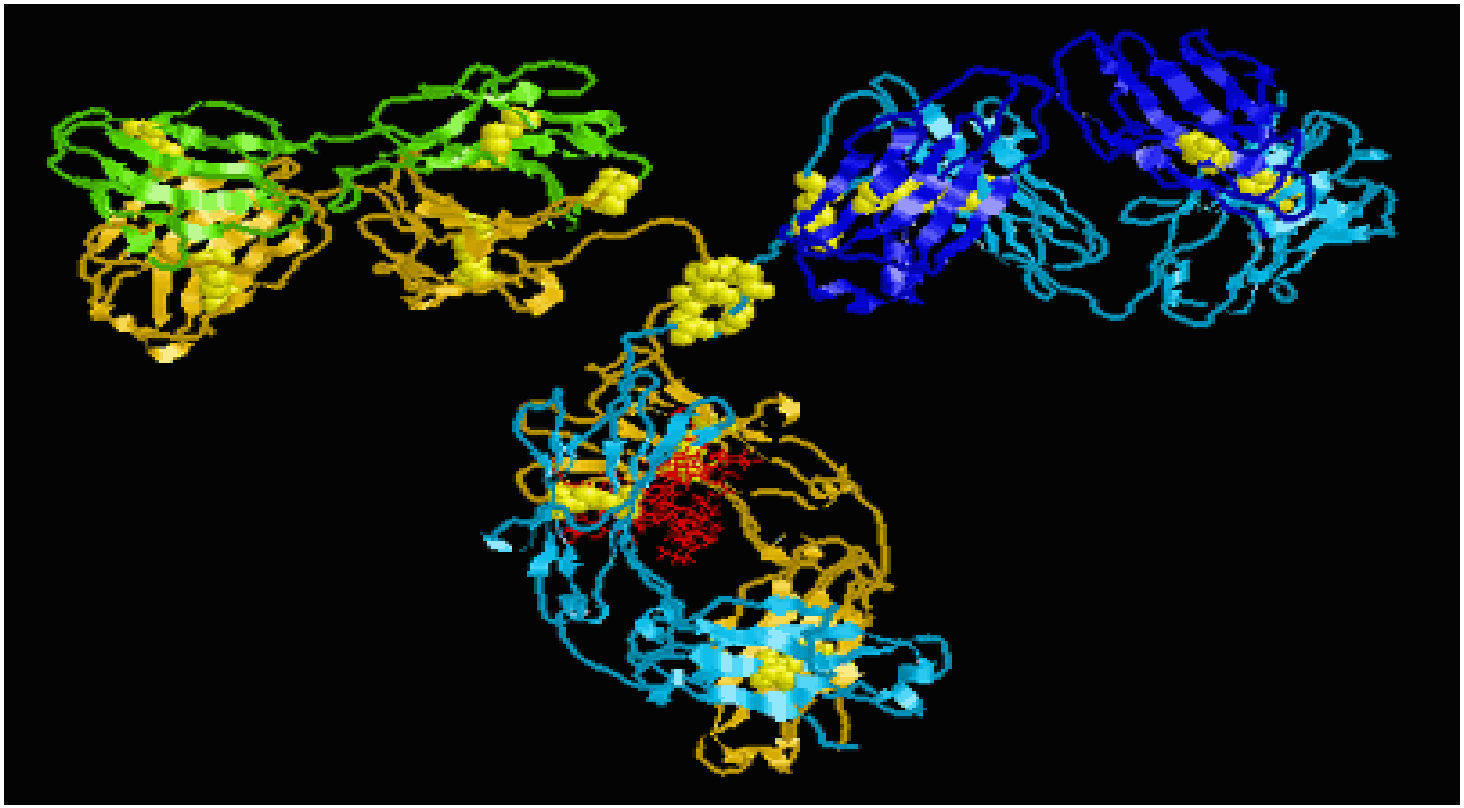
ANTIBODIES AS DRUG



Emerging focus

- Antibodies are naturally occurring
- Discovery of their innate properties hinted at great therapeutic potential
  - High-specificity in binding
  - Already present in the body
  - Can activate and couple components of the immune system
- Modification to structure and refinement in production methods have made antibodies a viable modern drug

At the turn of the 20th century:

- Emil Adolf von Behring
  - Developed serum therapy as an effective treatment against diphtheria and tetanus
  - For this, he received the first ever Noble Prize in Physiology or Medicine in 1901
  - The serum derived from immunized animals was latter shown to be effective because of the antibodies it contained
- Paul Ehrlich
  - Side-chain theory:
    - Toxins and antitoxins were chemical substances
    - Antitoxins were side-chains on cells that could bind with a toxin like a lock and key
  - Predicted autoimmunity or “horror autotoxicus”
  - Received the 1908 Nobel Prize in Physiology or Medicine for his work in immunity

- **"Discovery" of antibody chemical structure**
  - **Gerald Edelman and Rodney Porter, circa 1961**
  - **Received the 1972 Nobel Prize in Physiology or Medicine**

- **Development of hybridoma technology**
  - **Jerne, Kohler, and Milstein, 1975**
  - **Received the 1984 Nobel Prize in Physiology or Medicine**

- **Production of the 1st monoclonal antibody**
  - **In 1986, OKT-3 was approved for use in organ transplant rejection**

Important Terms

- **Antibody – immunoglobulin secreted by B cells**
- **Antigen (antibody generator) – any substance capable of eliciting an adaptive immune response**
- **Monoclonal antibodies (mAbs) – antibodies secreted from a single B cell, have identical paratopes**
- **Epitope – region of the antigen recognized by an antibody**
- **Paratope – region of the antibody that binds the epitope**

The Structure of an Antibody

2 identical light chains (~220 amino acids long)

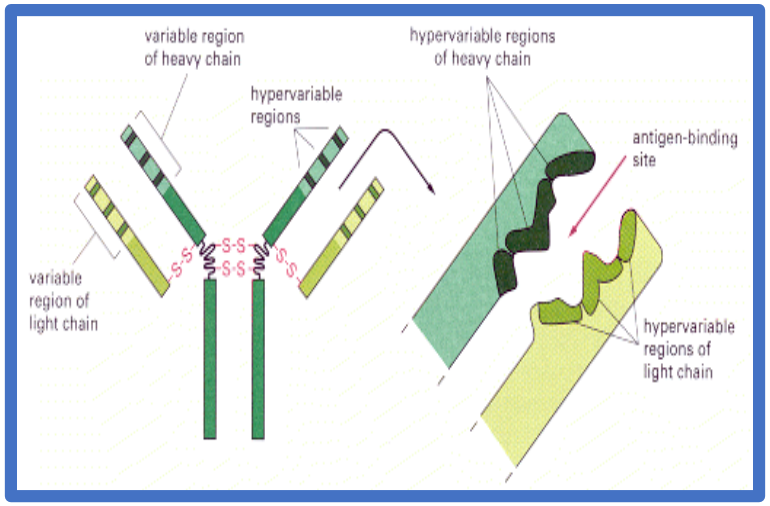
- Variable domain: V_L
- Constant domain: C_L

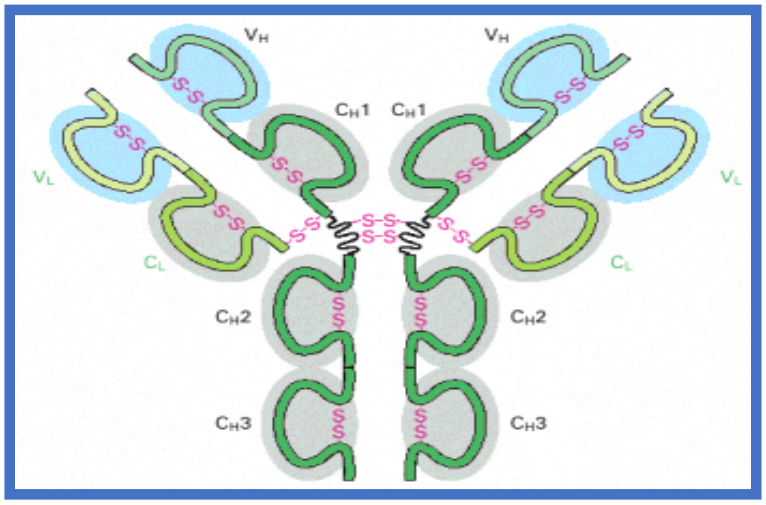
2 identical heavy chains (~440 amino acids long)

- Variable domain: V_H
- 3 Constant domains: C_H1, C_H2, C_H3

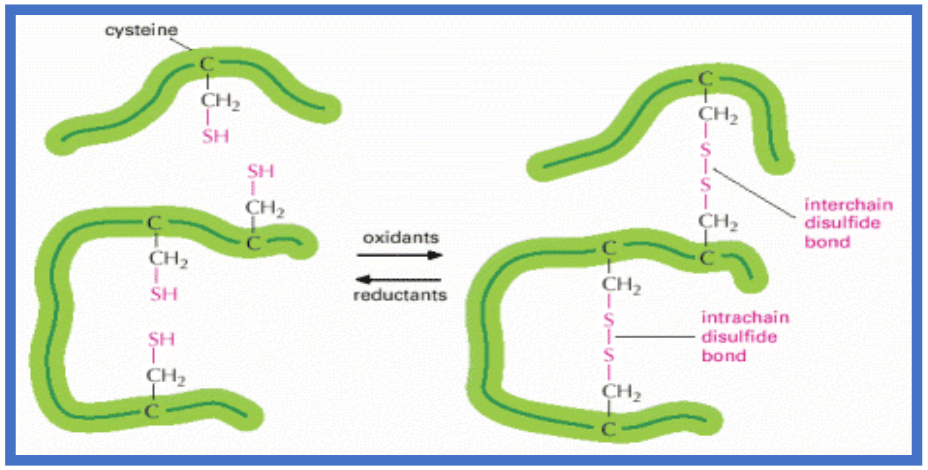
Covalent, disulfide bonds between cysteine residues

Flexible "hinge region"

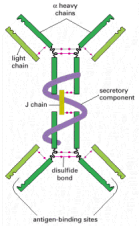
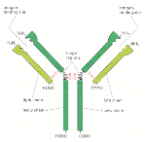
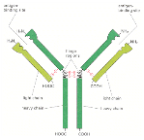
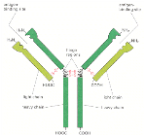
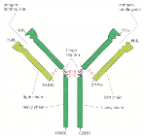
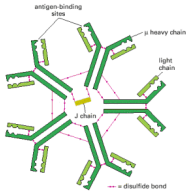




