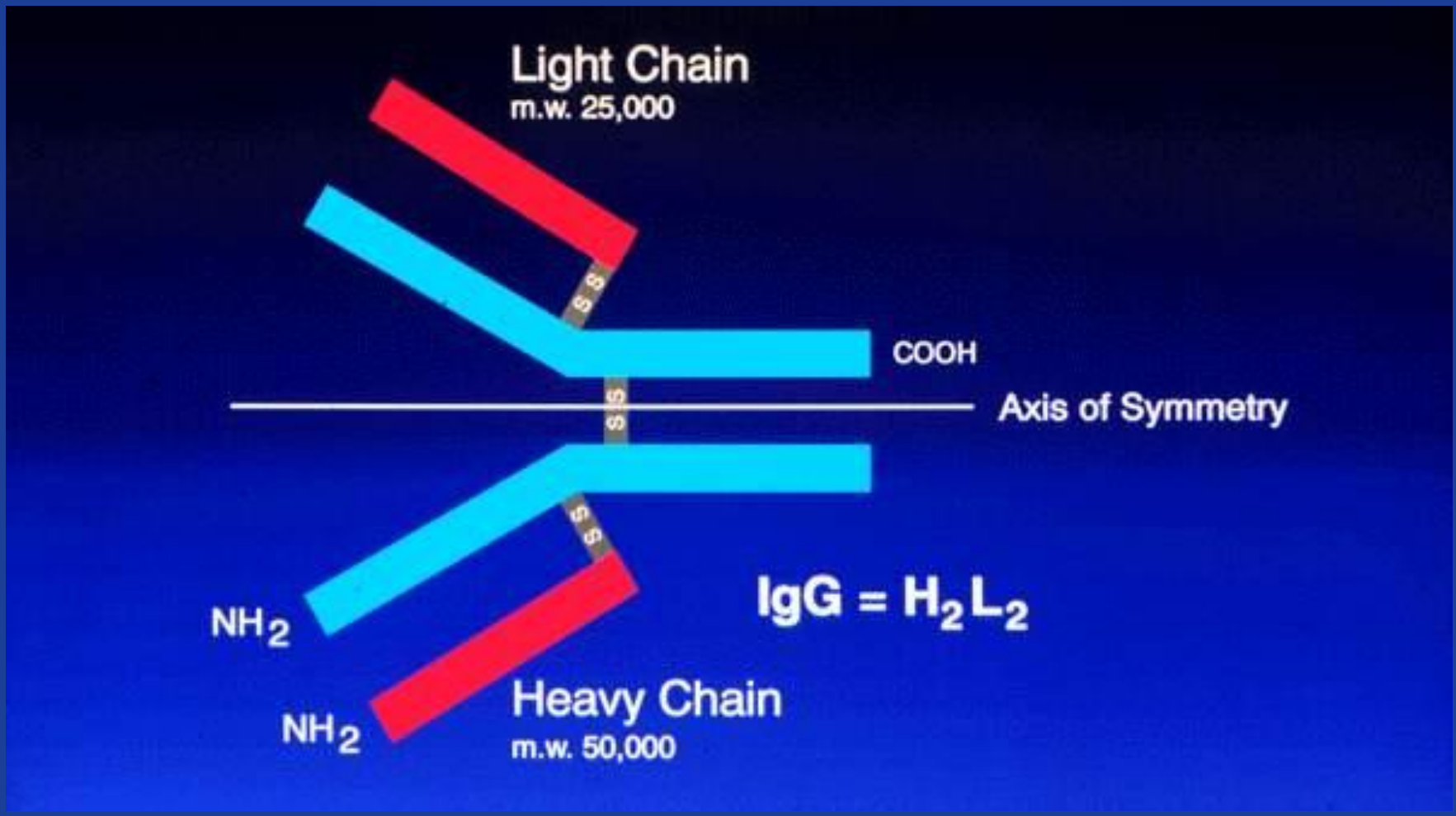
