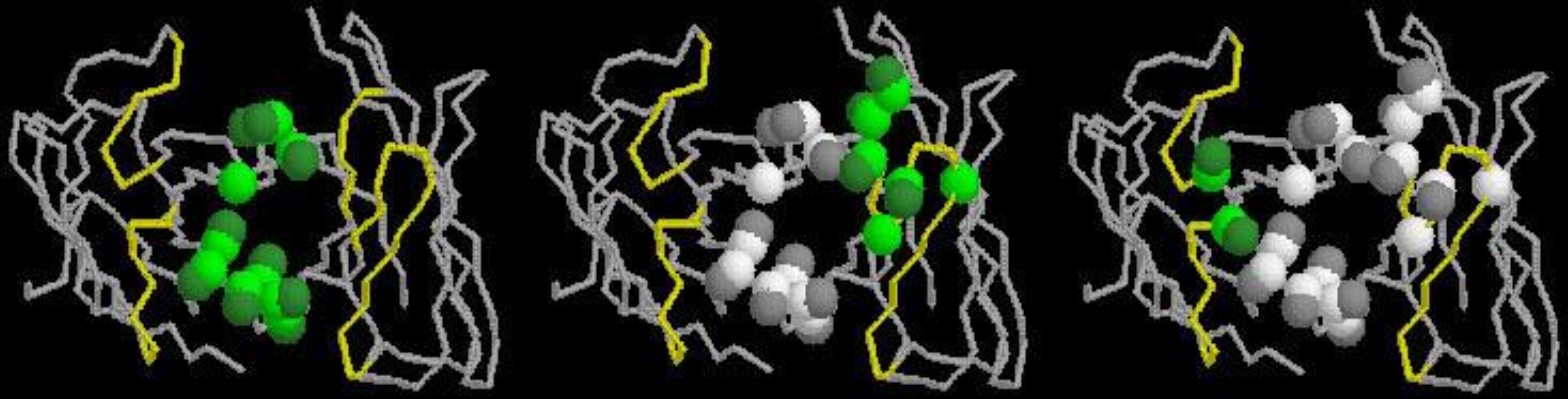


3-3 amino acids determine the idiotype of an immunoglobulin

Antibody affinity maturation

Pini et al. (1998) J. Biol. Chem. *273, 21769-21776*





1st library 2nd library 3rd library

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-γ	Inhibits	Induces	Inhibits		Induces	Inhibits	
TGF-β	Inhibits	Inhibits		Induces			Induces

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

IL-4 leads to IgG and IgE production (TH2)
IFN-γ leads to IgG production (TH1)
TNF-α leads to IgG and IgA production (TH1)
They will also inhibit the production of other isotypes

Antibody production

- Polyclonal antibodies - antisera

  immunization

  antibody purification

- Hybridomas and monoclonal antibodies for therapeutic use

  antibody designe and production

  humanization

  large scale fermentation

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
- Standard (during the bench)

Immunoglobulin purification

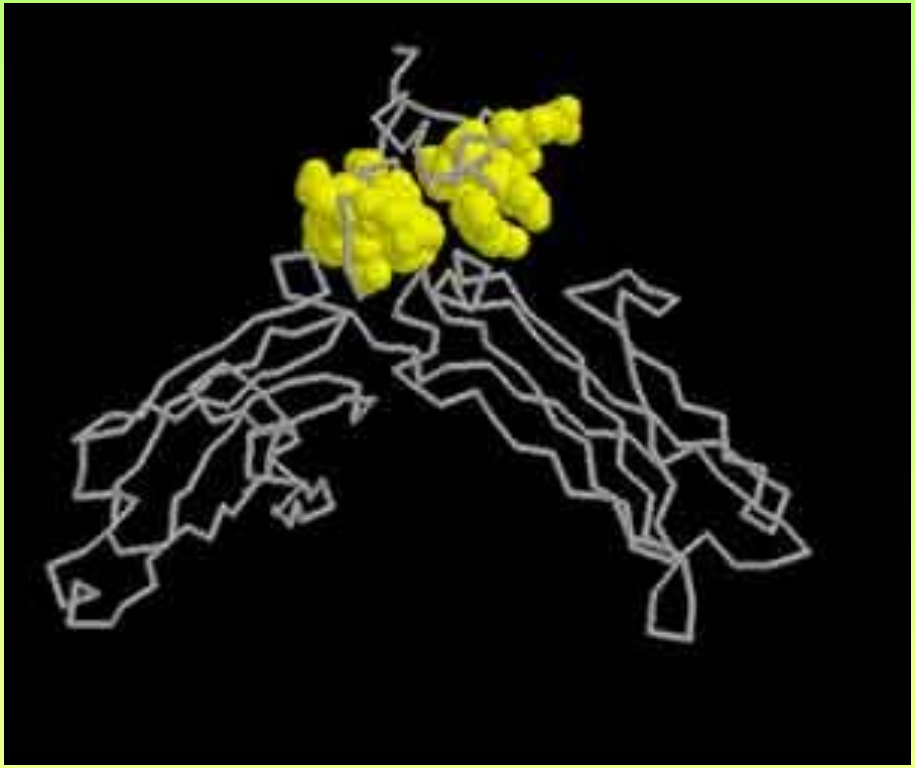
Salt precipitation $(NH_4)_2SO_4$ precipitation

Liquid chromatography

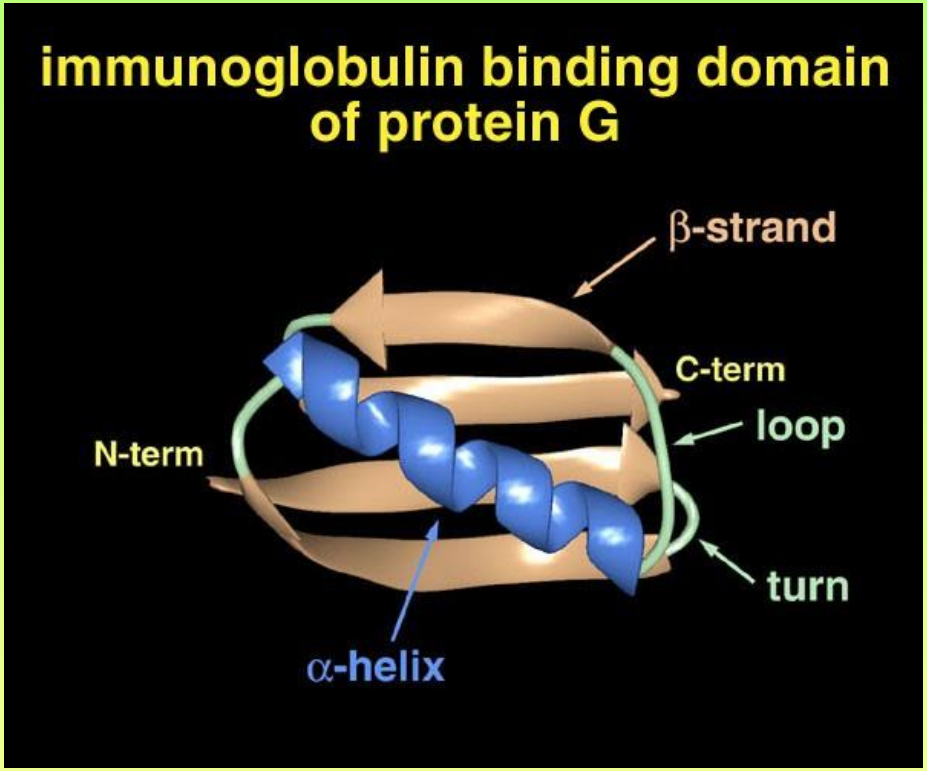
Affinity chromatography (Fc end, antigen)

Affinity purification

Protein A



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.”