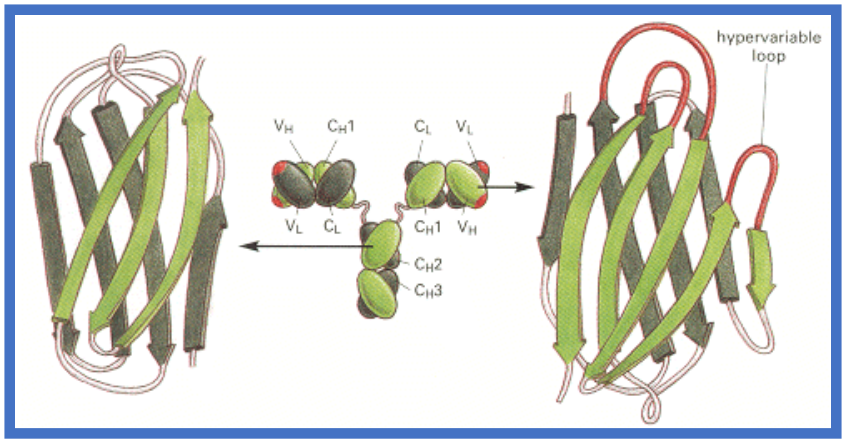
Formation of Disulfide Bonds



- Catalyzed in the Endoplasmic Reticulum
- Do *not* change the protein's conformation
  - Reinforce a favored conformation
- Disulfide bonds are sensitive to reduction

Immunoglobulin (Ig) Class	Heavy Chain	Diagram	Distribution	Biological Activity
IgA	α	 or 	External Secretions	
IgD	δ		B Cell surface receptor	
IgE	ε		Cells that secrete histamines	
IgG	γ		Main antibody in serum Most Stable	Promotes antibody-dependent cellular cytotoxicity (ADCC) Compliment fixation
IgM	μ		First antibody secreted in development	Compliment fixation

Hypervariable Loops



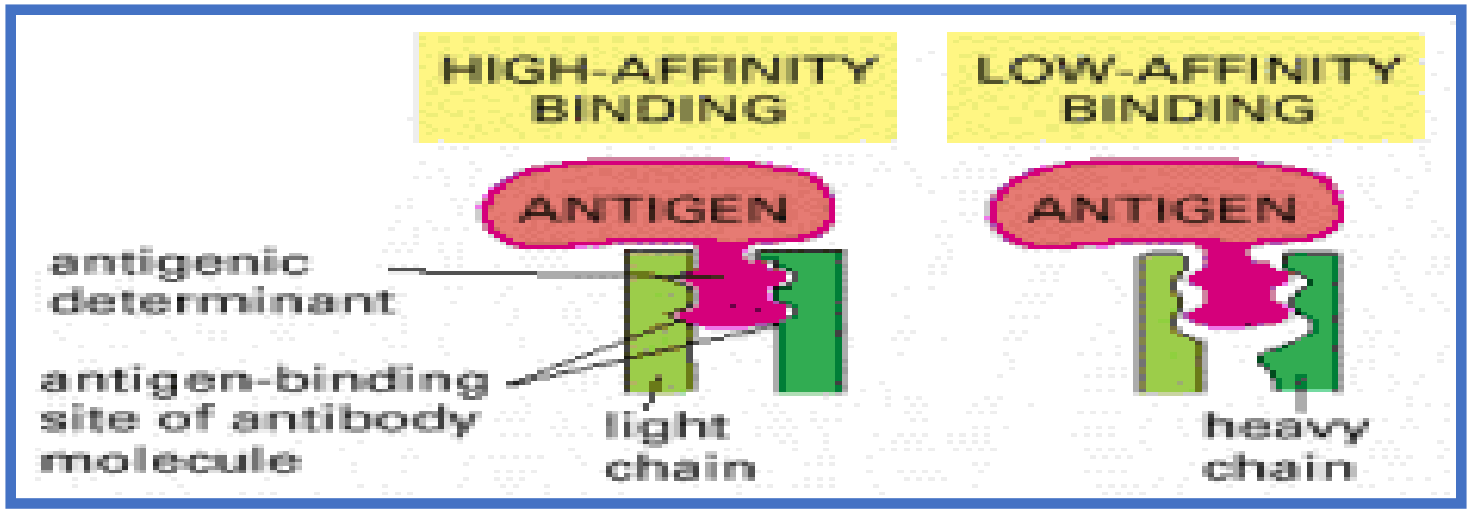
- A.k.a: Complementarity-Determining Regions (CDRs)
- Regions of increased amino acid sequence variability
- In each variable region,
  - 3 CDRs interspersed in between framework regions
  - Each CDR is between 5-10 amino acids long

A Dynamic Binding Site

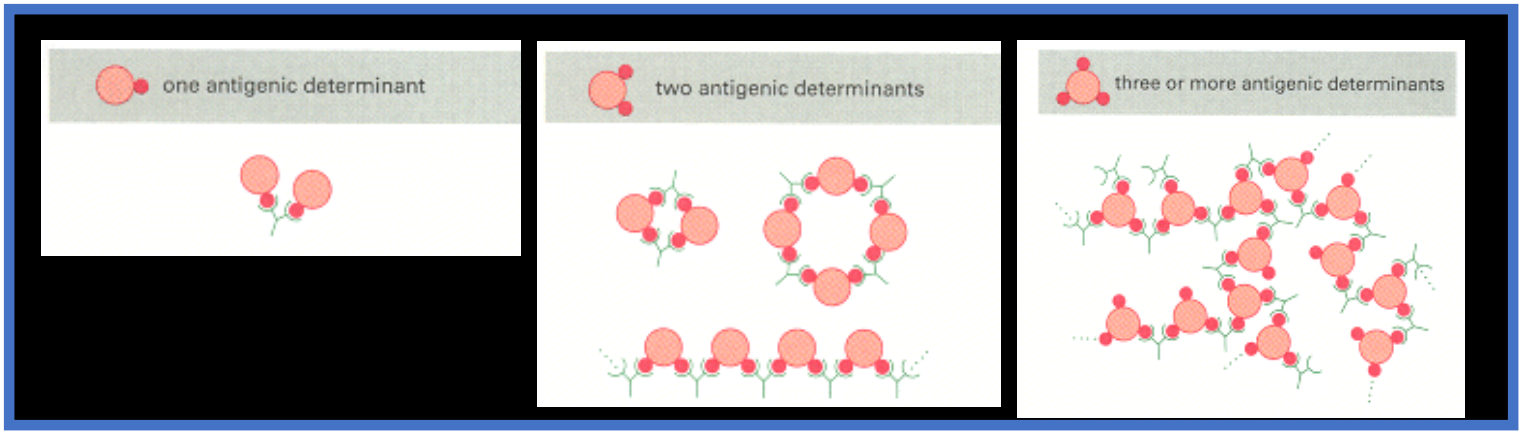
- The functional groups of the paratope (Fab) interact with the epitope (antigen)
  - Hydrogen bonding
  - Van der Waals forces
  - Ionic interactions
- The CDRs are necessary for antigen binding
- The tertiary structure of this region can contain pockets, undulating flatter surfaces, and even protrusions
- Small antigens typically bind in deep pockets

Antibody Pharmokinetics

- Antigen binding is reversible
  - Antigen (Ag) + Antibody (Ab) ↔ AntigenAntibody (AgAb) [bound]
  - $K_{affinity} = \frac{[AgAb]}{[Ag][Ab]}$
- For some therapeutic mAbs, the affinity must be balanced so that effective antigen binding occurs while tissue penetration is allowed



Cross-linking



- All antibodies are at least bivalent
  - Two paratopes can bind with two epitopes
- With 2 epitopes on a single antigen, cyclic or linear cross-linking can occur.
- Three or more epitopes on an antigen leads to formation of large three-dimensional lattices

Mechanisms of Action

1. **Blocking action of molecular targets**
   - **Can work antagonistically by binding a *receptor* to prevent activation**
   - **Can also bind the *antigen* and prevent activation**
2. **“Magic Bullet”**
   - **Compound with target specificity is coupled with various effector groups**
     - **Toxins, radionuclei, enzymes, DNA**
3. **Signal molecules**
   - **Coupled to mediators of apoptosis, cell division, etc.**

Monoclonal & Polyclonal

- ✓**Monoclonal Antibodies** are antibodies that are identical because they were produced by one type of immune cell (B cell), all clones of a single parent cell
- ✓**Polyclonal Antibodies** represent the antibodies from multiple clones of B lymphocytes, and therefore bind to a number of different epitopes (specific piece of the antigen that an antibody binds to)

  e.g. IV Immunoglobulin

ANTIBODIES

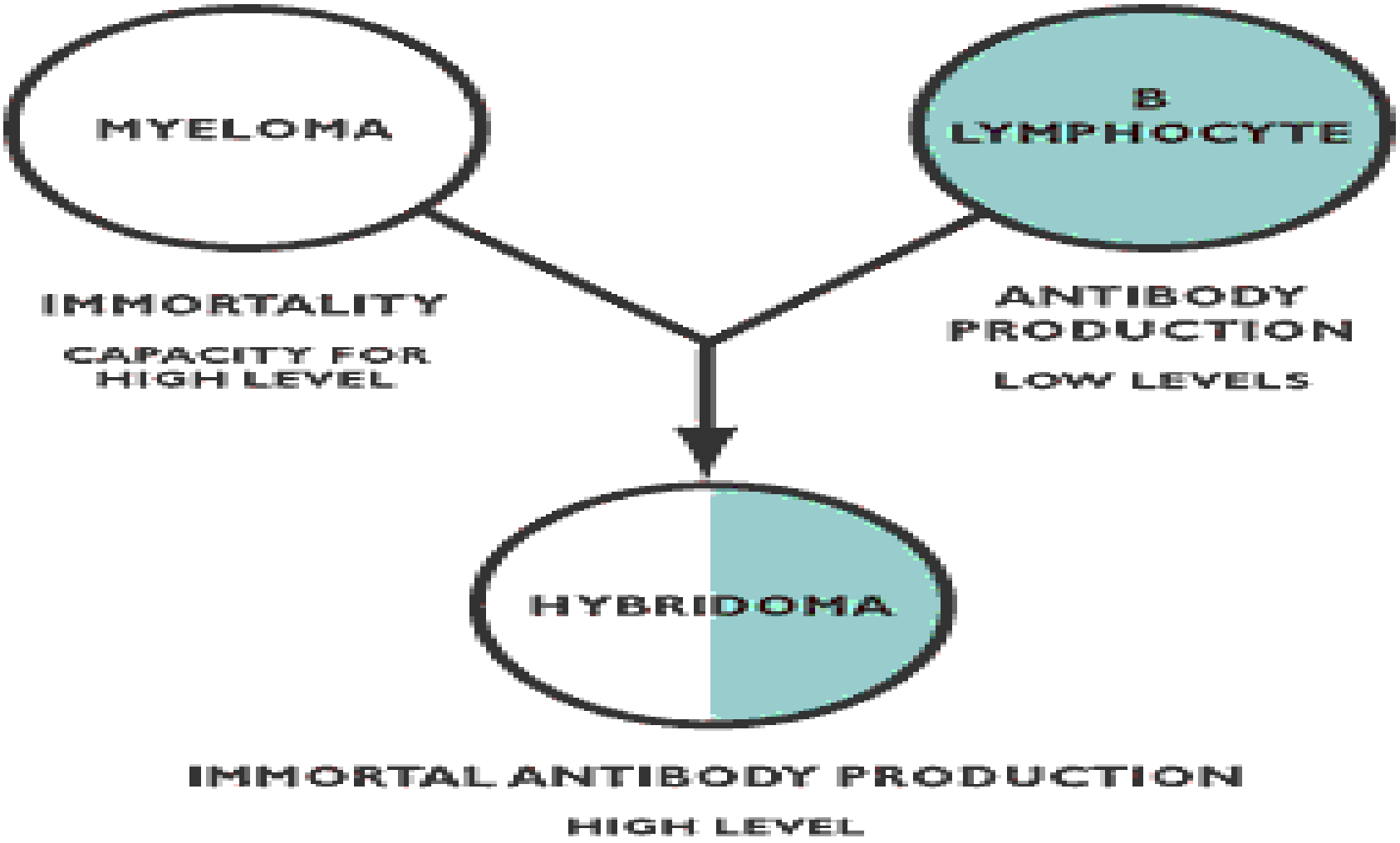
POLYCLONAL.	MONOCLONAL.
Derived from different B Lymphocytes cell lines	Derived from a single B cell clone
Batch to Batch variation affecting Ab reactivity & titre	mAb offer Reproducible, Predictable & Potentially inexhaustible supply of Ab with exquisite specificity
NOT Powerful tools for clinical diagnostic tests	Enable the development of secure immunoassay systems.