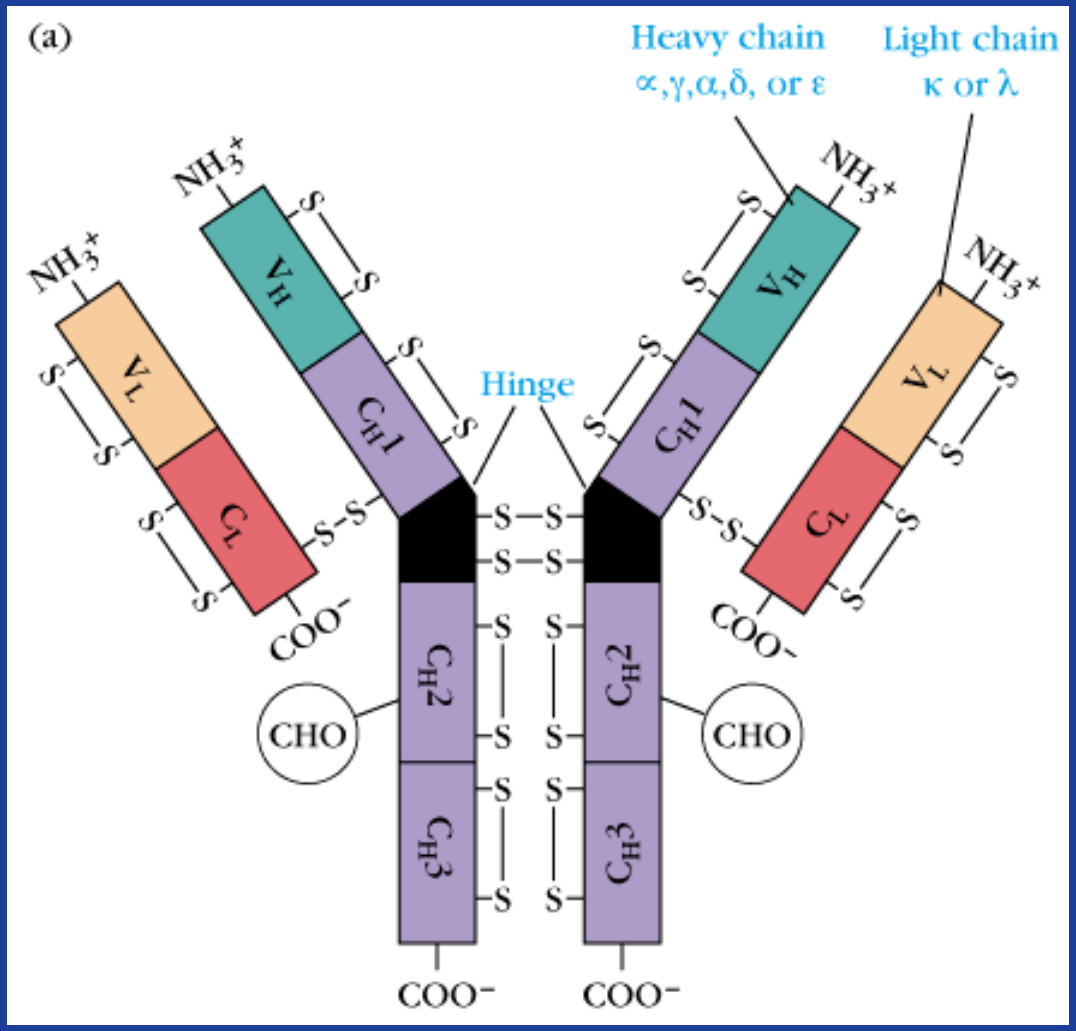
Monoclonal antibodies

