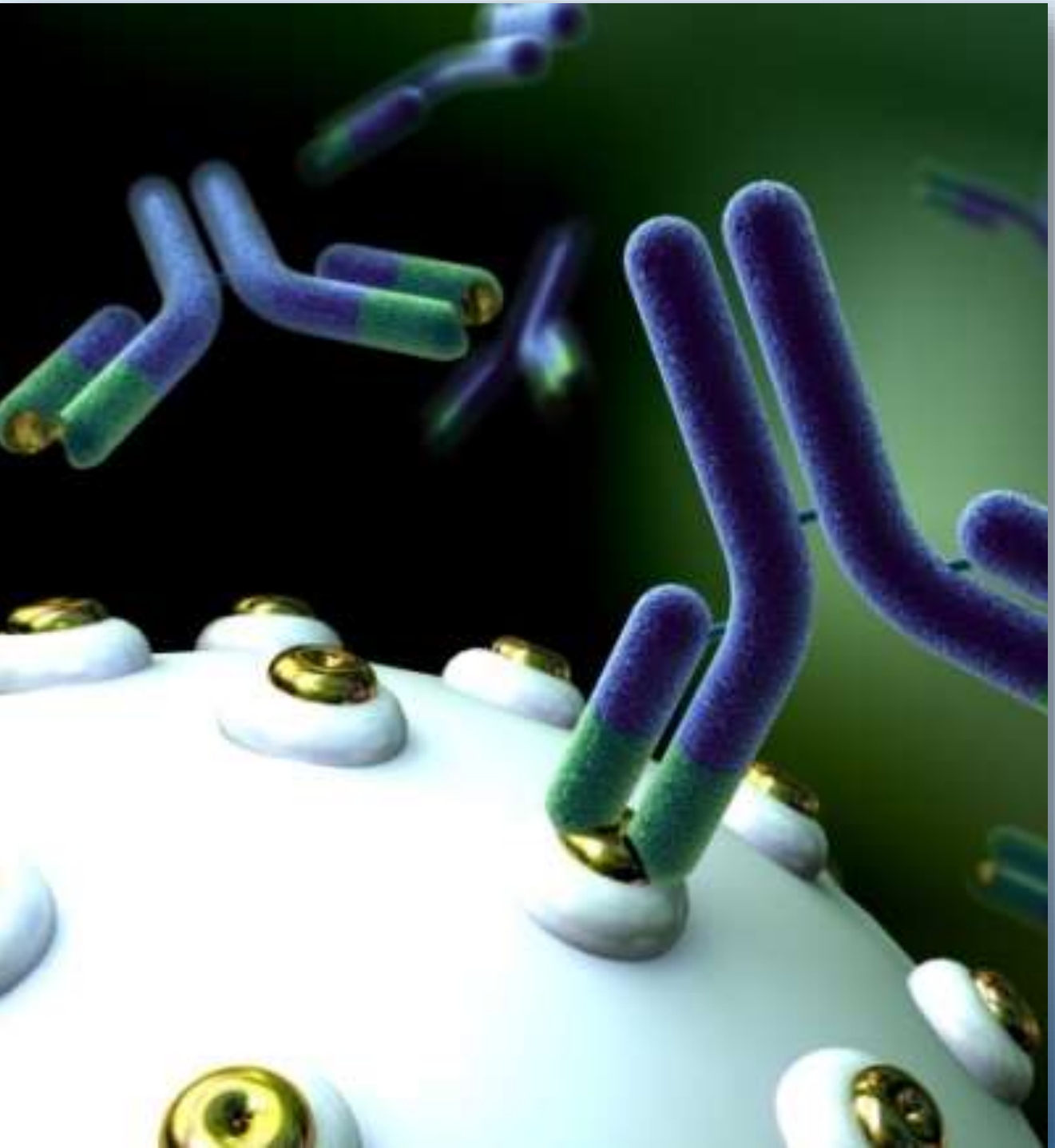


Application of monoclonal antibodies in medicine – in disease diagnosis and therapy

Types of antibodies/Monoclonal antibodies

Content

Antibodies (Immunoglobulins)