Discovery

The idea of a "magic bullet" was first proposed by Paul Ehrlich who at the beginning of the 20th century postulated that if a compound could be made that selectively targeted a disease-causing organism, then a toxin for that organism could be delivered along with the agent of selectivity.

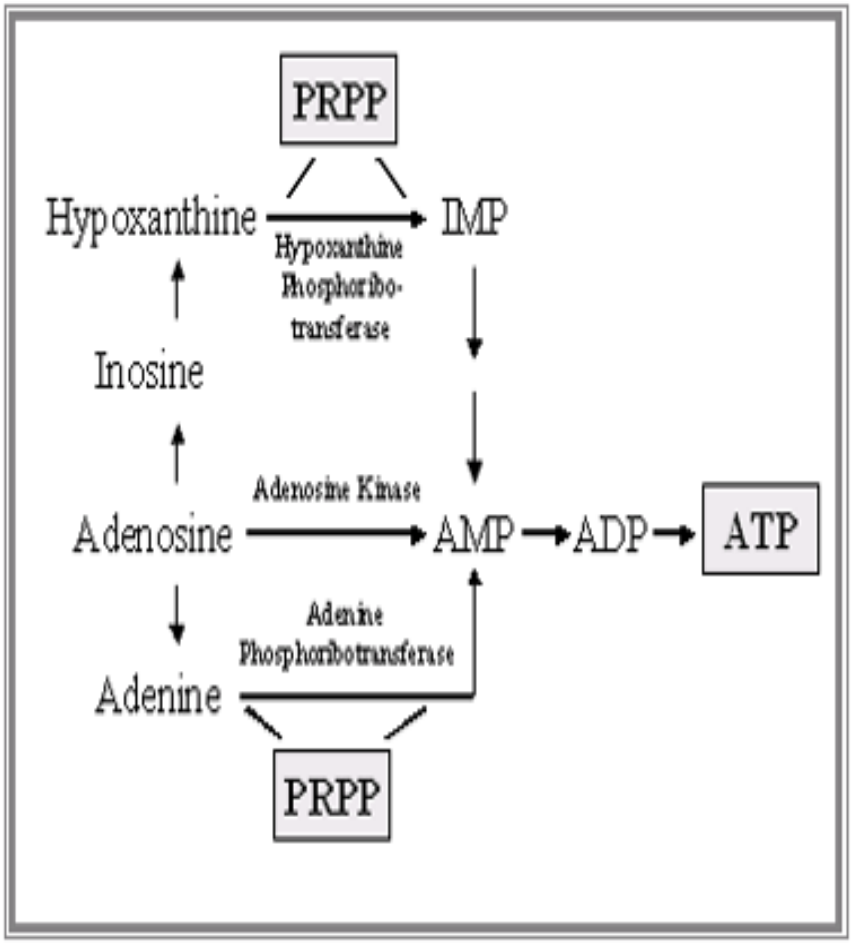
In 1988 Greg Winter and his team pioneered the techniques to humanize monoclonal antibodies, removing the reactions that many monoclonal antibodies caused in some patients.

HYBRIDOMA TECHNOLOGY

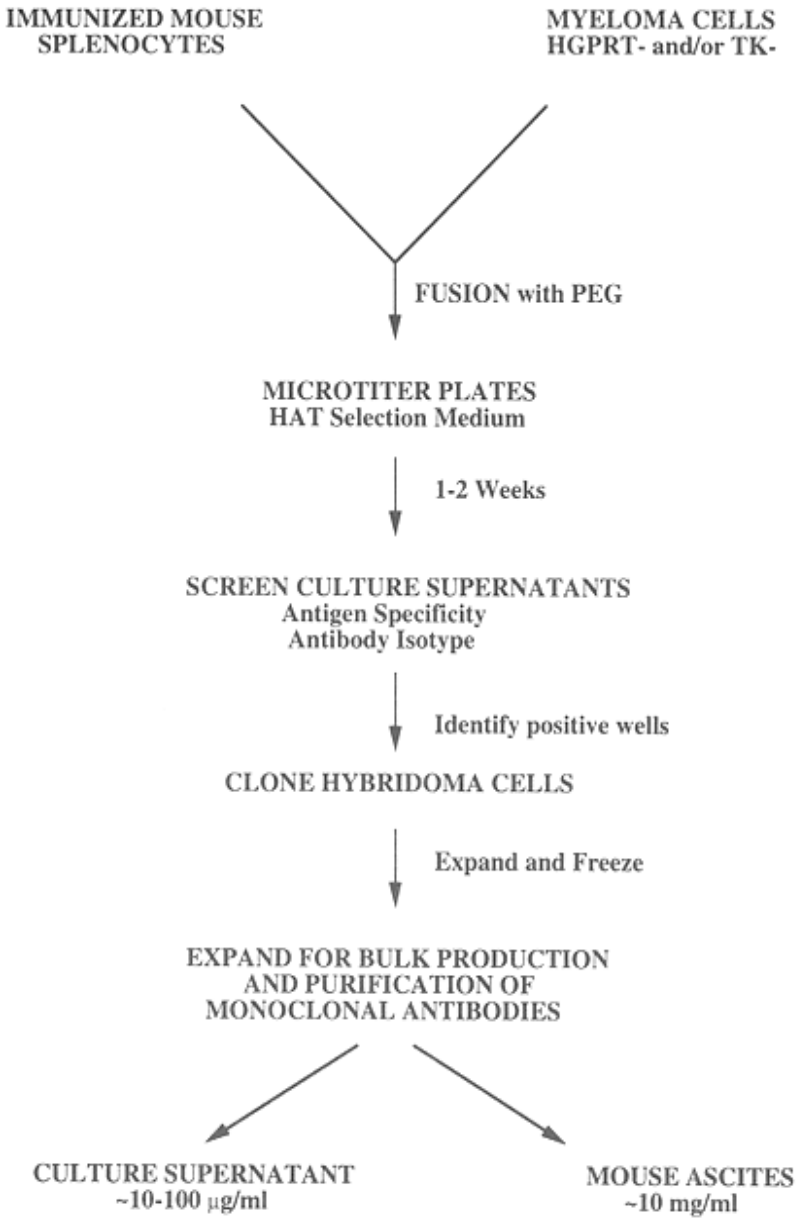


Principle

- **MYELOMA CELLS HAVE LOST** the ability to synthesize hypoxanthine - guanine-phosphoribosyl transferase (HGPRT), an enzyme necessary for the **salvage synthesis** of nucleic acids
- Which enables cells to synthesize purines by the **salvage pathway** here using an extracellular source of hypoxanthine as a precursor



- **The selective culture medium is called HAT medium (Hypoxanthine, Aminopterin, and Thymidine).**
- **Unfused myeloma cells cannot grow because they lack HGPRT.**
- **Unfused normal spleen cells cannot grow indefinitely because of their limited life span.**



PRODUCTION OF MONOCLONAL ANTIBODY

HYBRIDOMA TECHNOLOGY

Step 1: - Immunization Of Mice & Selection Of Mouse Donor For Generation Of Hybridoma cells

ANTIGEN (Intact cell / Whole cell membrane / micro-organisms) + ADJUVANT (emulsification)

Ab titre reached in Serum

PRODUCTION OF MONOCLONAL ANTIBODY

HYBRIDOMA TECHNOLOGY

Step 2: - Screening Of Mice For Antibody Production

After several weeks of immunization

Serum Antibody Titre Determined

(Technique: - ELISA / Flow cytometery)

Titre too low

BOOST
(Pure antigen)

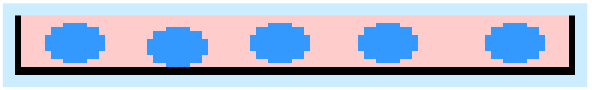
Titre High

BOOST 2 weeks
(Pure antigen)