Four chain structure of IgG




(a)
Heavy chain
α,γ,α,δ, or ε
Light chain
κ or λ
Hinge
NH_3^+
V_H
C_H1
V_L
C_L
C_H2
C_H3
CHO
COO^-

Some functions of Fc part

- Binds to Fc receptors
- Binds and activates complement
- Activates macrophages
- Induces Antibody Dependent Cellular Toxicity (ADCT)

Biological function IgG isotypes

	IgG1	IgG2	IgG3	IgG4
Complement binding	+	+/-	+	-
Phagocytosis/ADCC	+	-	+	+/-
Ig regulation	+	+/-	+	-
Half-life	long	long	short	long

ADCC, antibody-dependant cellular toxicity

eurahp
European Academy of Hospital Pharmacy

Developing antibodies

From polyclonal to monoclonal

European Association
of Hospital Pharmacists

Antibody production

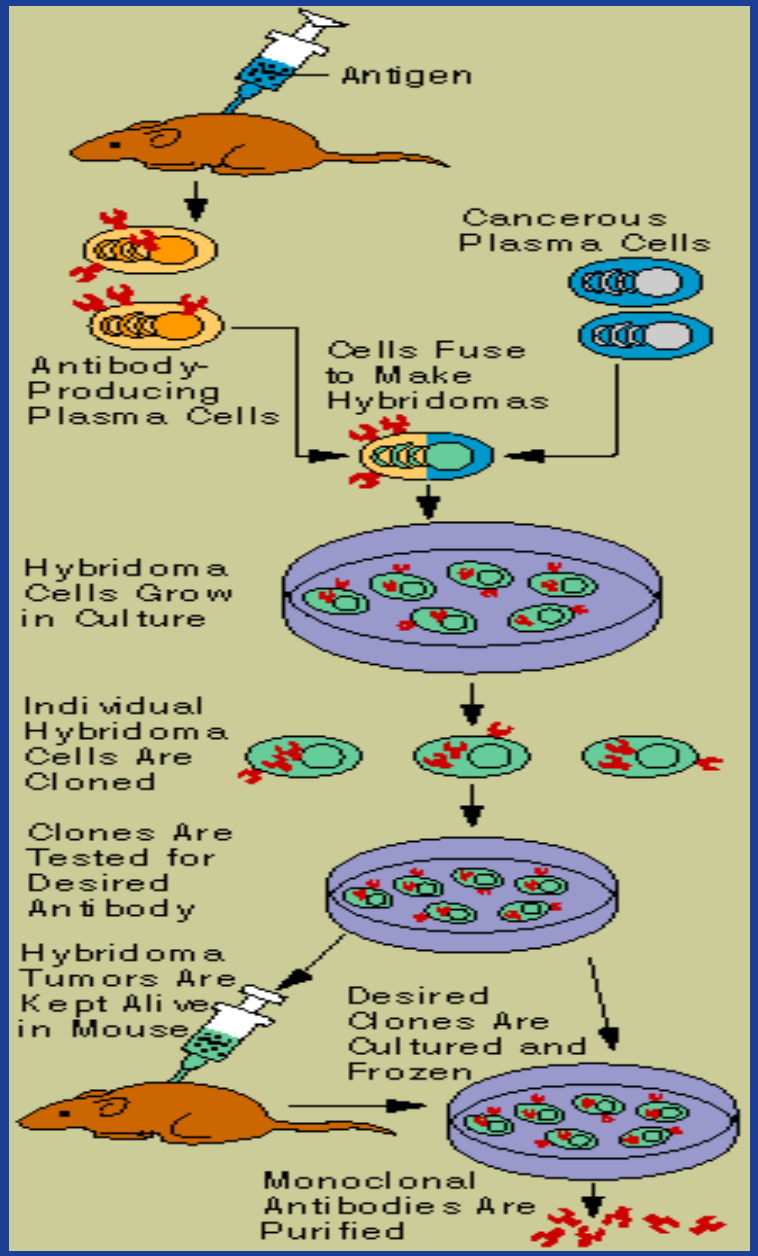
- Polyclonal:
  - Antibodies are collected from sera of exposed animals

  Or
  - A combination of monoclonal colonies is combined (can be any animal: rabbit, goat horse, rat, sheep)

- Suite of antibodies recognising multiple antigenic sites of injected biochemical sites