Research articles in the NCBI PubMed

	„monoclonal antibody”	*„therapeutic monoclonal antibody”*
	355.933	153.281
• 1976	169	18
• 1977	186	31
• 1978	204	23
• 1979	331	45
• 1980	705	95
• 1985	6839	245
• 1995	9698	1.628
• 2005	8429	4.080
• 2013	11356	7.048
• 2014	12728	8.798

2021

More than 2000 therapeutic monoclonal antibodies are under clinical trial

Preliminaries

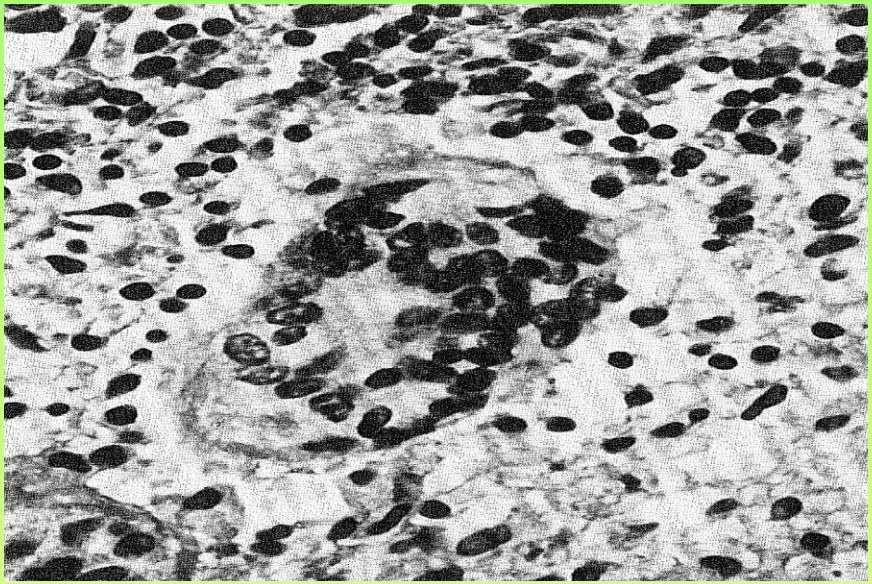
Johannes Müller (1801-1858): firstly described the fusion of somatic cells

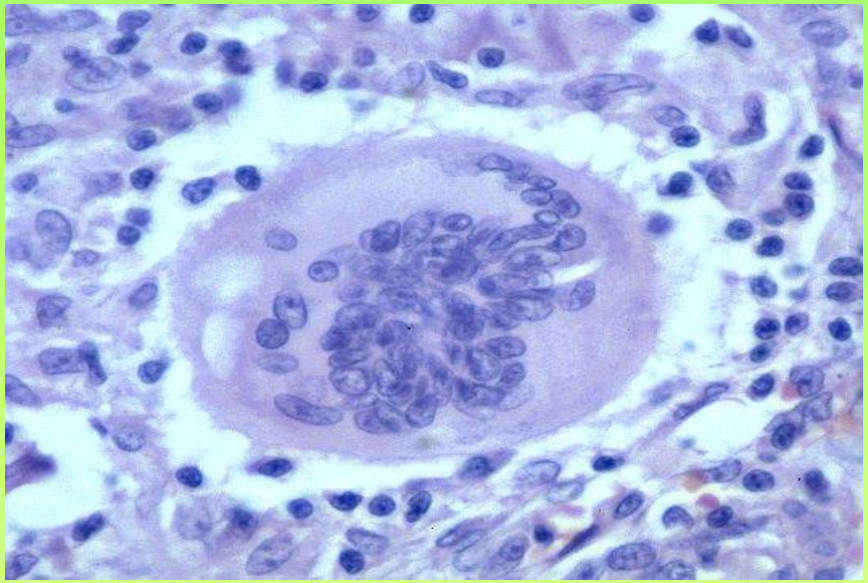
Virchow (1821-1902) and **Langerhans** (1847-1888): published cell fusions in pathologic tissues

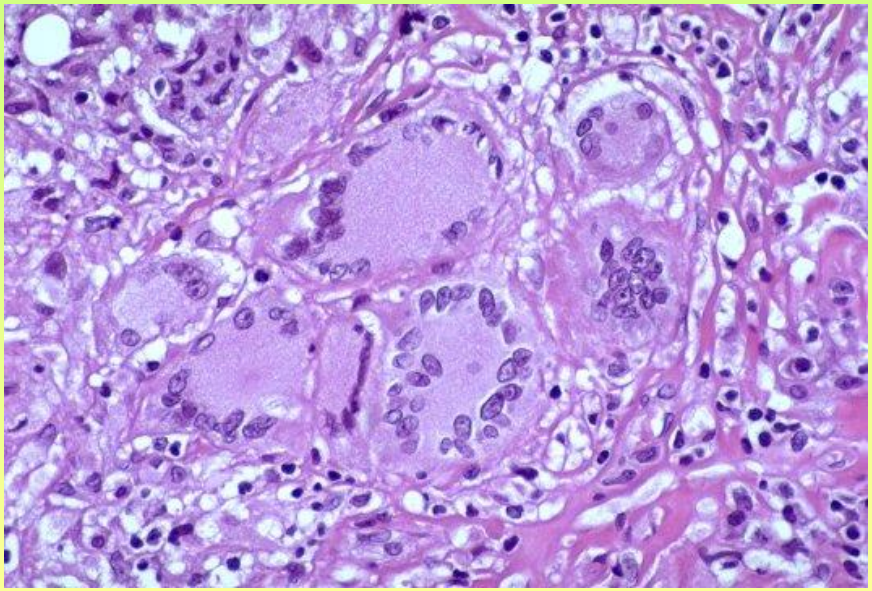
Ringertz (1876): described the giant cell formations caused by somatic cell fusions in pathologic conditions

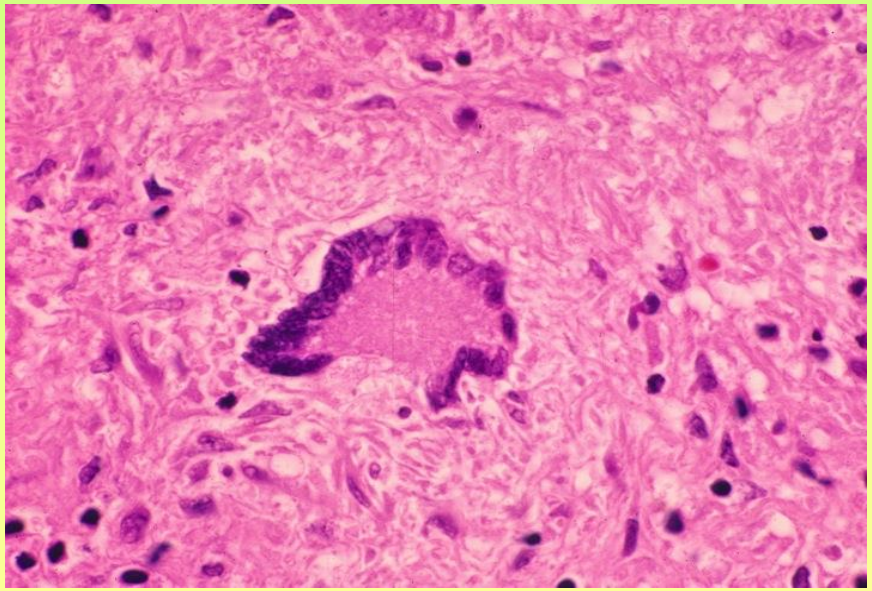
Lewis (1927) spontaneous cell fusions in *in vitro* cultured tumor cells

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

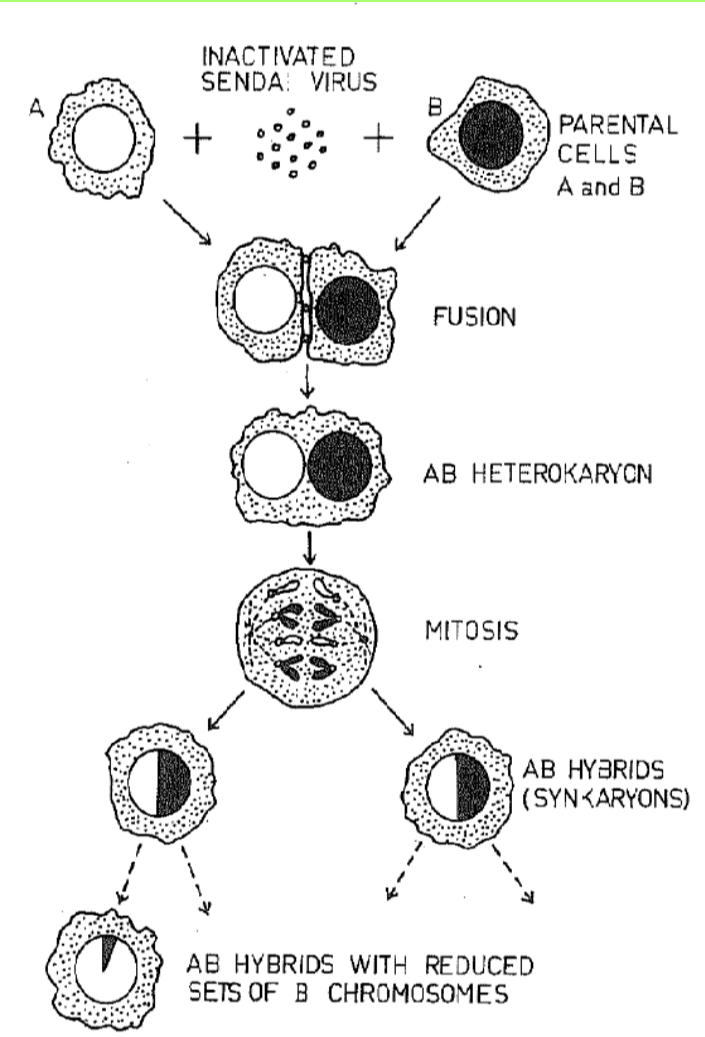
Littlefield (1964, 1966): selection of cell hybrids by the use of enzyme (HGPRT, TK) deficient mutant cell lines