PRODUCTION OF MONOCLONAL ANTIBODY

HYBRIDOMA TECHNOLOGY

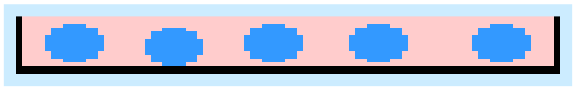
Step 3: - Preparation of Myeloma Cells



Myeloma Cells

Immortal Tumor Of Lymphocytes

\+ **8 - Azaguanine**



Myeloma Cells

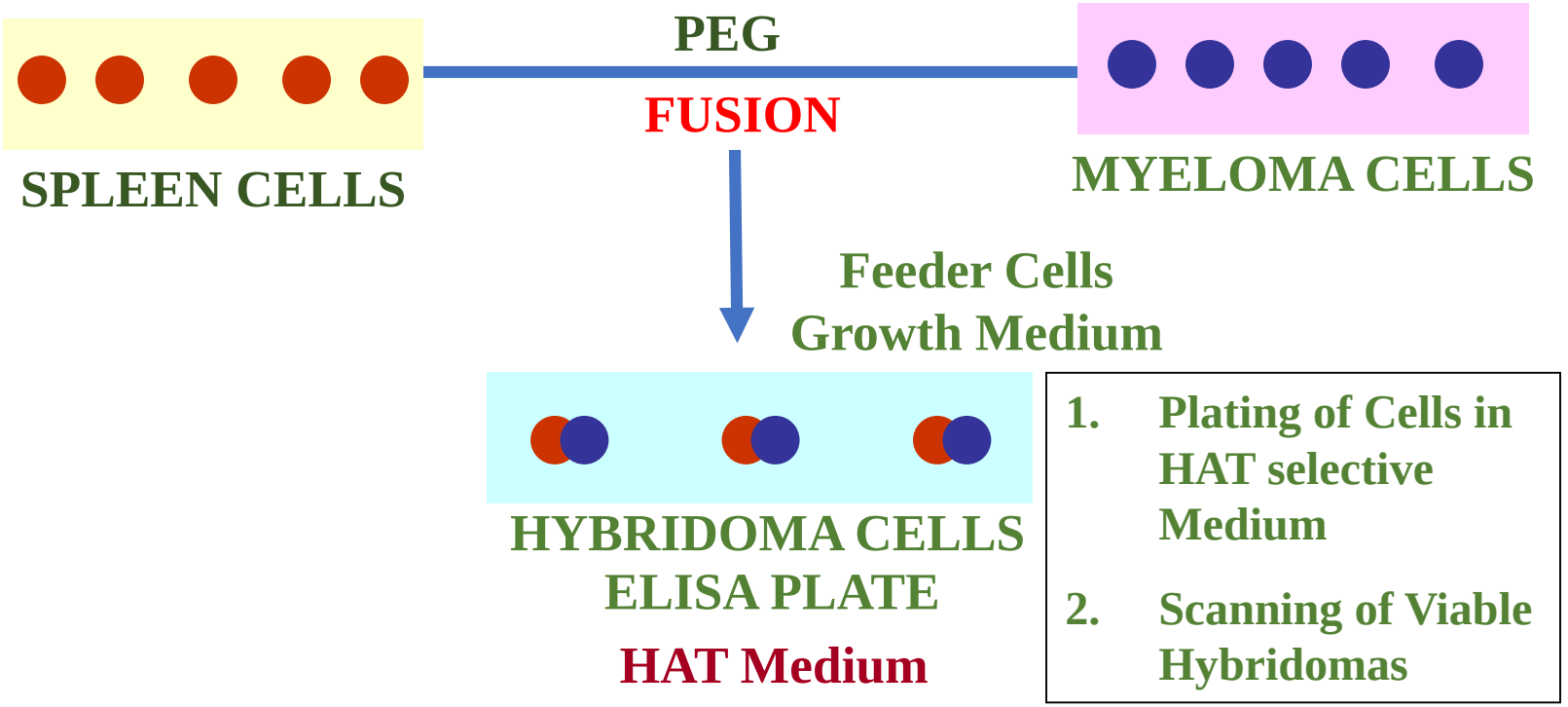
HGPRT^{-}

High Viability & Rapid Growth

PRODUCTION OF MONOCLONAL ANTIBODY

HYBRIDOMA TECHNOLOGY

Step 4: - Fusion of Myeloma Cells with Immune Spleen Cells & Selection of Hybridoma Cells