Antibody production

- Individual B lymphocyte hybridoma is cloned and cultured
- Secreted antibodies are collected from culture media
- Typically BALBc mice
- Sometimes Rat (ascites fluid)
- Antibodies recognise one antigenic binding site of the antigen


Antigen
Cancerous Plasma Cells
Antibody-Producing Plasma Cells
Cells Fuse to Make Hybridomas
Hybridoma Cells Grow in Culture
Individual Hybridoma Cells Are Cloned
Clones Are Tested for Desired Antibody
Hybridoma Tumors Are Kept Alive in Mouse
Desired Clones Are Cultured and Frozen
Monoclonal Antibodies Are Purified

NRDC
National Research Development Corporation
PO box 236 Kingsgate House 66/74 Victoria Street London SW1 E 6SL
Telephone 01-828 3400 Telegrams Nardec London SW1 Telex 23580

Your ref

Our ref EJT/AED

7th October 1976.

Mr. L.D. Hamlyn,
Medical Research Council,
20 Park Crescent,
London, W1N 4AL.

Dear Jimmy,

Continuous Cultures of Fused Cells

We have now had an opportunity to study the paper by Kohler and Milstein to which you referred in your letter of 24th September addressed to Ron Homer.

Although the authors suggest that the cultures which they have developed, or rather similar cultures, could be valuable for medical and industrial use, I think this statement should be taken as a matter of long term potential rather than immediate application. It is certainly difficult for us to identify any immediate practical applications which could be pursued as a commercial venture, even assuming that publication had not already occurred. I would add that the general field of genetic engineering is a particularly difficult area from the patent point of view and it is not immediately obvious what patentable features are at present disclosed in the Nature paper.

In summary, therefore, unless further work indicates a diagnostic application or industrial end product which we can protect, despite the disclosure in the Nature paper, we would not suggest taking any further action ourselves.

Kind regards,

Yours sincerely,

Eric.

E. [redacted]
Biosciences Group

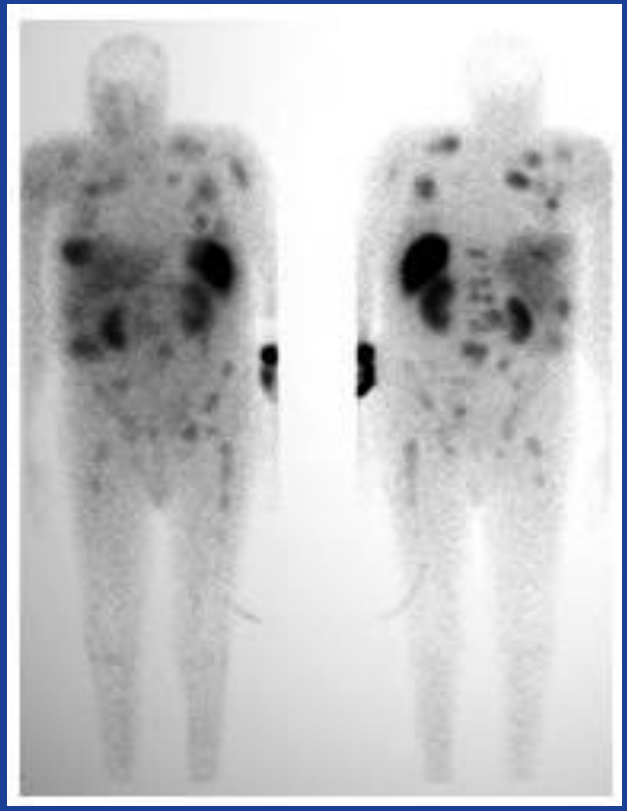
Application of monoclonal antibodies

- Therapy
  - Infectious diseases
  - Transplantation
  - Auto immune diseases
  - Cancer
- Diagnostics
  - In vitro
  - In vivo

Monoclonal antibodies and cancer

- Direct use
- Drug delivery (isotopes, toxins and cytostatic drugs)
- Delivery enzymes to activate prodrugs
- Delivery of antisense DNA
- To induce an immune reaction/inflammation
- Delivery of immune cells