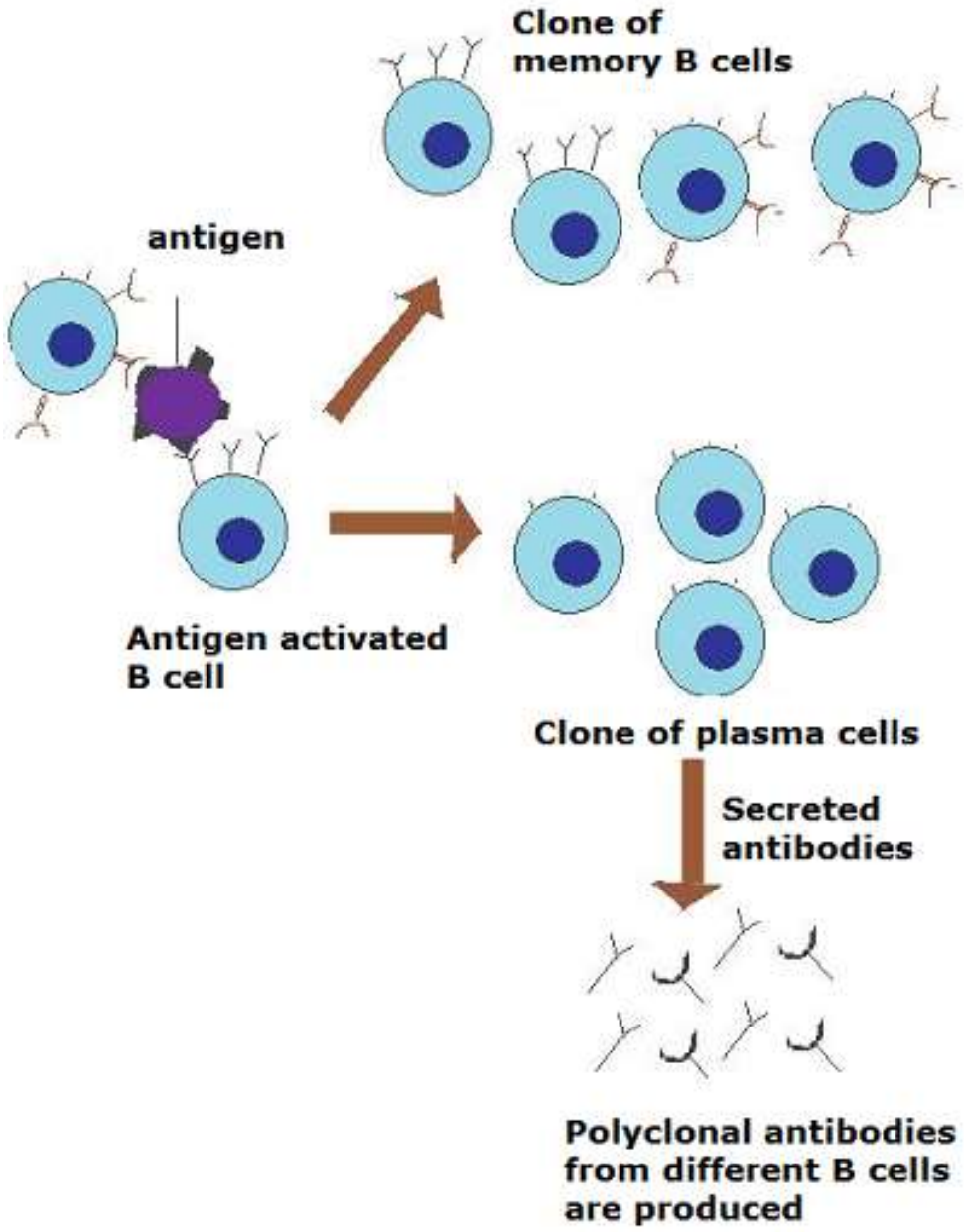
1. What is antibody
2. Antibody Structure
3. Antibody Functions
4. Antibody Classes:
   - IgA
   - IgG
   - IgM
   - IgD
   - IgE
5. Immunoglobulin analysis
6. Immunoglobulin therapy