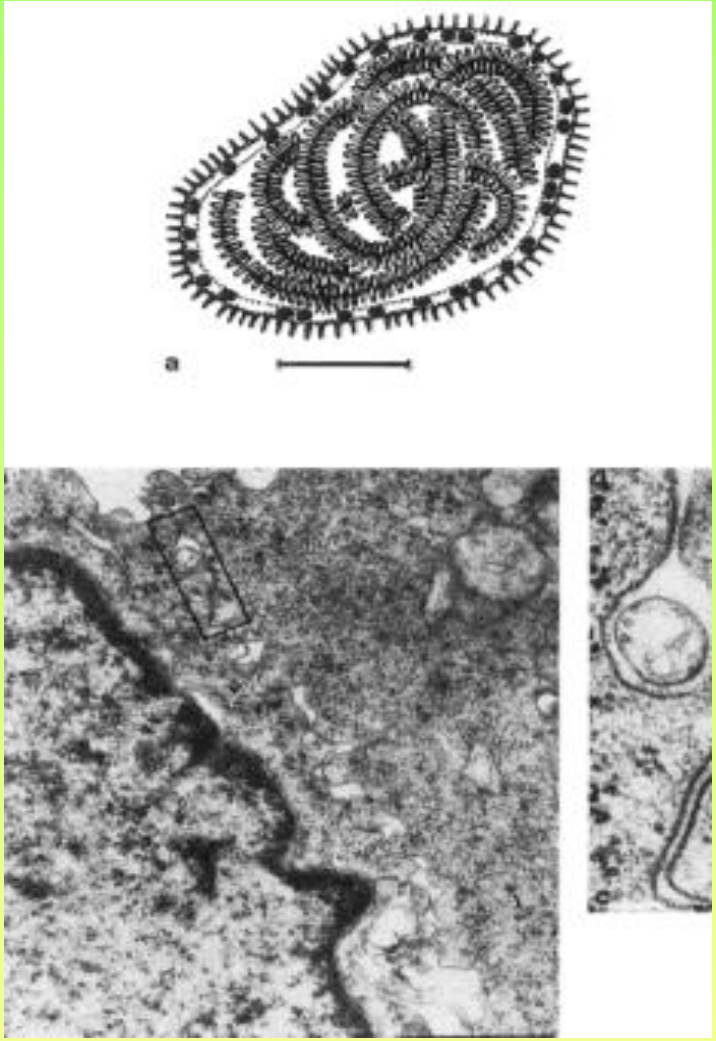
Harris and **Watkins** (1965, 1969), and **Okada** and **Murayama**: first interspecies hybrids

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

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

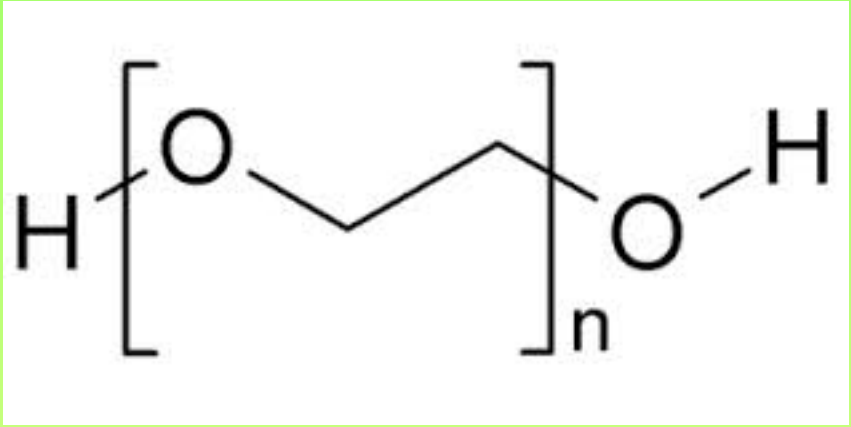
Sendai virus induced somatic cell hybrids