DNA, deoxyribonucleic acid

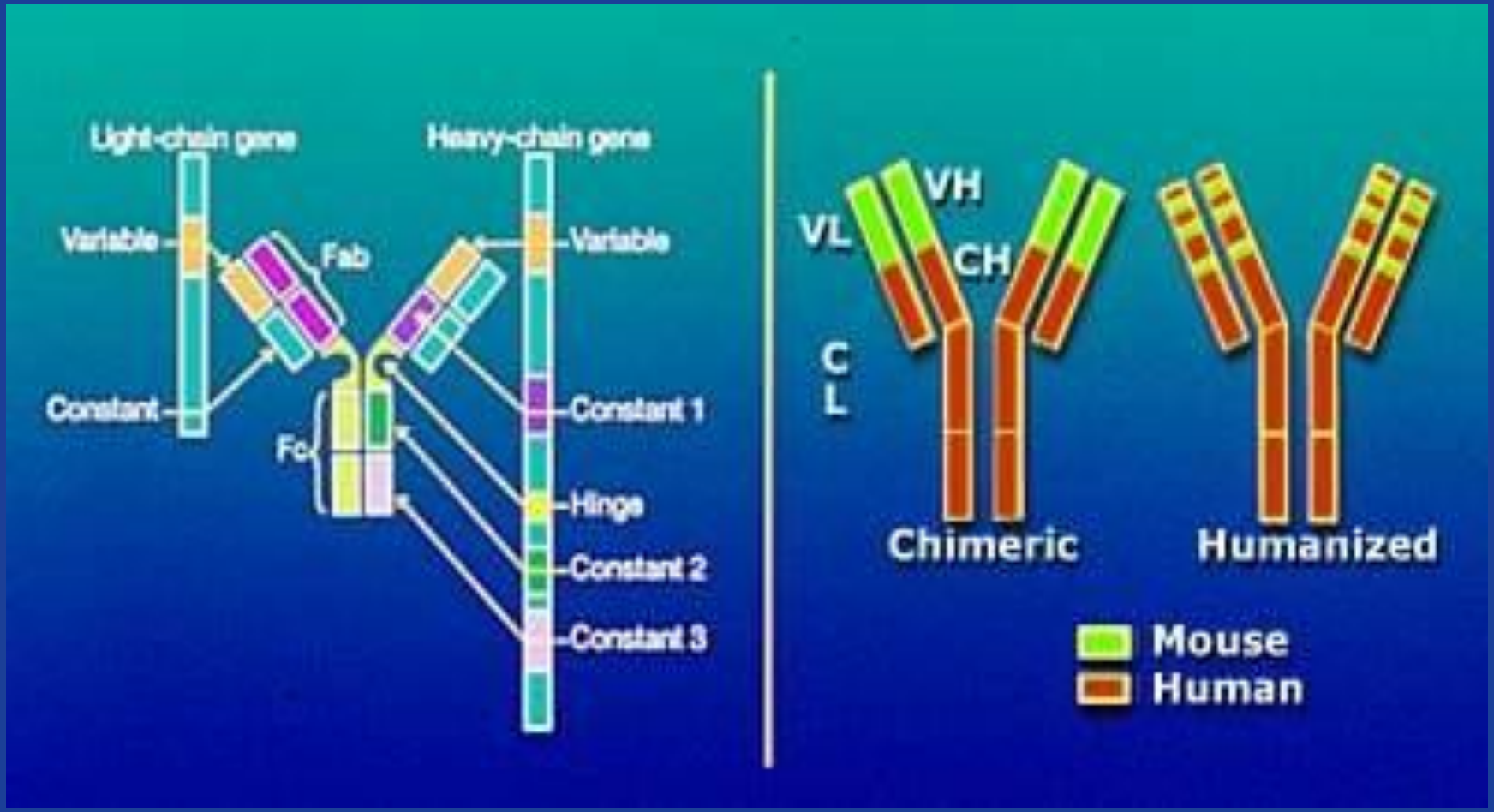


Problems with murine Mab's

- Inappropiate Fc functions
- Immunogenicity

Mab, monoclonal antibody

Chimerised and humanised antibodies

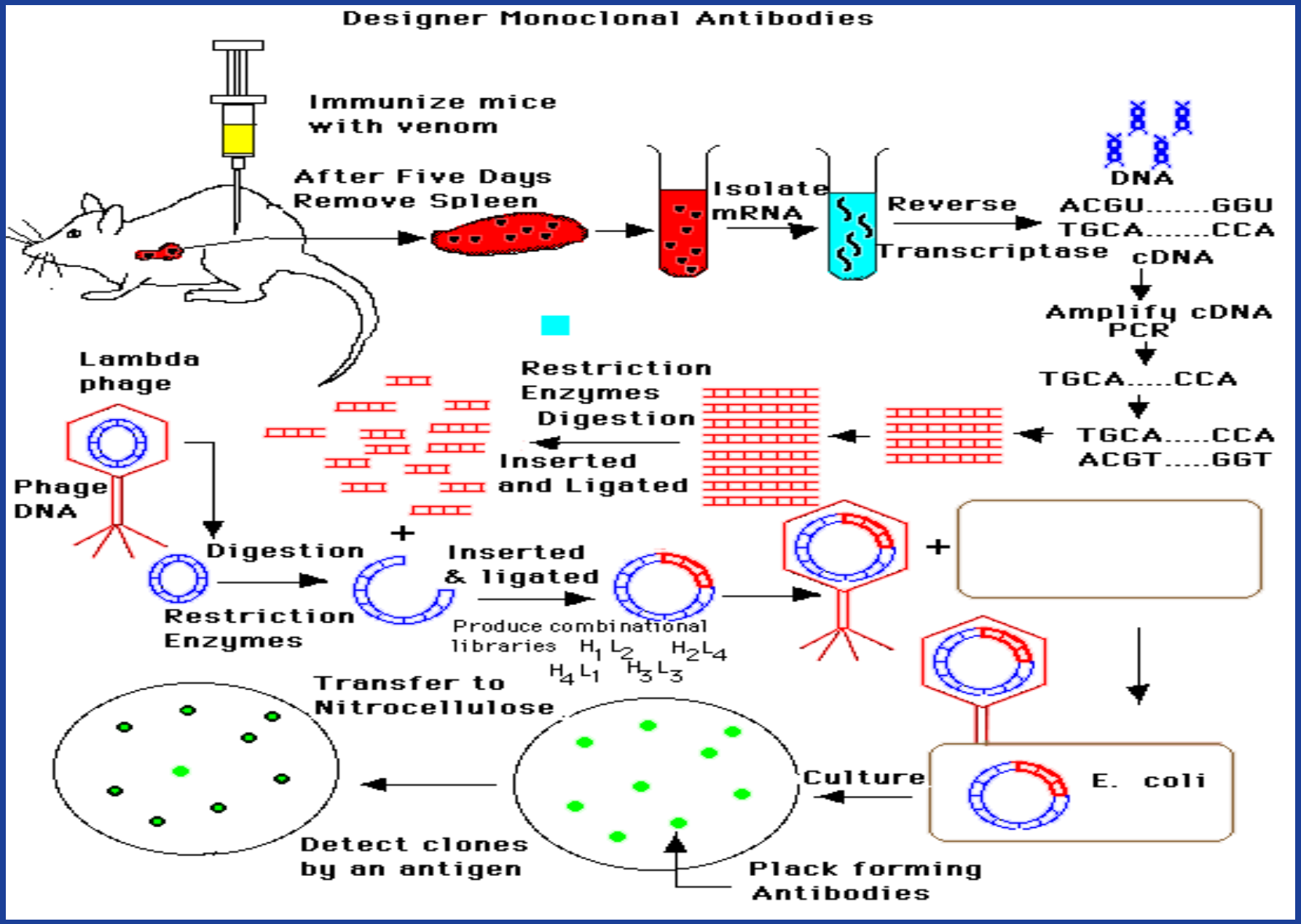


Effect of aglycosylation on Fcγ activities

huFcγRI binding	abolished
huFcγRII binding	abolished
huFcγRIII mediated killing	abolished
C1q binding	reduced 3x
C' activation	abolished
Rheumatoid factor binding	unaffected
Protein A binding	unaffected