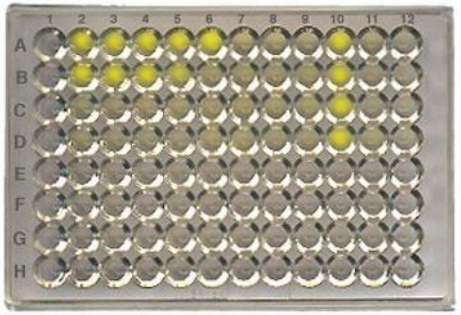
PRODUCTION OF MONOCLONAL ANTIBODY

HYBRIDOMA TECHNOLOGY

Step 5: - Cloning of Hybridoma Cell Lines by " Limiting Dilution" or Expansion

A. Clone Each +VE Culture

B. Test Each Supernatant for Antibodies



C. Expand +ve Clones

Tissue Culture Method

Propagate

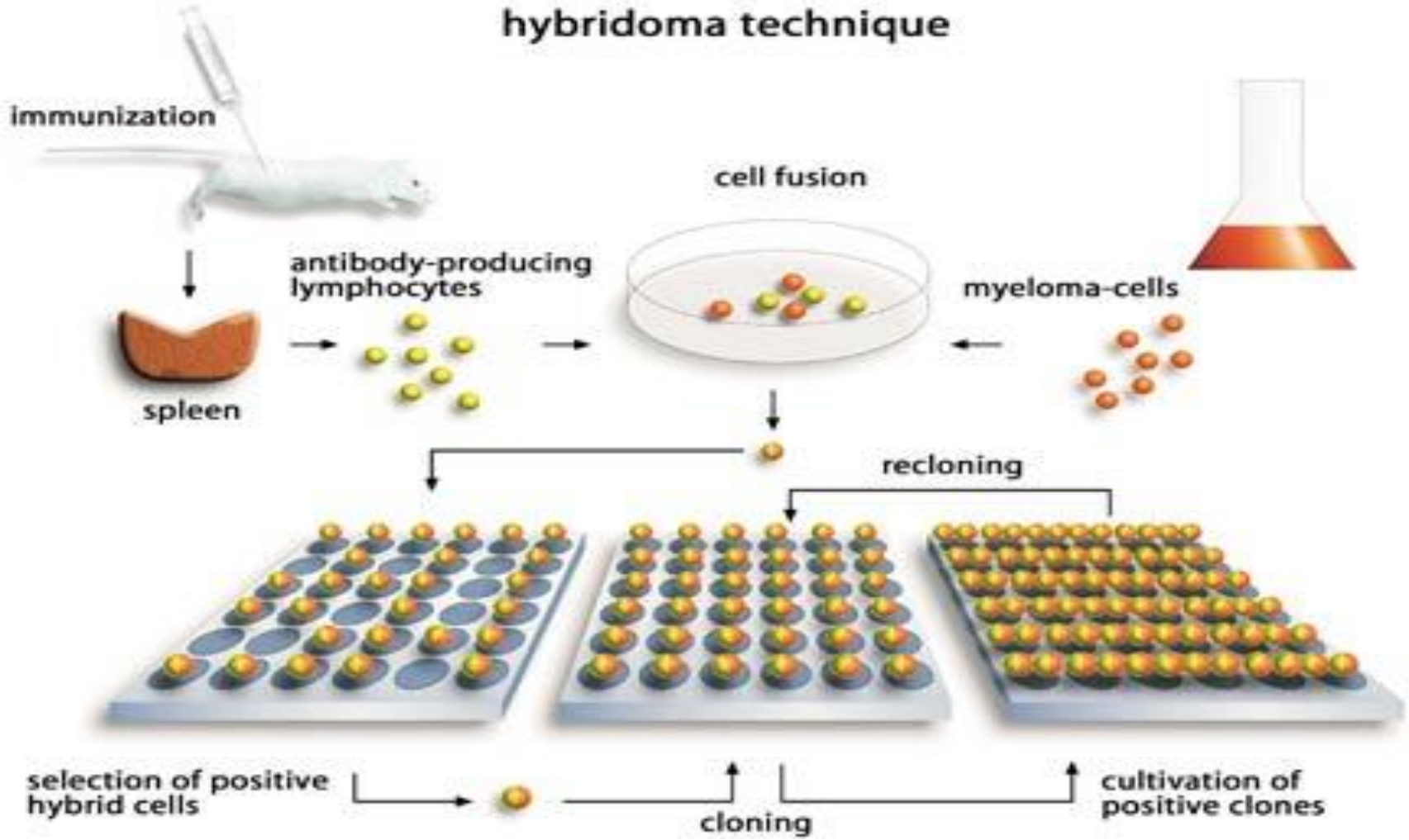
or

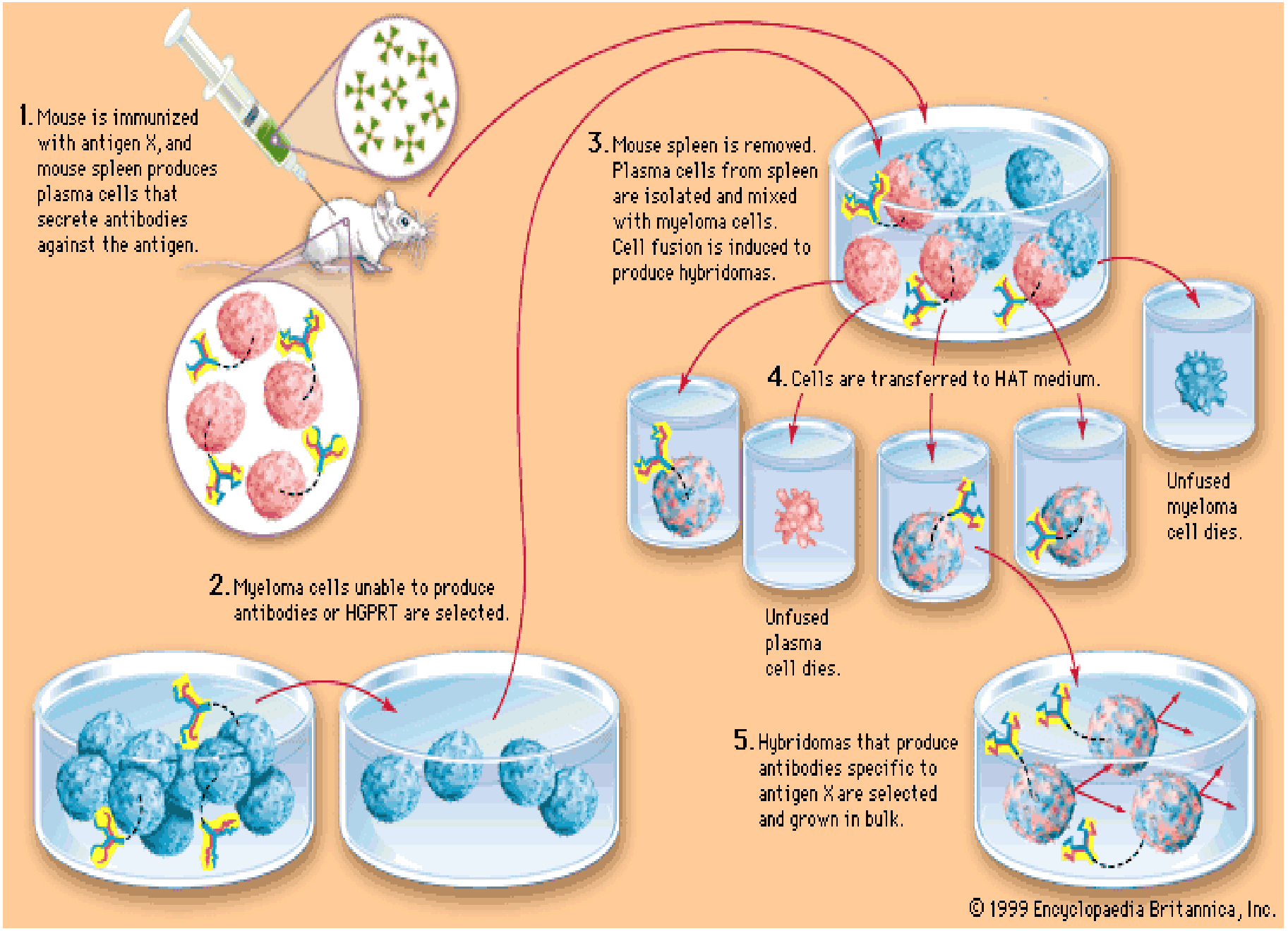
Mouse Ascites Method

Harvest monoclonal antibodies

PRODUCTION OF MONOCLONAL ANTIBODY

HYBRIDOMA TECHNOLOGY




1. Mouse is immunized with antigen X, and mouse spleen produces plasma cells that secrete antibodies against the antigen.
2. Myeloma cells unable to produce antibodies or HGPRT are selected.
3. Mouse spleen is removed. Plasma cells from spleen are isolated and mixed with myeloma cells. Cell fusion is induced to produce hybridomas.
4. Cells are transferred to HAT medium.
Unfused myeloma cell dies.
Unfused plasma cell dies.
5. Hybridomas that produce antibodies specific to antigen X are selected and grown in bulk.
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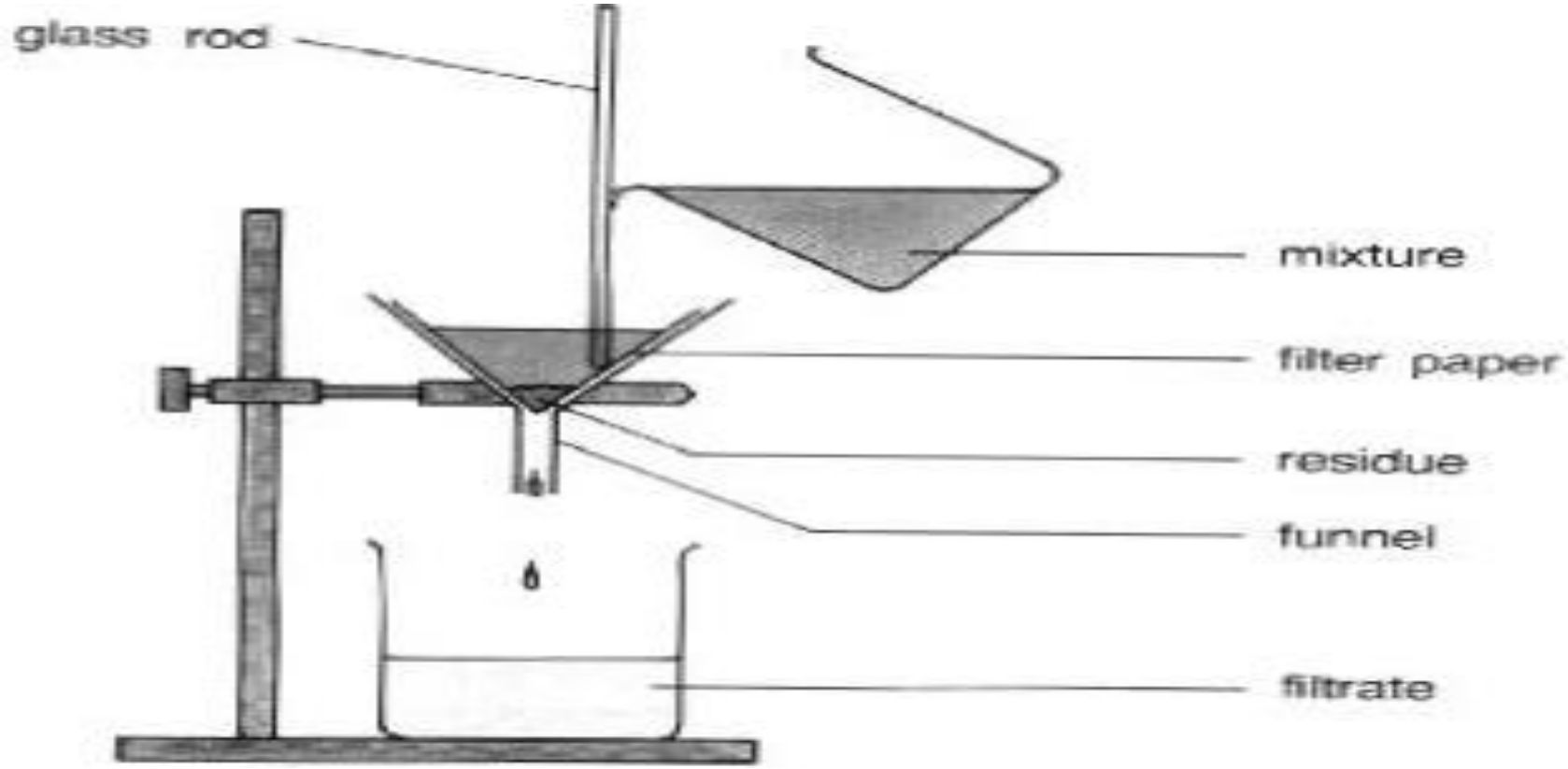
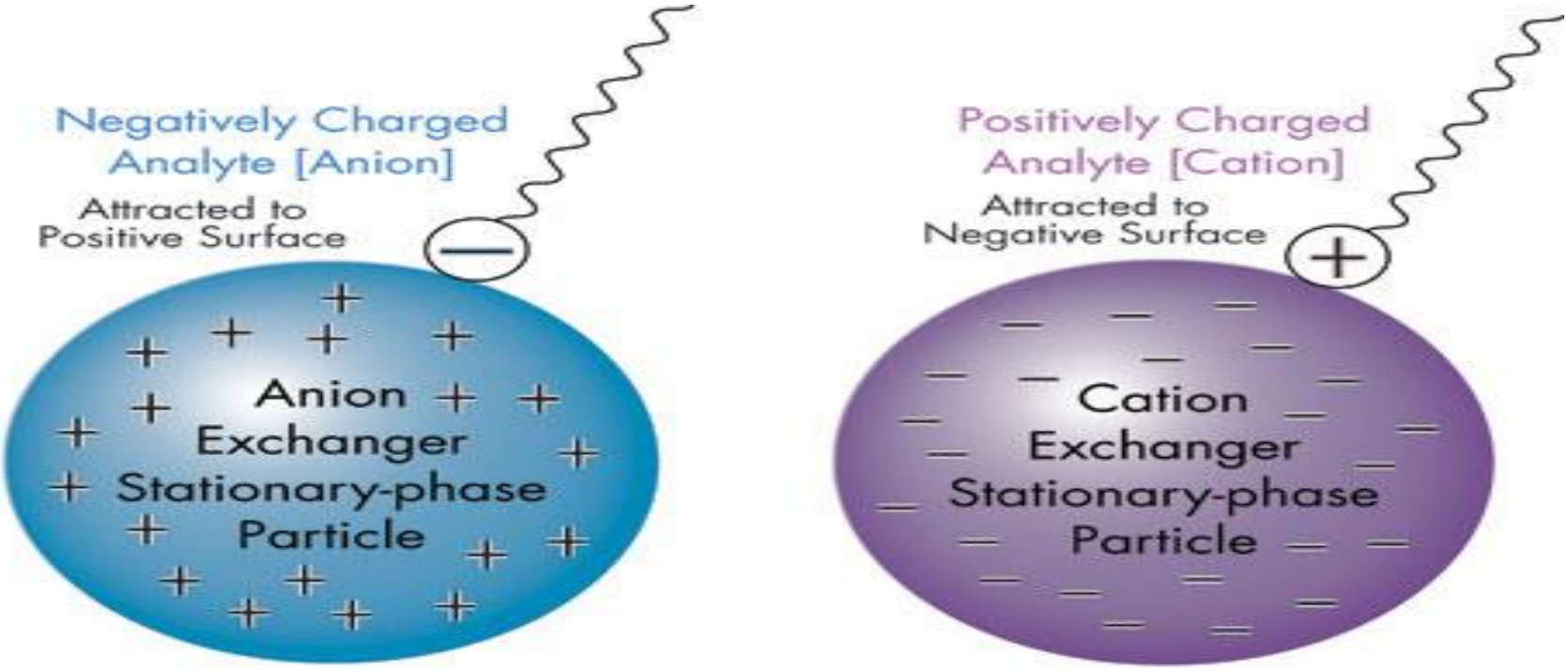
PURIFICATION TECHNIQUES

Cells, cell debris, lipids, and clotted material are first removed, typically by filtration with a 0.45 um filter.



Chromatography

Most of the charged impurities are usually anions such as nucleic acids and endotoxins. These are often separated by ion exchange chromatography.

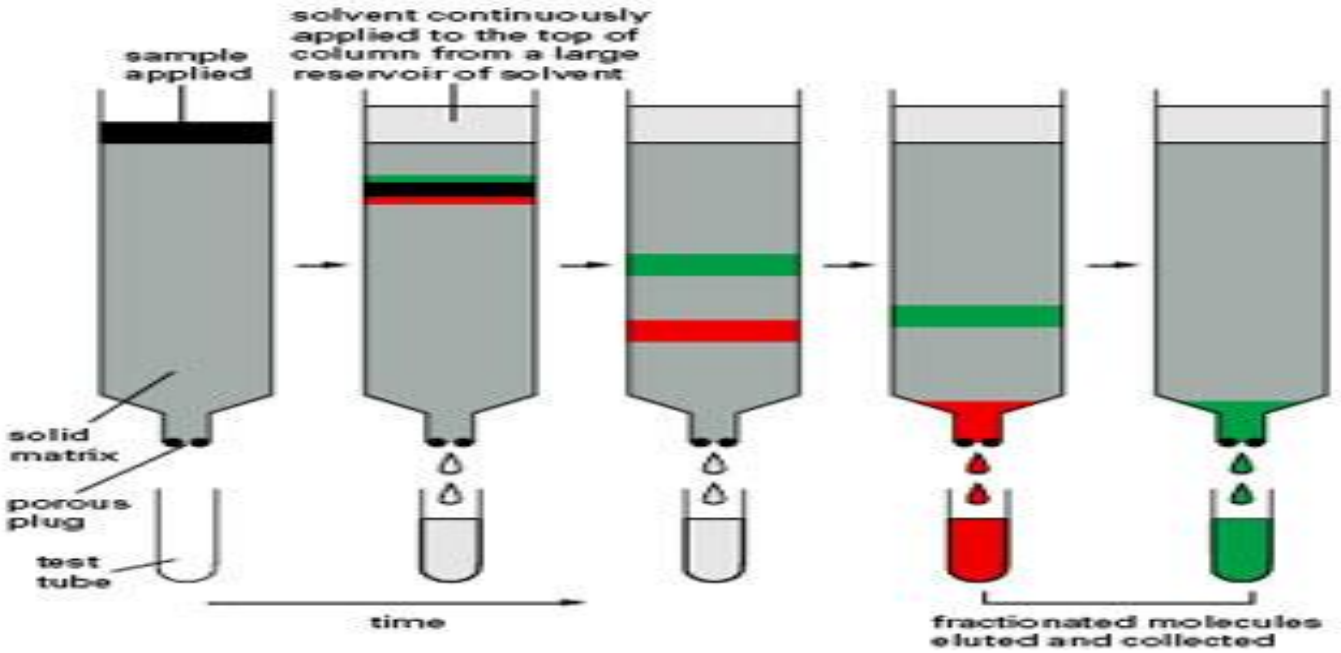


A much quicker method of separation is Protein A affinity chromatography. The antibody selectively binds to Protein A, so a high level of purity is obtained.