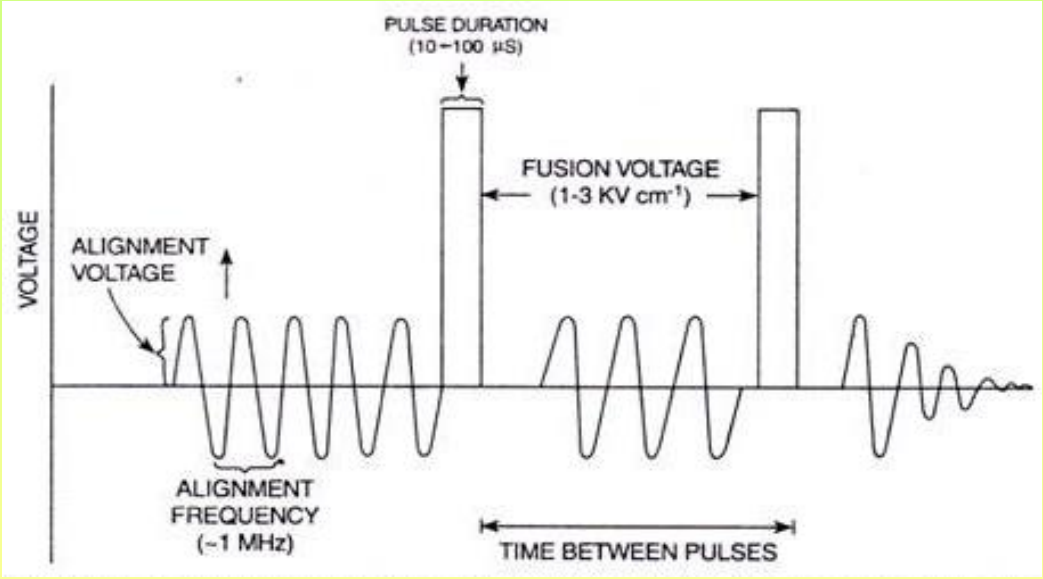


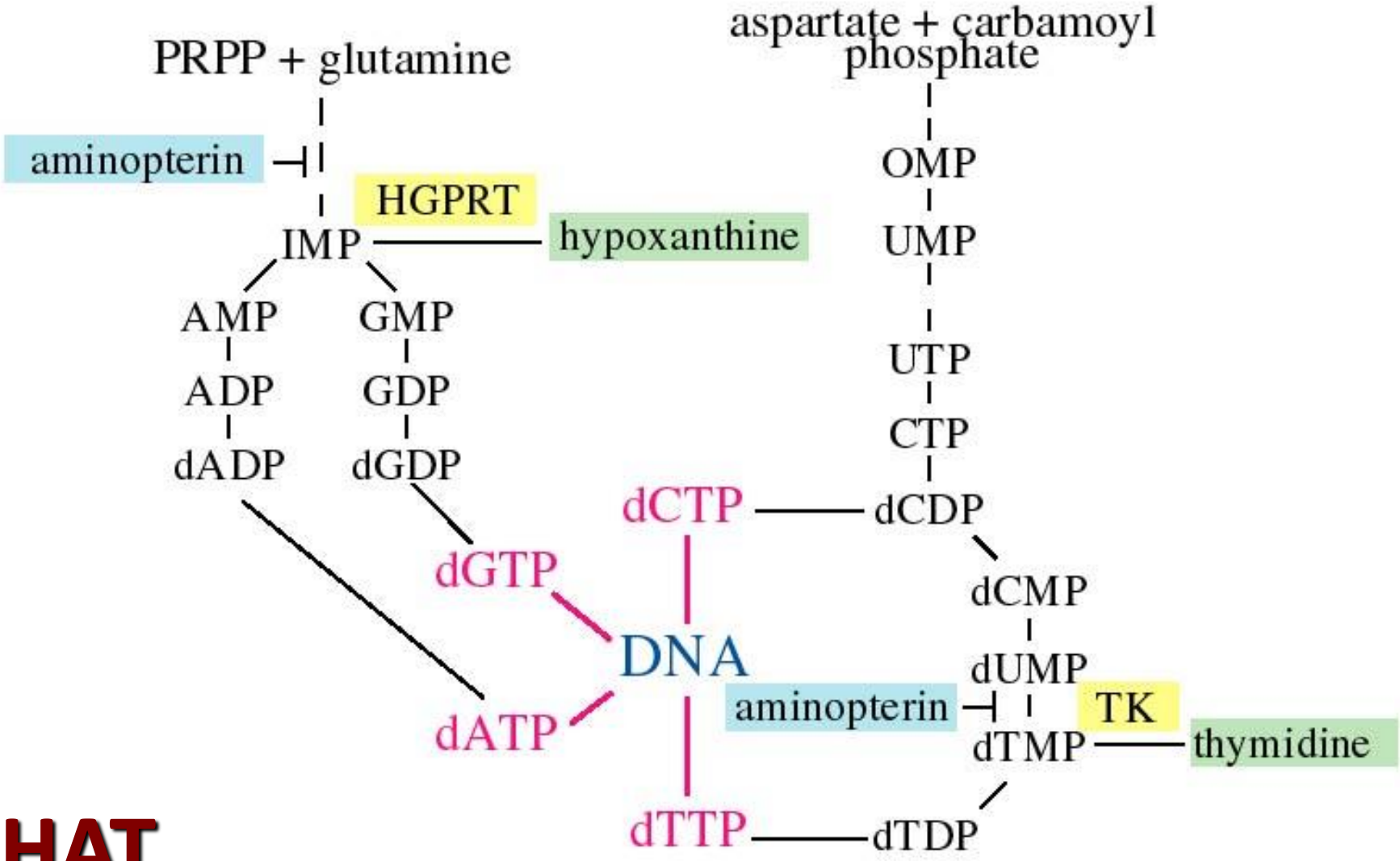
Polyethylene glycol

$H\text{-}(O\text{-}CH_2\text{-}CH_2)_n\text{-}OH$



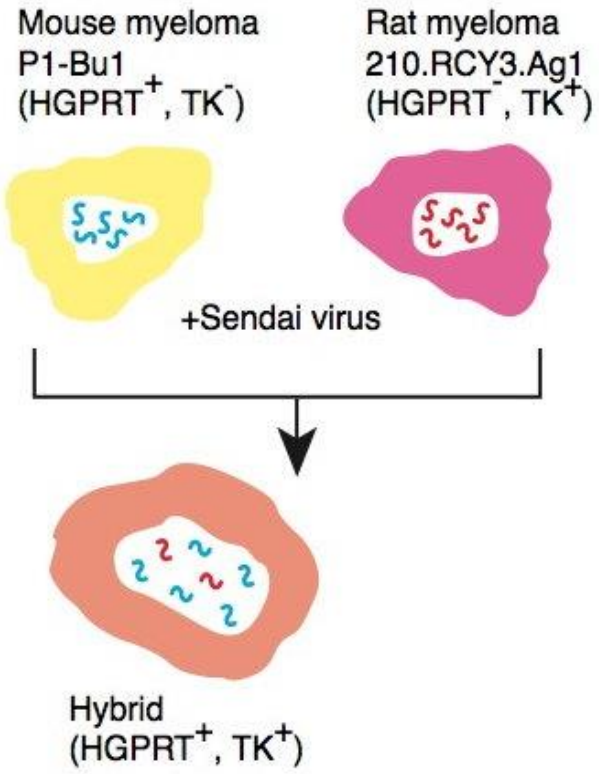
Electrofusion

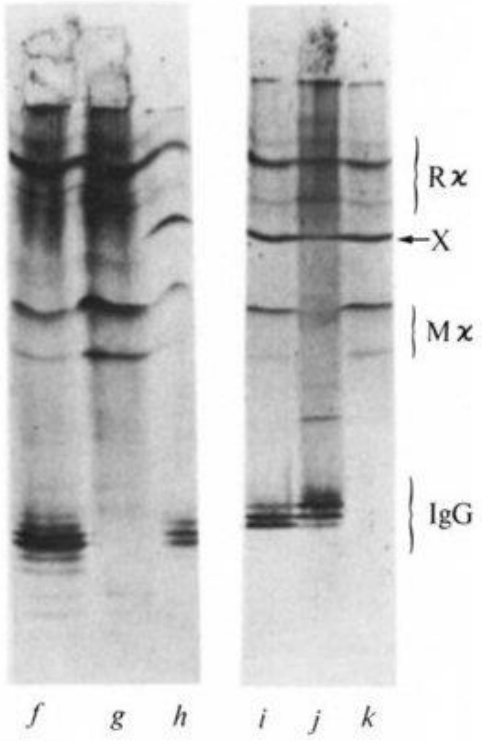



PRPP + glutamine
aspartate + carbamoyl phosphate
aminopterin
HGPRT
IMP
hypoxanthine
OMP
UMP
AMP
GMP
UTP
ADP
GDP
CTP
dADP
dGDP
dCTP
dCDP
dGTP
dCMP
DNA
dUMP
aminopterin
TK
dATP
dTMP
thymidine
dTTP
dTDP

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

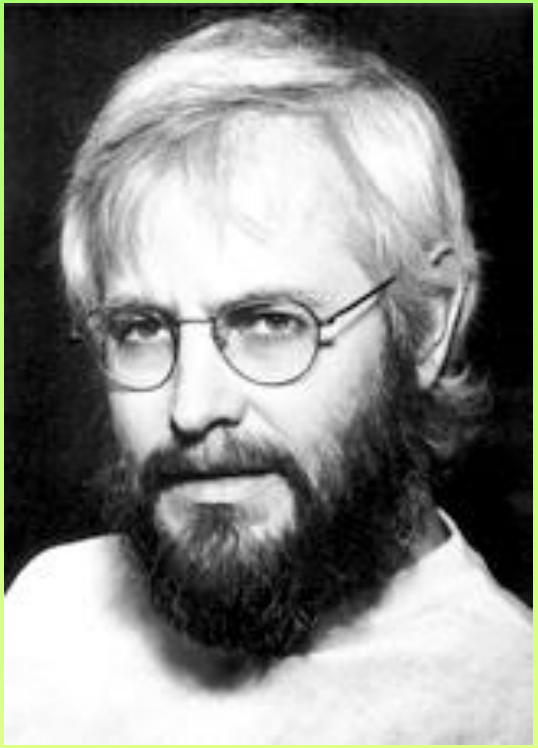
RGH Cotton and C Milstein
Nature 244: 42 (1973)

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[1,2] 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.

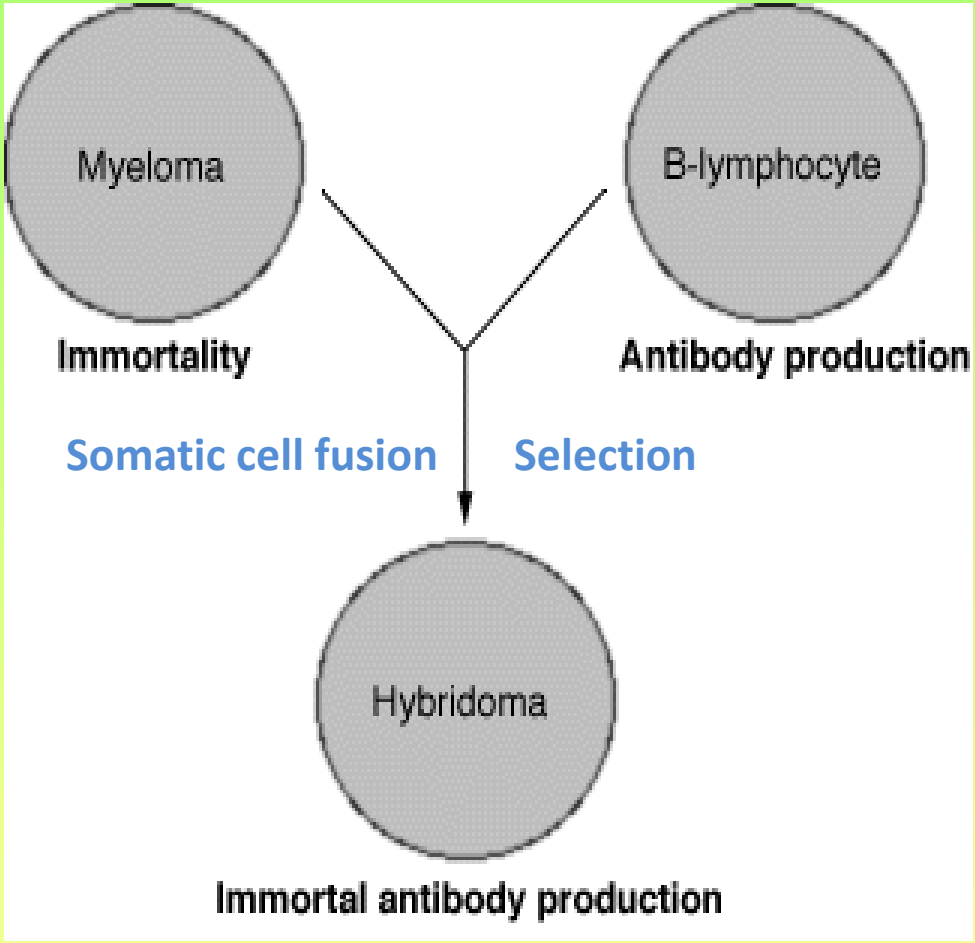
References
1.Potter, M., *Physiol. Rev.*, **52**, 631–719 (1972).2.Horibata, K., and Harris, A. W., *Expl Cell Res.*, **60**, 61–70 (1970).3.Milstein, C., and Munro, A. J., in *Defence and Recognition* (edit. by Porter, R. R.), 199–228 (MTP Int. Rev. Sci., Butterworth, London, 1973).4.Cotton, R. G. H., 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**