Human monoclonal antibodies

- Human hybridoma’s
- HuMab mice
- Phage display


Designer Monoclonal Antibodies
Immunize mice with venom
After Five Days Remove Spleen
Isolate mRNA
Reverse Transcriptase
DNA
ACGU.....GGU
TGCA.....CCA
cDNA
Amplify cDNA PCR
TGCA.....CCA
TGCA.....CCA
ACGT.....GGT
Restriction Enzymes Digestion
Inserted and Ligated
Lambda phage
Phage DNA
Digestion
Restriction Enzymes
Inserted & ligated
Produce combinational libraries H_1L_2 H_2L_4 H_4L_1 H_3L_3
Transfer to Nitrocellulose
Culture
E. coli
Detect clones by an antigen
Plack forming Antibodies

The immunogenicity of monoclonal antibodies

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Immunogenicity of monoclonal antibodies

- Considered the biggest hurdle for the therapeutic use of monoclonal antibodies
- The incentive for the humanising

Number of murine Mab injection and HAMA response

# of inj.	# of studies	#pos/n	% positive
1	13	84/182	46
2-5	15	141/187	75
>5	18	117/213	55

HAMA, human anti-mouse antibodies

Kuus-Reichel et al. 1994

Dose of murine Mab and HAMA response

Dose(mg)	# of studies	#pos/n	% positive
<1	4	23/47	49
2-10	3	6/11	55
11-50	7	39/64	61
51-200	5	20/32	62
>200	6	84/90	93

Kuus-Reichel et al. 1994

Form of murine Mab and HAMA response

Antibody	# of studies	#pos/n	% positive
Intact	52	441/774	57
$F(ab')_2$	2	34/41	83
Fab	4	19/965	2

Kuus-Reichel et al. 1994

Effects of antibodies to Mab's

- No effect
- Reduction of efficacy
- Increase of efficacy
- General immune effects

“”Fully human mAbs are anticipated to be nonimmunogenic and thus to allow repeated administration without human anti-human antibody response.””

Yang X, et al. Development of ABX-EGF, a fully human anti-EGF receptor monoclonal antibody, for cancer therapy. Crit Rev in Oncol/Hematol 2001;38:17–23

Immunogenicity of marketed therapeutic mAbs

Trade name	Generic name	type of Mab	Ig type	% antibodies
Humira	adalimumab	human	IgG1	12
Remicade	infliximab	chimeric	IgG1	24
Reopro	abciximab	chimeric	Fab	6
Herceptin	trastuzumab	humanized	IgG1	1
Mabthera	rituximab	chimeric	IgG1	1
Xolair	omalizumab	humanized	IgG1	0
Simulect	basiliximab	chimeric	IgG1	0
Synagis	palivizumab	humanized	IgG1	1
Campath	alemtuzumab	humanized	IgG1	2
Zenapax	daclizumab	humanized	IgG1	9

Factors influencing immunogenecity of Mabs

- Presence of foreign sequences
- Methods and frequency of administration
- Dose
- Complete/incomplete antibody
- Patient characteristics
- Specificity of antibody
- Formation of immune complexes
- Fc functions
- Purity and formulation ?

Strategies to reduce the immunogenicity of Mabs

- Chimeric antibodies: murine variable regions and human constant regions
- Humanization: murine CDR sequences grafted on a human IgG backbone
- Human antibodies

  phage display

  transgenic mice with human Ig repertoire

CDR, complementarity-determining region; Ig, immunoglobin