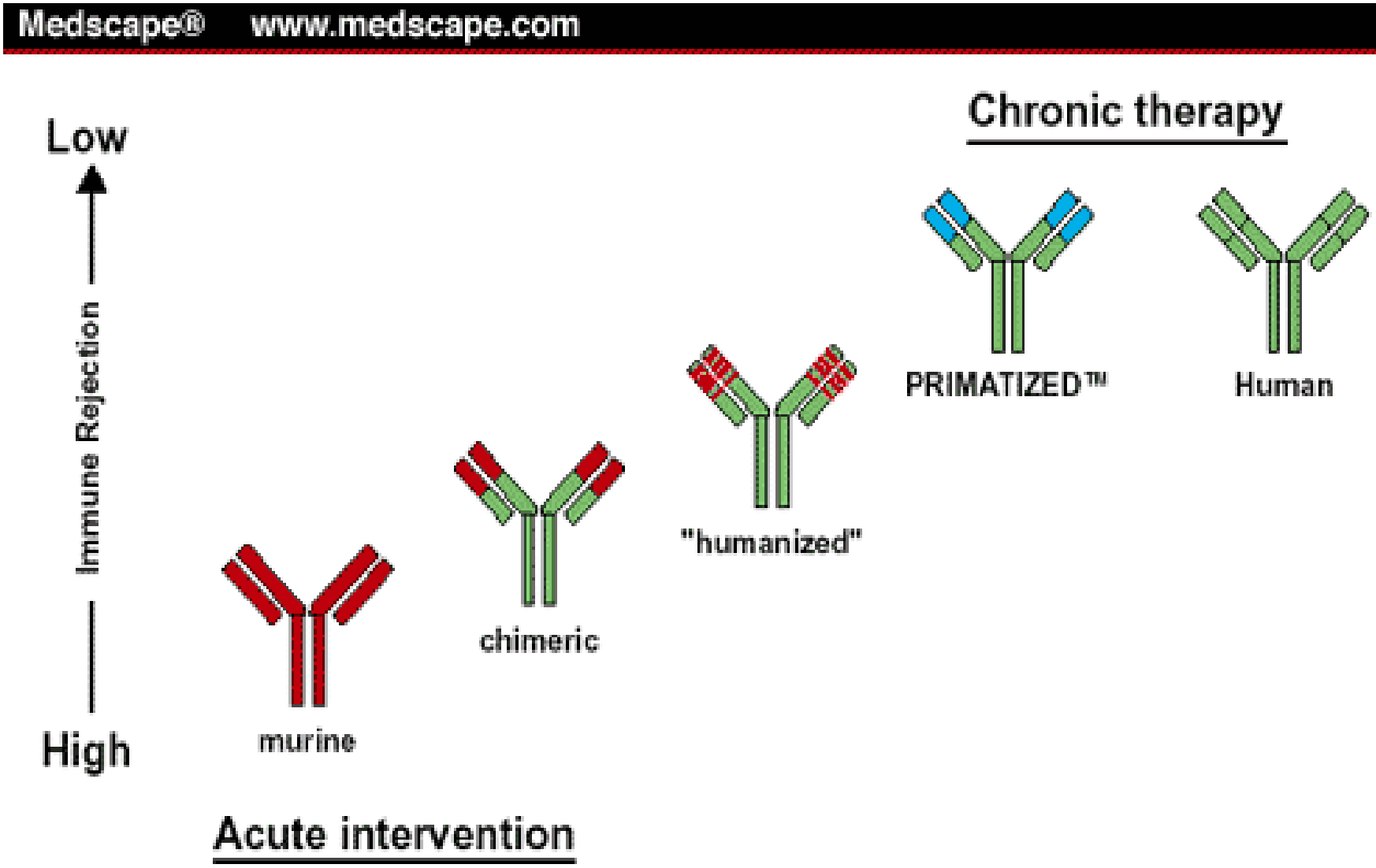
However, this method is not advisable for antibodies that are easily damaged

COLUMN CHROMATOGRAPHY

Proteins are often fractionated by column chromatography. A mixture of proteins in solution is applied to the top of a cylindrical column filled with a permeable solid matrix immersed in solvent. A large amount of solvent is then pumped through the column. Because different proteins are retarded to different extents by their interaction with the matrix, they can be collected separately as they flow out from the bottom. According to the choice of matrix, proteins can be separated according to their charge, hydrophobicity, size, or ability to bind to particular chemical groups (see *below*).

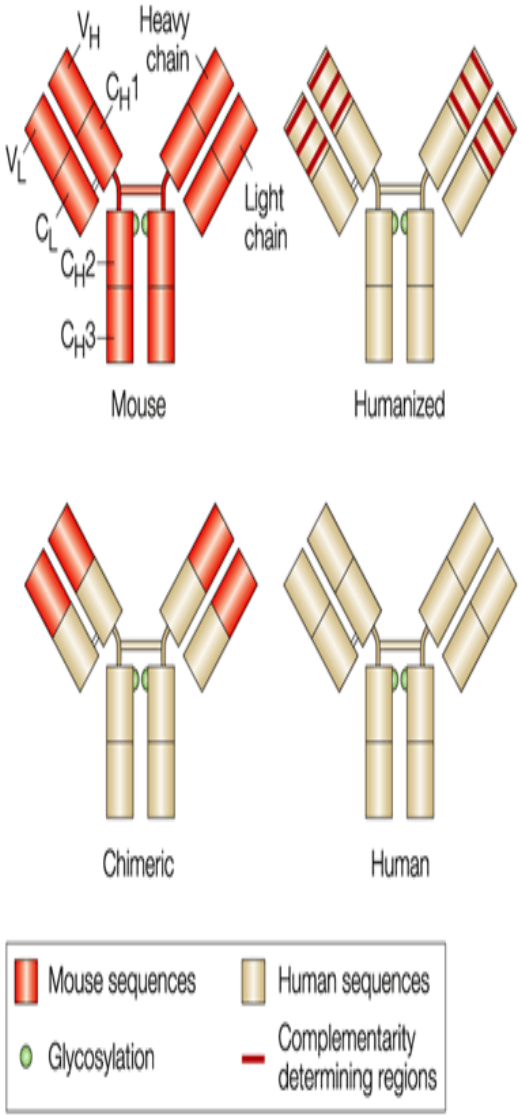


Types of Monoclonal Antibodies



Murine antibody

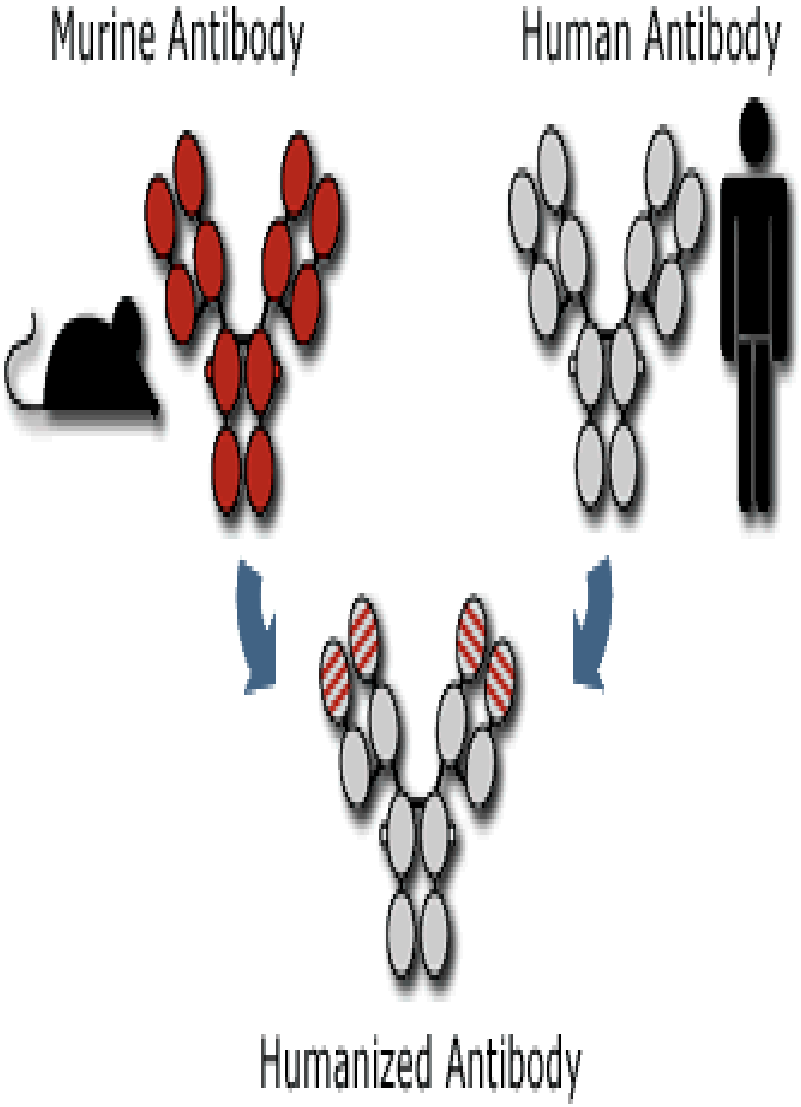
- **Whole of the antibody is of murine origin**
- **Major problems associated with murine antibodies include**
  1. **Reduced stimulation of cytotoxicity**
  2. **Formation of complexes after repeated administration**
  3. **Allergic reactions**
  4. **Anaphylactic shock**



- Chimeric antibodies are composed of murine variable regions fused onto human constant regions.
- Antibodies are approximately 65% human.
- This reduces immunogenicity and thus increases serum half-life.