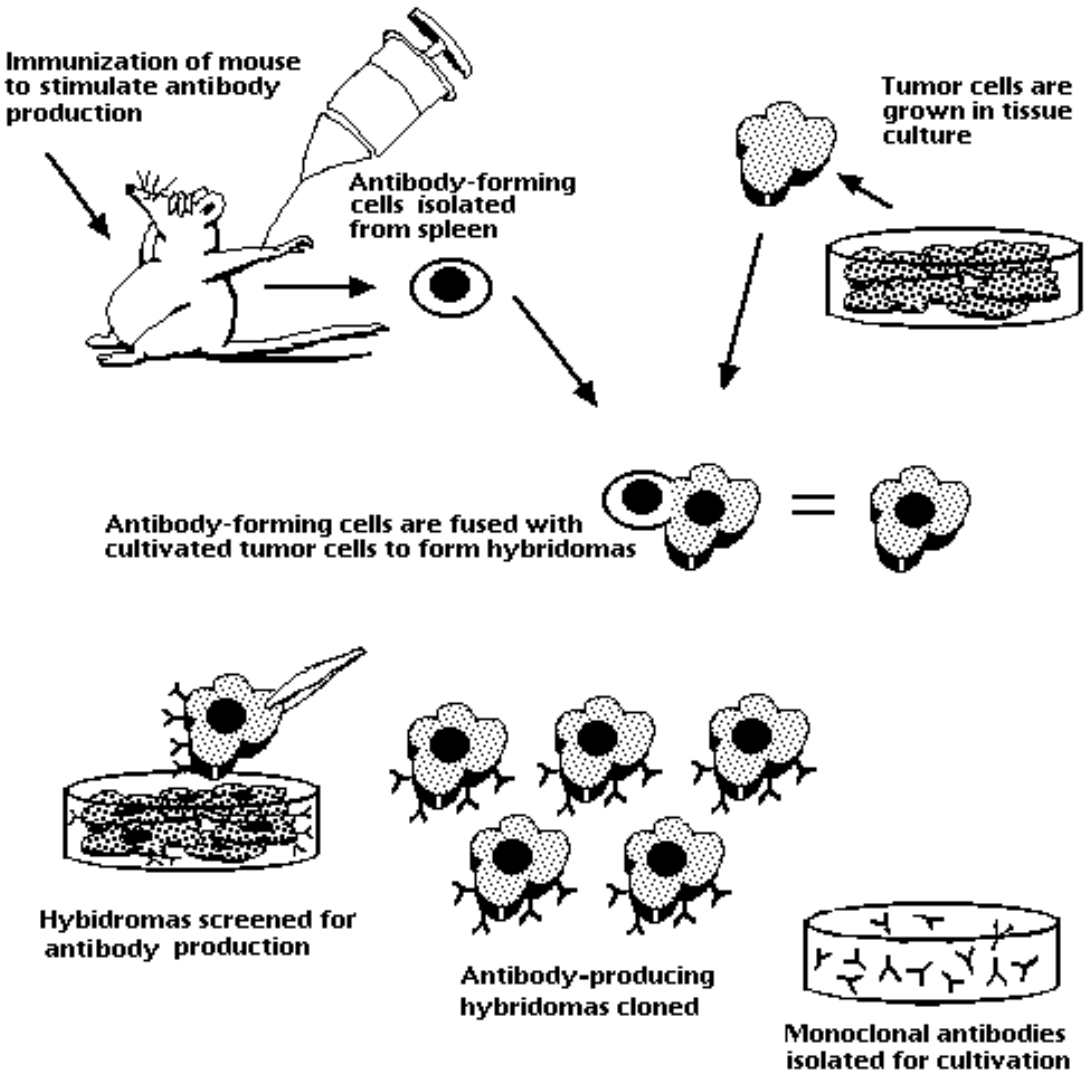
Nobel prize,**1984**: ***"for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies"***

Hybridoma

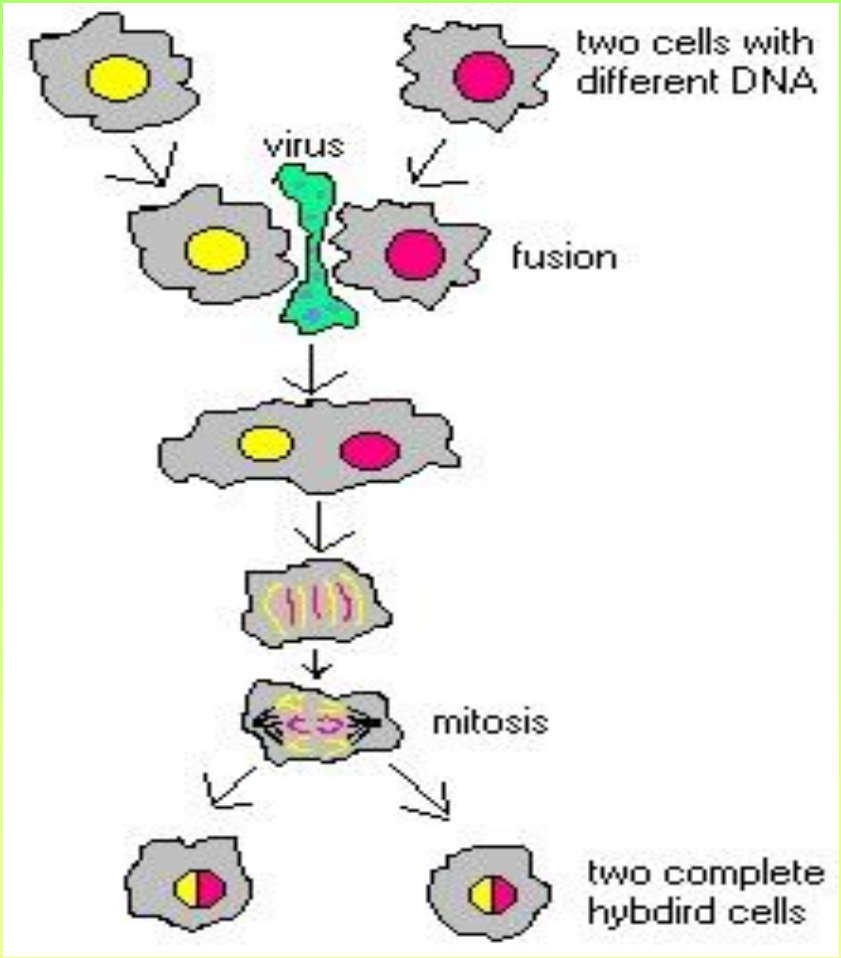

Myeloma
B-lymphocyte
Immortality
Antibody production
Somatic cell fusion
Selection
Hybridoma
Immortal antibody production