Humanised Mab

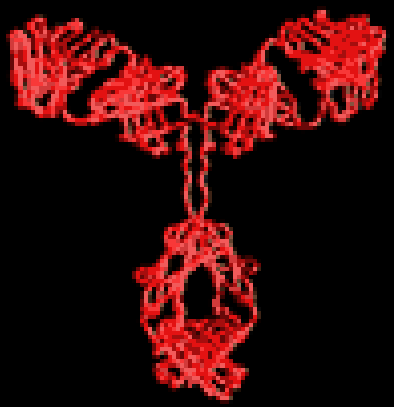
- **Humanised antibodies are produced by grafting murine hypervariable amino acid domains into human antibodies.**
- **This results in a molecule of approximately 95% human origin**
- **These bind weakly to the antigens**



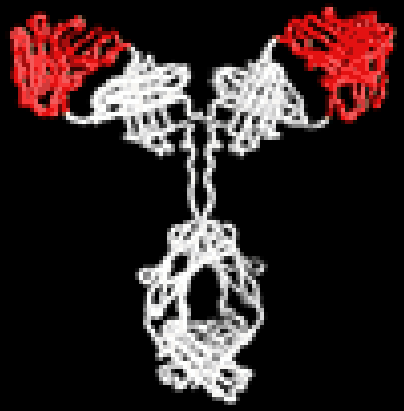
Human Monoclonal antibody

Human monoclonal antibodies are produced by transferring human immunoglobulin genes into the murine genome, after which the transgenic mouse is vaccinated against the desired antigen, leading to the production of monoclonal antibodies

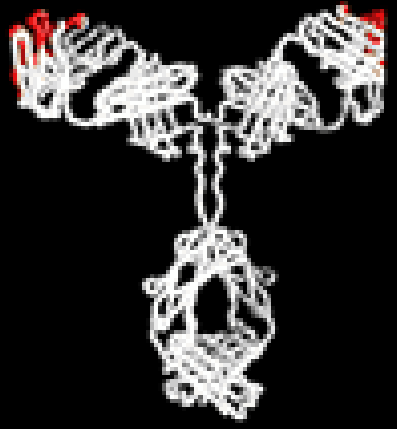
Approved therapeutic antibodies



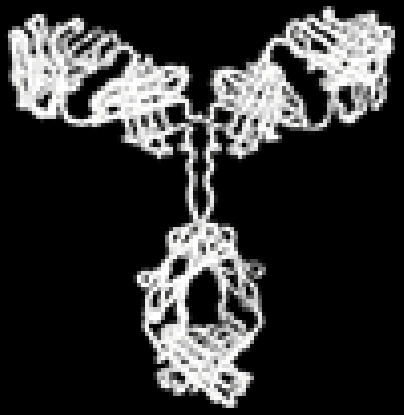
MOUSE
OKT3
BEXXAR
Zevalin



CHIMERIC
Rituxan
Remicade
Reopro
Simulect
Erbitux



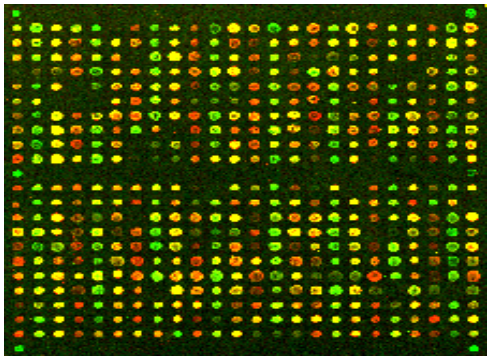
HUMANIZED
Synagis
Herceptin
Zenapax
Myelotarg
Campath
Xolair
Raptiva
Avastin
Tsyabri
(Actemra-Japan)

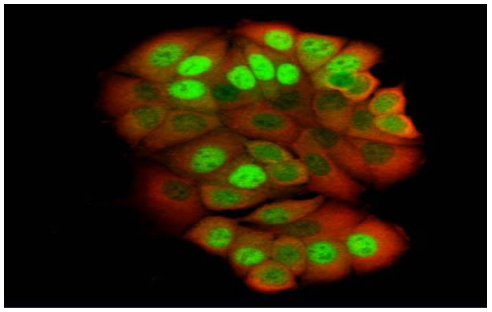


HUMAN
Humira
Vectibix

Applications of Monoclonal Antibodies

- Diagnostic Applications
  Biosensors & Microarrays
- Therapeutic Applications
  Transplant rejection
  Cardiovascular disease
  Cancer
  Infectious Diseases
  Inflammatory disease
- Clinical Applications
  Purification of drugs,
  Imaging the target
- Future Applications
  Fight against Bioterrorism





Example FDA approved therapeutic monoclonal antibodies[1]

Antibody	Brand name	Approval date	Type	Target	Indication (What it's approved to treat)
Abciximab	ReoPro	1994	chimeric	inhibition of glycoprotein IIb/IIIa	Cardiovascular disease
Adalimumab	Humira	2002	human	inhibition of TNF-α signaling	Several auto-immune disorders
Alemtuzumab	Campath	2001	humanized	CD52	Chronic lymphocytic leukemia
Basiliximab	Simulect	1998	chimeric	IL-2Rα receptor (CD25)	Transplant rejection
Bevacizumab	Avastin	2004	humanized	Vascular endothelial growth factor (VEGF)	Colorectal cancer, Age related macular degeneration
Cetuximab	Erbitux	2004	chimeric	epidermal growth factor receptor	Colorectal cancer, Head and neck cancer
Certolizumab pegol	Cimzia	2008	humanized	inhibition of TNF-α signaling	Crohn's disease
Daclizumab	Zenapax	1997	humanized	IL-2Rα receptor (CD25)	Transplant rejection
Eculizumab	Soliris	2007	humanized	Complement system protein C5	Paroxysmal nocturnal hemoglobinuria
Efalizumab	Raptiva	2002	humanized	CD11a	Psoriasis
Gemtuzumab	Mylotarg	2000	humanized	CD33	Acute myelogenous leukemia (with calicheamicin)
Ibritumomab tiuxetan	Zevalin	2002	murine	CD20	Non-Hodgkin lymphoma (with yttrium-90 or indium-111)

Infliximab	Remicade	1998	chimeric	inhibition of TNF-α signaling	Several autoimmune disorders
Muromonab-CD3	Orthoclone OKT3	1986	murine	T cell CD3 Receptor	Transplant rejection
Natalizumab	Tysabri	2006	humanized	alpha-4 (α4) integrin,	Multiple sclerosis and Crohn's disease
Omalizumab	Xolair	2004	humanized	immunoglobulin E (IgE)	mainly allergy-related asthma
Palivizumab	Synagis	1998	humanized	an epitope of the RSV F protein	Respiratory Syncytial Virus
Panitumumab	Vectibix	2006	human	epidermal growth factor receptor	Colorectal cancer
Ranibizumab	Lucentis	2006	humanized	Vascular endothelial growth factor A (VEGF-A)	Macular degeneration
Rituximab	Rituxan, Mabthera	1997	chimeric	CD20	Non-Hodgkin lymphoma
Tositumomab	Bexxar	2003	murine	CD20	Non-Hodgkin lymphoma
Trastuzumab	Herceptin	1998	humanized	ErbB2	Breast cancer

Main category	Type	Application	Mechanism/Target	Mode
Anti-inflammatory	**infliximab**[23]	■ rheumatoid arthritis ■ Crohn's disease ■ Ulcerative Colitis	inhibits TNF-α	chimeric
	adalimumab	■ rheumatoid arthritis ■ Crohn's disease ■ Ulcerative Colitis	inhibits TNF-α	human
	etanercept[23]	■ rheumatoid arthritis	Contains decoy TNF receptor	fusion protein
	basiliximab[23]	■ Acute rejection of kidney transplants	inhibits IL-2 on activated T cells	chimeric
	daclizumab[23]	■ Acute rejection of kidney transplants	inhibits IL-2 on activated T cells	humanized
	omalizumab	■ moderate-to-severe allergic asthma	inhibits human immunoglobulin E (IgE)	humanized

Anti-cancer	**gemtuzumab**[23]	■ relapsed acute myeloid leukaemia	targets myeloid cell surface antigen CD33 on leukemia cells	humanized
	alemtuzumab[23]	■ B cell leukemia	targets an antigen CD52 on T- and B-lymphocytes	humanized
	rituximab[23]	■ non-Hodgkin's lymphoma	targets phosphoprotein CD20 on B lymphocytes	chimeric
	trastuzumab	■ breast cancer with HER2/neu overexpression	targets the HER2/neu (erbB2) receptor	humanized
	nimotuzumab	■ Approved in squamous cell carcinomas, Glioma ■ Clinical trials for other indications underway	EGFR inhibitor	Humanized
	cetuximab	■ Approved in squamous cell carcinomas, colorectal carcinoma	EGFR inhibitor	Chimeric
	bevacizumab	■ Anti-angiogenic cancer therapy	inhibits VEGF	humanized

Other	**palivizumab**[23]	■ RSV infections in children	inhibits an RSV fusion (F) protein	humanized
	abciximab[23]	■ Prevent coagulation in coronary angioplasty	inhibits the receptor GpIIb/IIIa on platelets	chimeric

Side effects

more common side effects

- **Allergic reactions, such as hives or itching**
- **Flu-like symptoms, including chills, fatigue, fever, and muscle aches and pains**
- **Nausea**
- **Diarrhea**
- **Skin rashes**

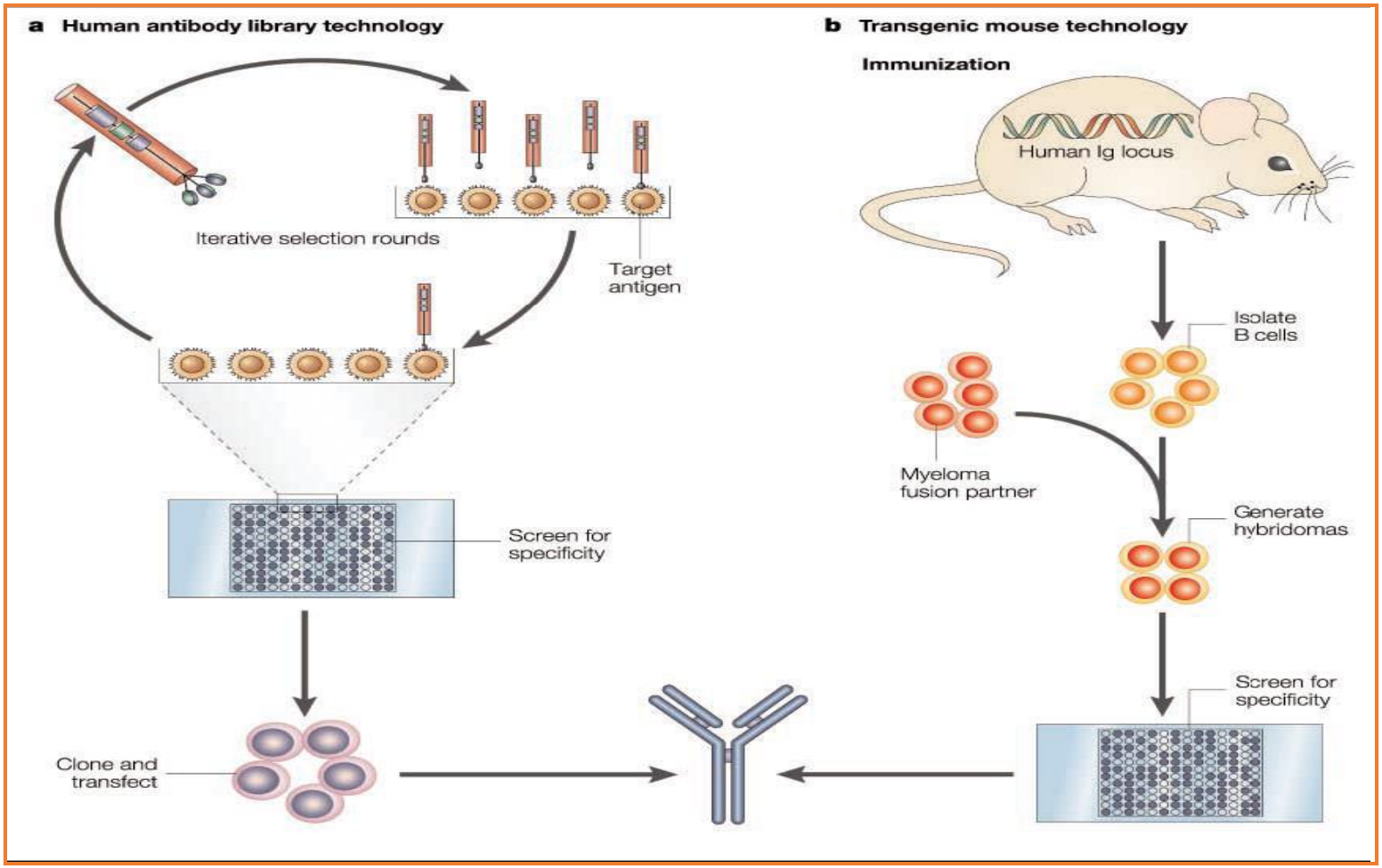
- Rare ---- more serious side effects
- Infusion reactions. Severe allergy-like reactions can occur and, in very few cases, lead to death
- Dangerously low blood cell counts. Decreased red blood cells, white blood cells and platelets
- Cardiac complications Certain monoclonal antibodies may cause heart failure and a small risk of MI
- Bleeding. Some of the monoclonal antibody drugs are designed to stop cancer from forming new blood vessels. There have been reports that these medications can cause bleeding.