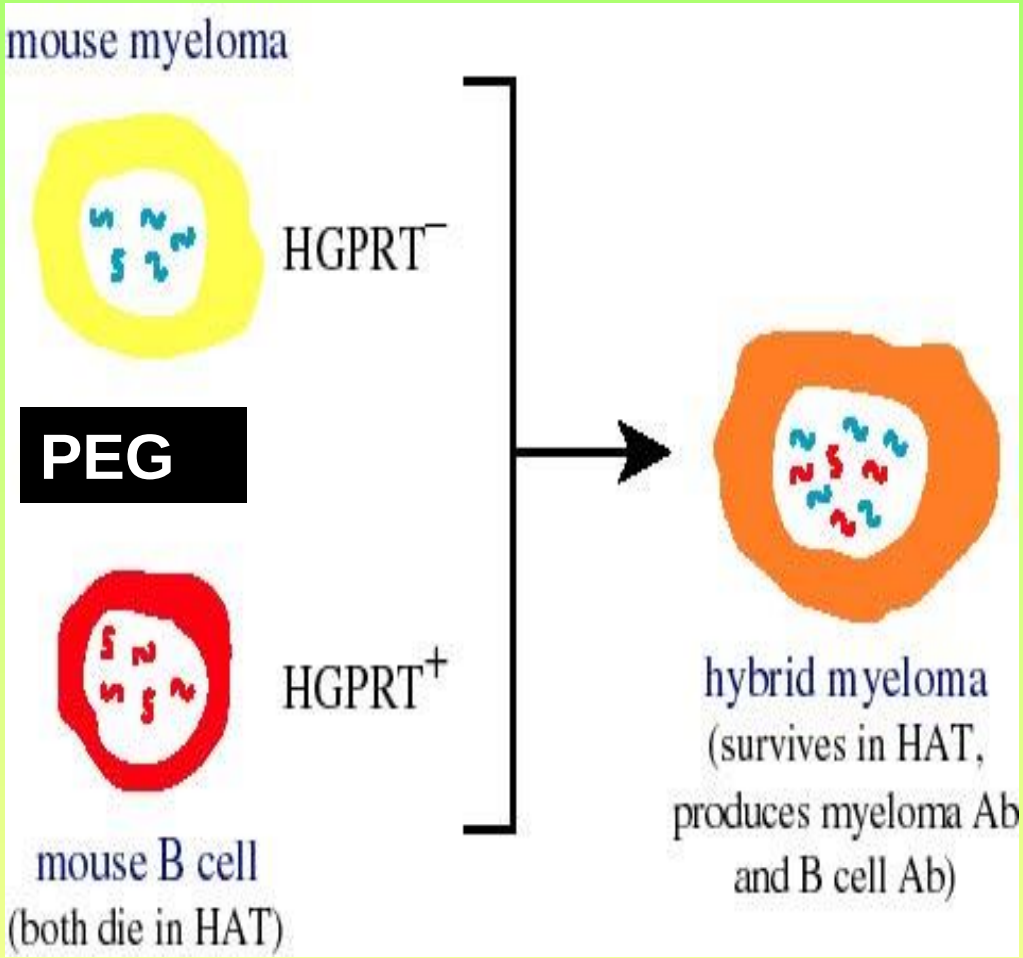

SCIENCE PHOTO LIBRARY

Monoclonal Antibody Production



Somatic cell hybridization and selection



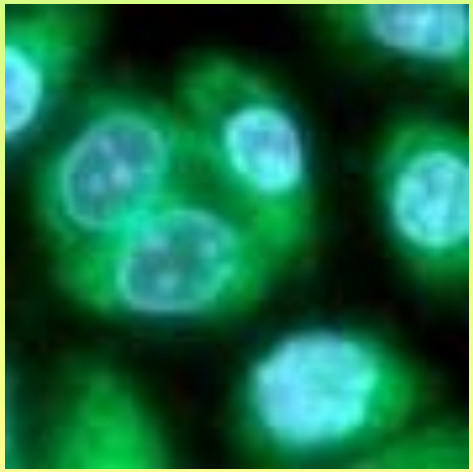


Main steps of monoclonal antibody production

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

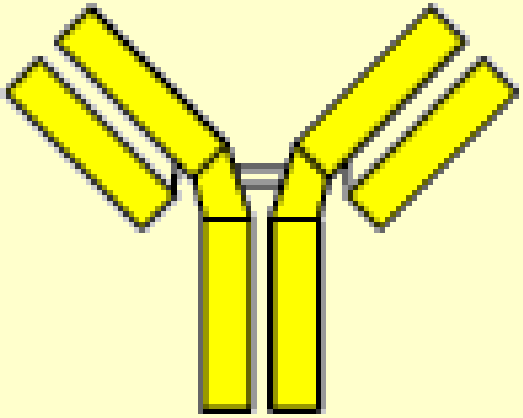
Main characteristics of monoclonal antibodies

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

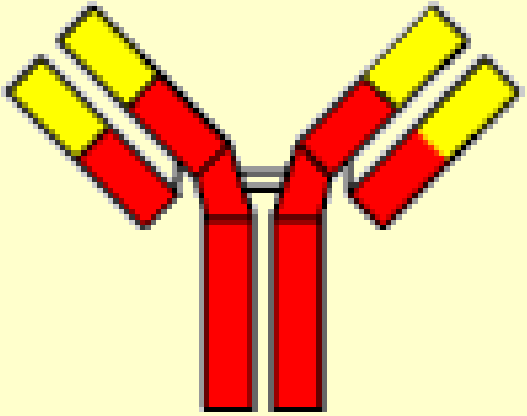


Recombinant monoclonal antibodies for therapeutic use

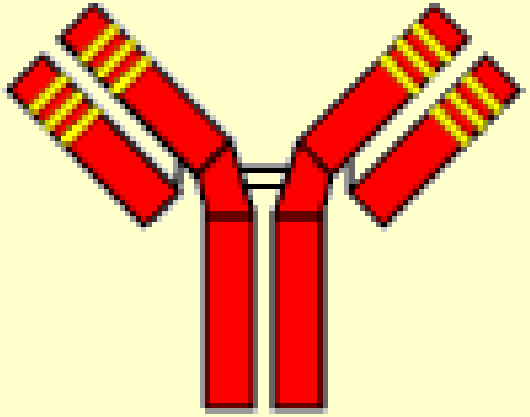
- Chimeric mabs
- Humanized mabs
- Human mabs
- Ig like constructions



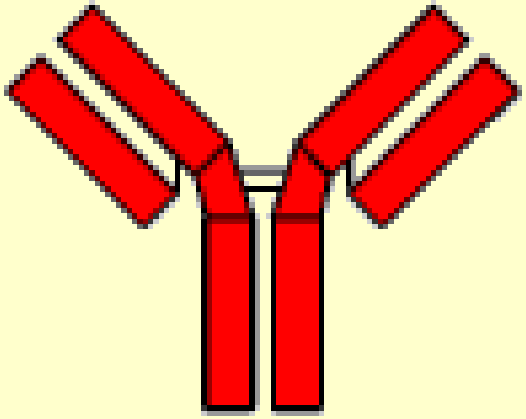
Murine



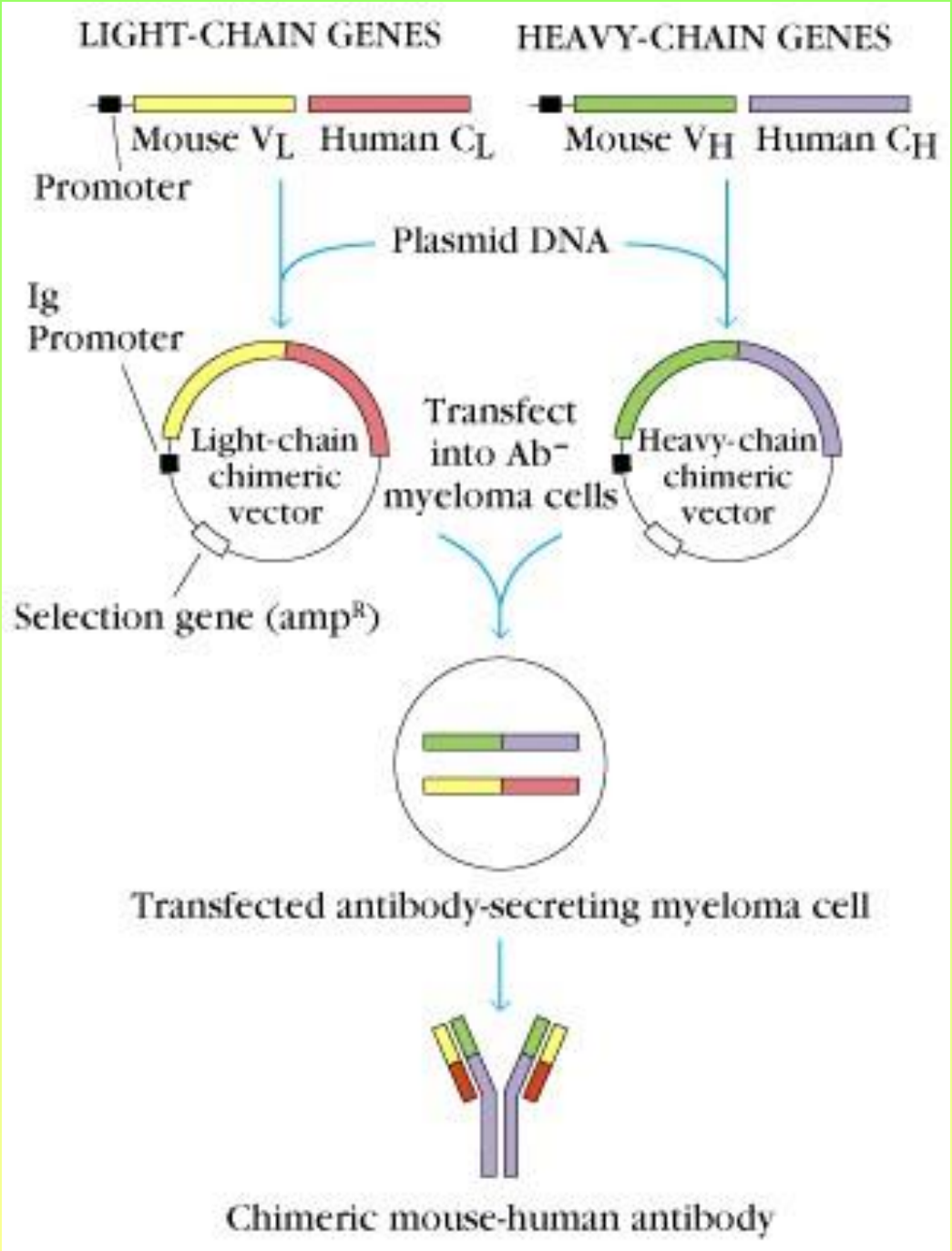
Chimaeric



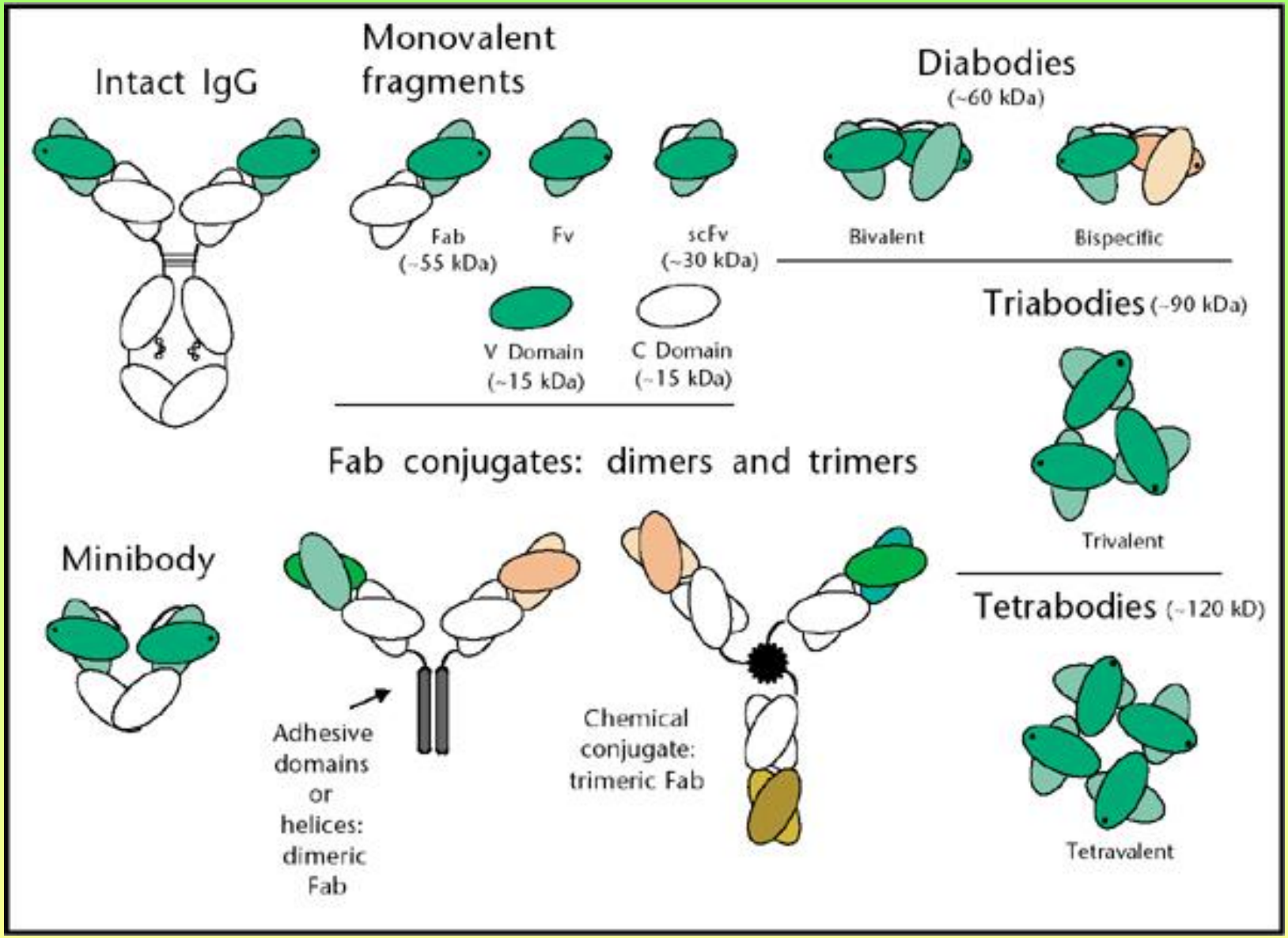
Humanised



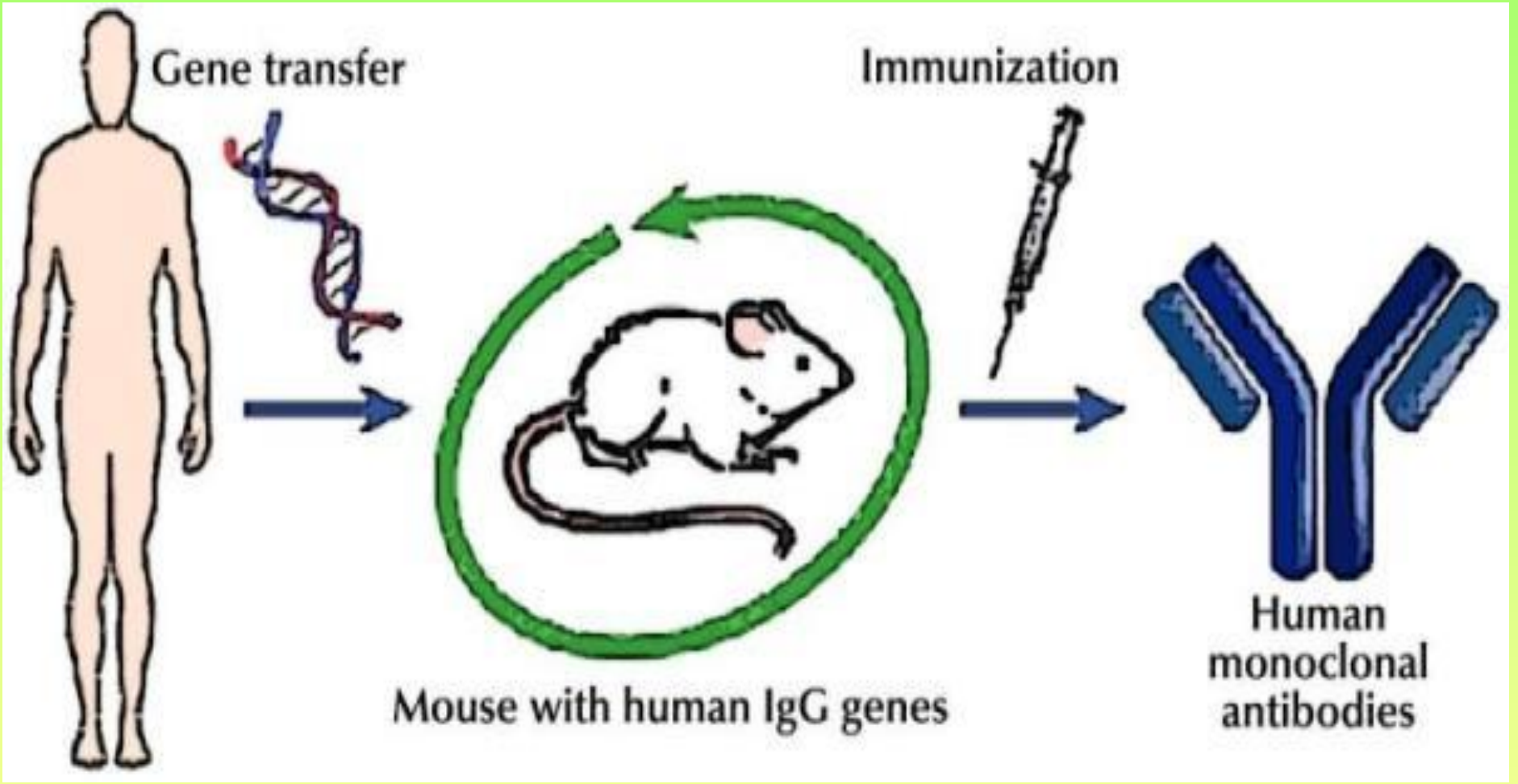
Human


LIGHT-CHAIN GENES
HEAVY-CHAIN GENES
Mouse V_L
Human C_L
Mouse V_H
Human C_H
Promoter
Plasmid DNA
Ig
Promoter
Light-chain
chimeric
vector
Transfect
into Ab⁻
myeloma cells
Heavy-chain
chimeric
vector
Selection gene (amp^R)
Transfected antibody-secreting myeloma cell
Chimeric mouse-human antibody