Monoclonal antibodies for cancer treatment

Mechanisms that could be responsible for the cancer treatment

- ✓ **Binding to a critical receptor and blocking down stream signaling**
- ✓ **Down regulation of receptors**
- ✓ **Immunomodulation**
- ✓ **ADCC (Antibody-dependent Cell-Medicated Cytotoxicity)**

Production of Human Antibodies



Pharmaceutical Antibodies

- The fastest growing segment of the biopharmaceutical market
  - $14 billion in sales for 2005
  - Expected to grow to $30 billion by 2010
- Today, 20 therapeutic mAbs are on the market in the US
- However, an estimated 500 antibody-based therapies are currently under development

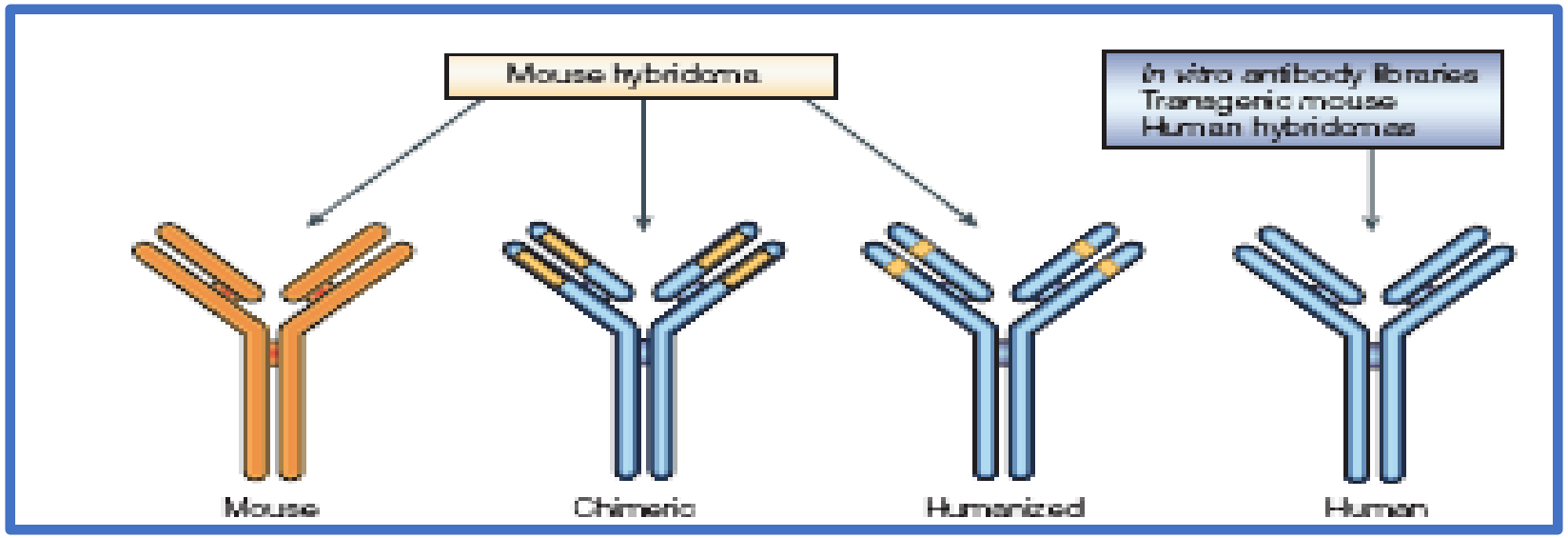
Nomenclature of Monoclonal Antibodies

Prefix	Target		Source		Suffix
variable	*-o(s)-*	bone	*-u-*	human	*-mab*
	-vi(r)-	viral	*-o-*	mouse	
	-ba(c)-	bacterial	*-a-*	rat	
	-li(m)-	immune	*-e-*	hamster	
	-le(s)-	infectious lesions	*-i-*	primate	
	-ci(r)-	cardiovascular	*-xi-*	chimeric	
	-mu(l)-	musculoskeletal	*-zu-*	humanized	
	-ki(n)-	interleukin	*-axo-*	rat/murine hybrid	
	-co(l)-	colonic tumor			
	-me(l)-	melanoma			
	-ma(r)-	mammary tumor			
	-go(t)-	testicular tumor			
	-go(v)-	ovarian tumor			
	-pr(o)-	prostate tumor			
	-tu(m)-	miscellaneous tumor			
	-neu(r)-	nervous system			
	-tox(a)-	toxin as target			

Autoimmune Disease

- An immune reaction against self
- Mechanism unknown, arises out of a failure in immune regulation
- Examples:
  - Rheumatoid arthritis
  - Systemic lupus erythematosus
  - Multiple sclerosis (MS)
  - Insulin-dependent diabetes mellitus
  - And the list goes on…

“Humanizing” Antibodies



- **Chimeric Antibodies**
  - **Murine Fv + human Fc**
  - **Human anti-chimeric antibodies (HACA) still observed**
- **Humanized Antibodies**
  - **Murine CDRs + human framework and Fc**

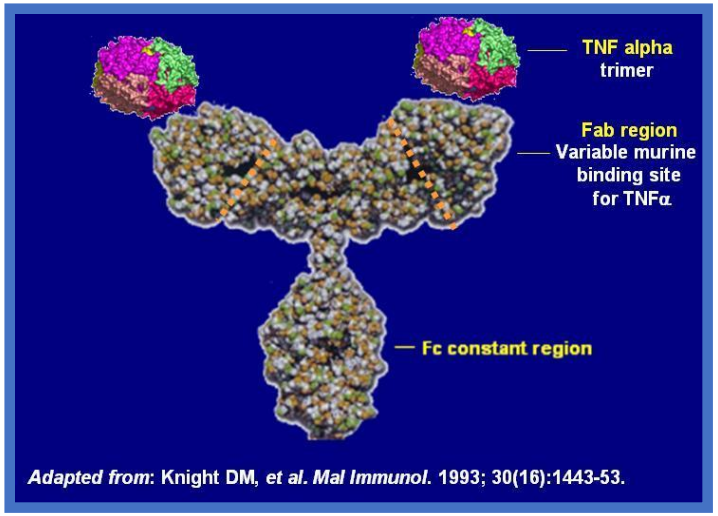
Rheumatoid Arthritis

- Chronic, autoimmune disease characterized by:
  - Severe joint inflammation
  - Increased synovial fluid and thickened synovial membrane
  - Destruction of bone and cartilage in several joints
  - Elevated levels of pro-inflammatory cytokines
    - TNF-α, IL-1, IL-6

- Affects 1% of the US population
- Women are 3 times more likely to develop
- If untreated for 2+ more years, irreversible damage occurs

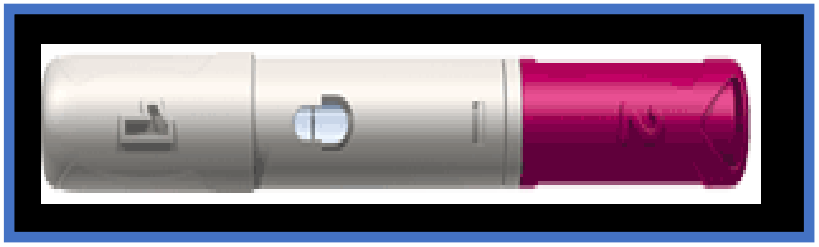
Infliximab

- Remicade® by Johnson & Johnson
- Chimeric mAb
- Anti TNF-α
- Approved by the FDA in 1998
- Administered intravenously
- Designated for use in patients who did not respond to methotrexate
- Proven to slow the clinical and radiological progression of rheumatoid arthritis



Adalimumab

- **Humira® by Abbott Laboratories**
- **Fully human IgG1 mAb**
- **Anti-TNF-α**
- **Approved by the FDA in 2002**
- **Available in 1 mL Humira pens and syringes for convenient use at home**



Rituximab

- Rituxan® by Genentech
- Anti-B cell (CD20) antibody
- First approved in 1997 for use in B-cell lymphoma
- Given in combination with Methotrexate
- Directed for patients who do not respond to Anti-TNF treatments
- Indicates the rheumatoid arthritis has a B cell component to its pathology

Interesting Variations

- **Small antibody fragments (Fv or Fab) are also effective in blocking cytokines**
  - **Benefit: More readily penetrate tissue**
- **Coupling of antibody fragments to form dimers and tetramers**
  - **Increases avidity and cross-linking**
- **Engineered Diabodies**
  - **Two different antigen specificities**
    - **One against the target**
    - **The other against effectors**
  - **Can cross-link effector cells**

Nanobodies

- 1989 - Raymond Hamers
- Discovered in camels
- Completely lack the light chain!
- Same antigen affinity as their four-chain counterparts
- Structure makes them more resistant to heat and pH
  - May lead to development of oral nanobody pills