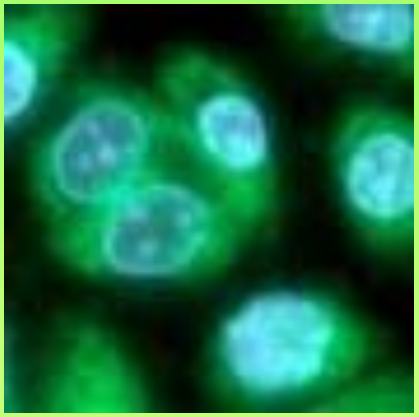
Ig like constructions

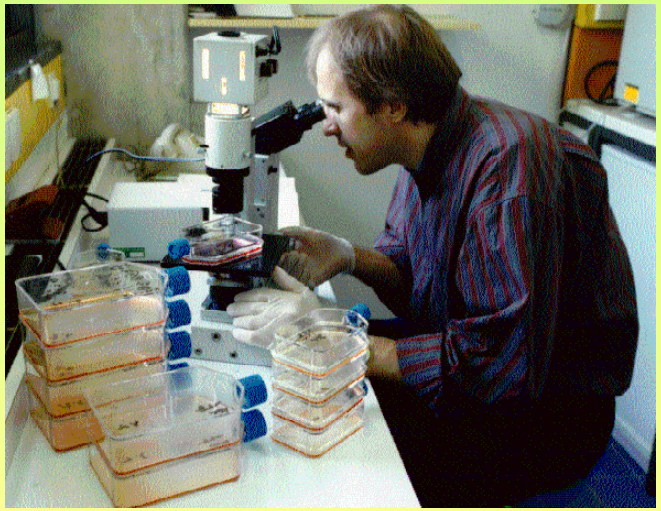


Human monoclonal antibody construction



Hybridoma culturing






CELLMAX
SPECTRUM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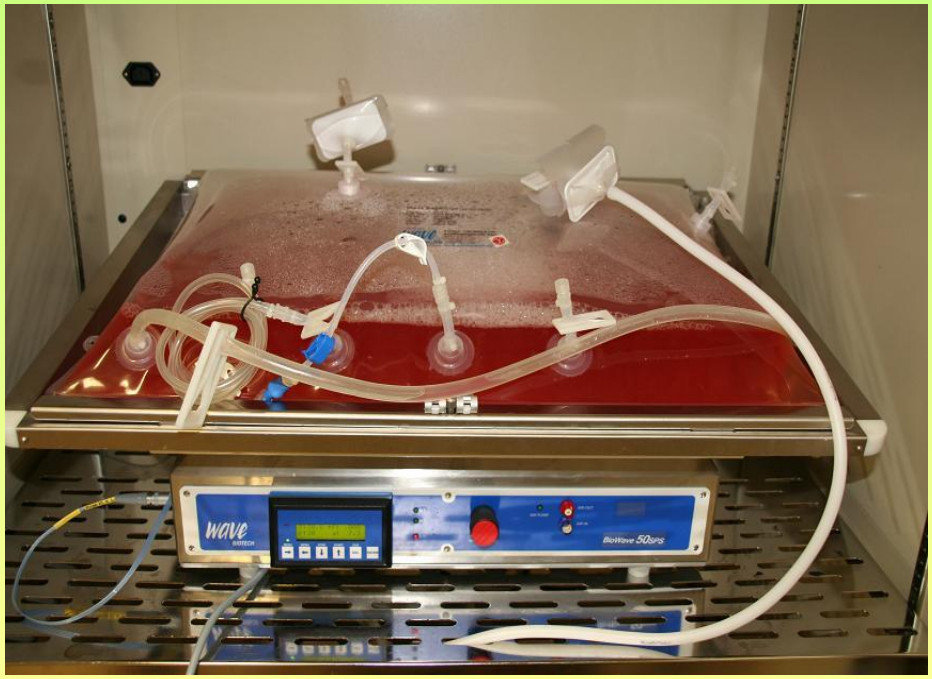
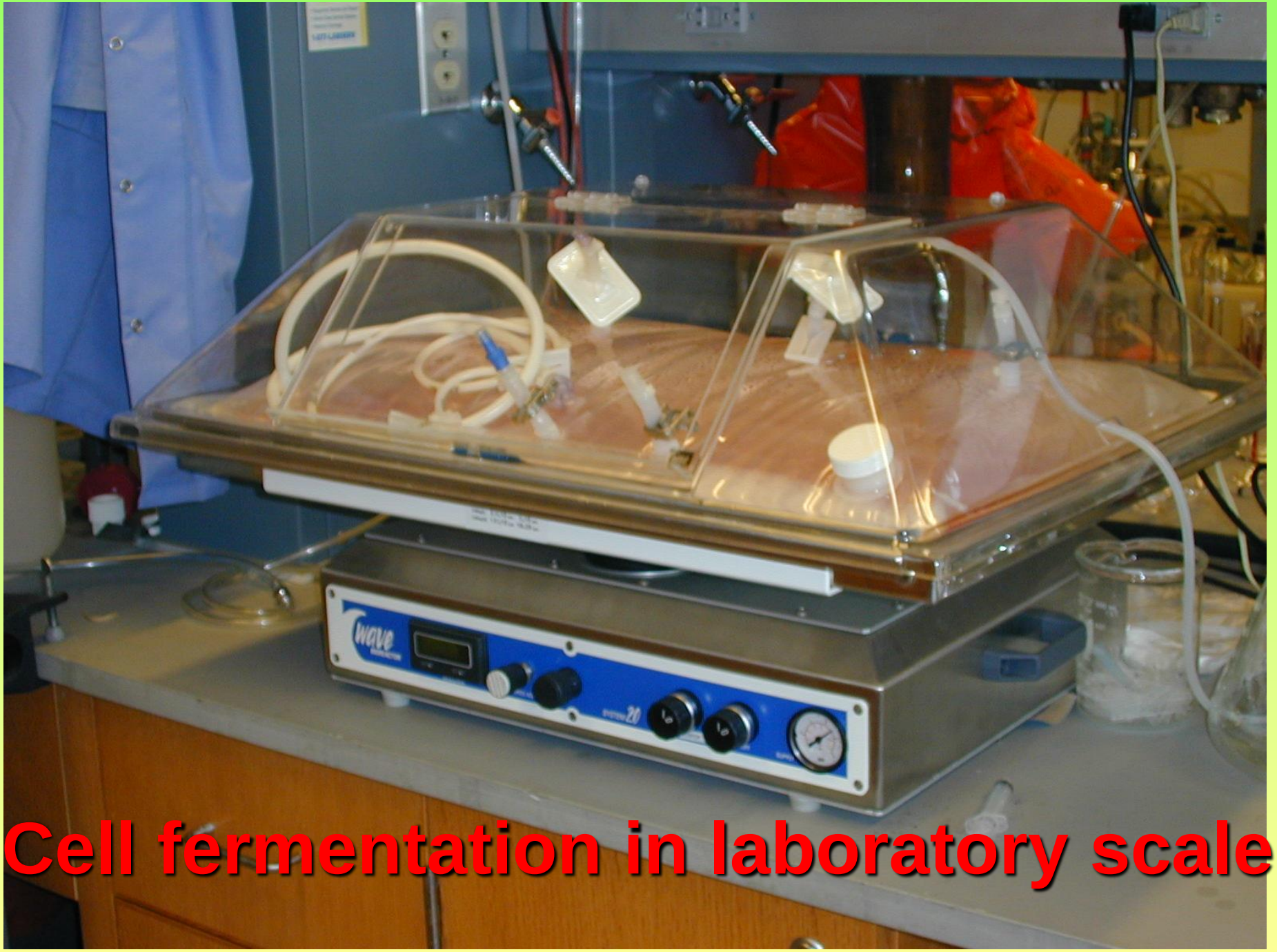
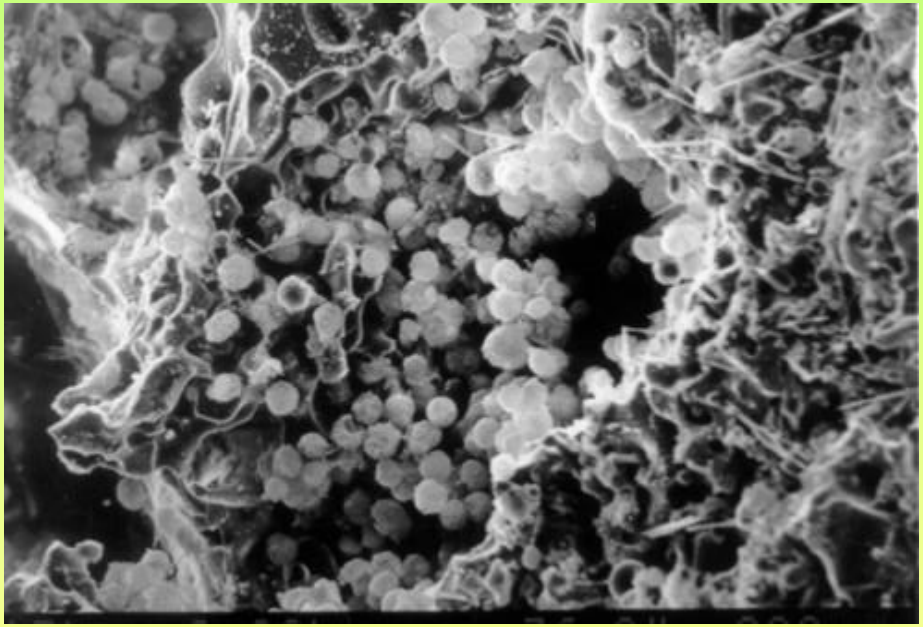

wave


Cell fermentation in laboratory scale

Cell fermantation in industrial scale


CMC


AMIT VERMA

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New production greenhouse facilities are also available to …… through a collaboration with the University of Arkansas at Fayetteville. These plant growth facilities will support cGMP compliant growth of **transgenic plants for the expression of monoclonal antibodies in plants.**