Monoclonal Antibodies

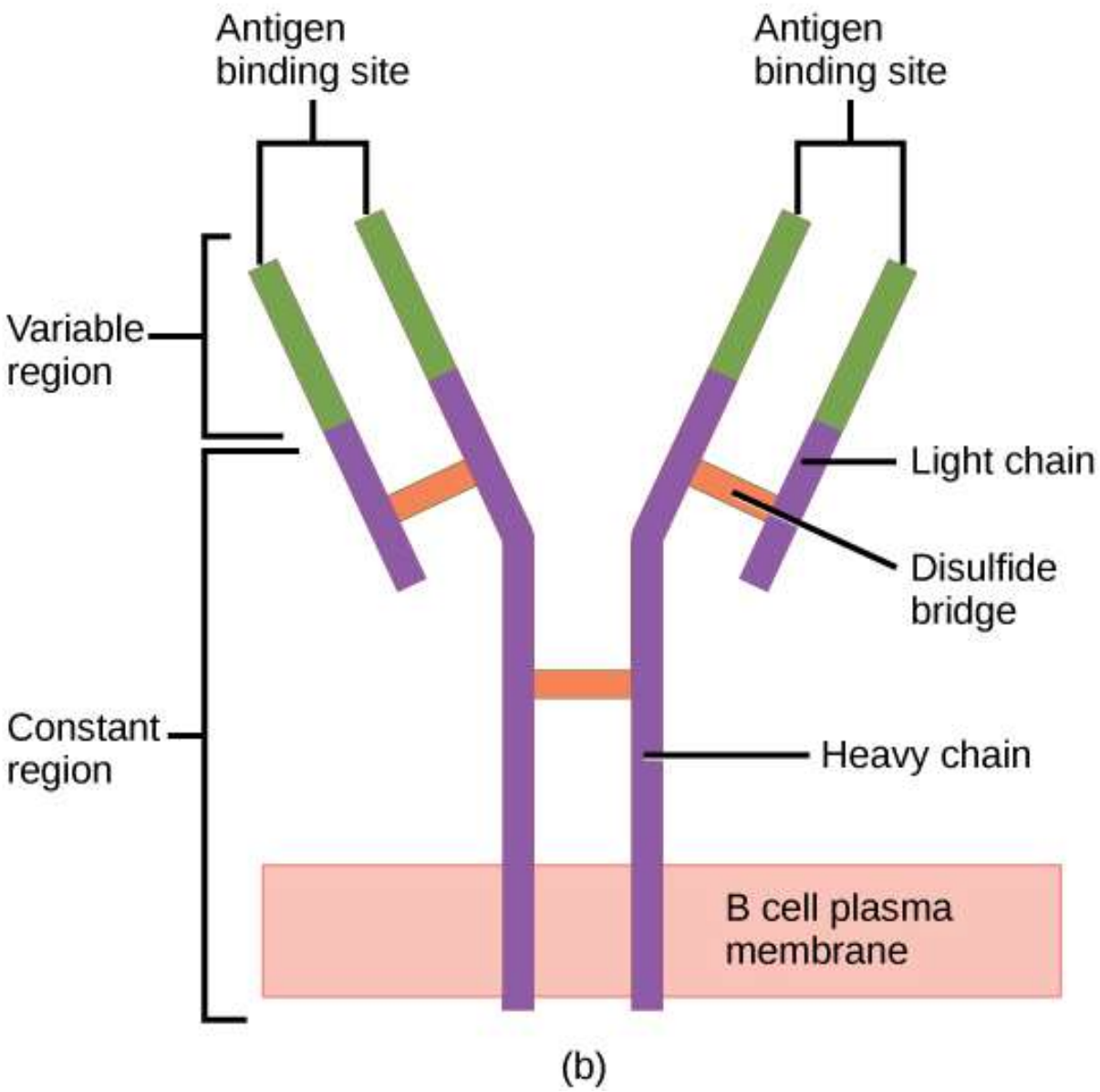
1. Polyclonal vs. Monoclonal Antibodies
2. Types of mAb:
   - Murine mAb
   - Chimera mAb
   - Humanized mAb
   - Human mAb

What is antibody

- ✓ An antibody, also known as an immunoglobulin (Ig), is a protein that is produced by plasma cells after stimulation by an antigen.
- ✓ Antibodies are the functional basis of humoral immunity.
- ✓ Antibodies occur in the blood, in gastric and mucus secretions, and in breast milk.
- ✓ Antibodies in these bodily fluids can bind pathogens and mark them for destruction by phagocytes before they can infect cells.



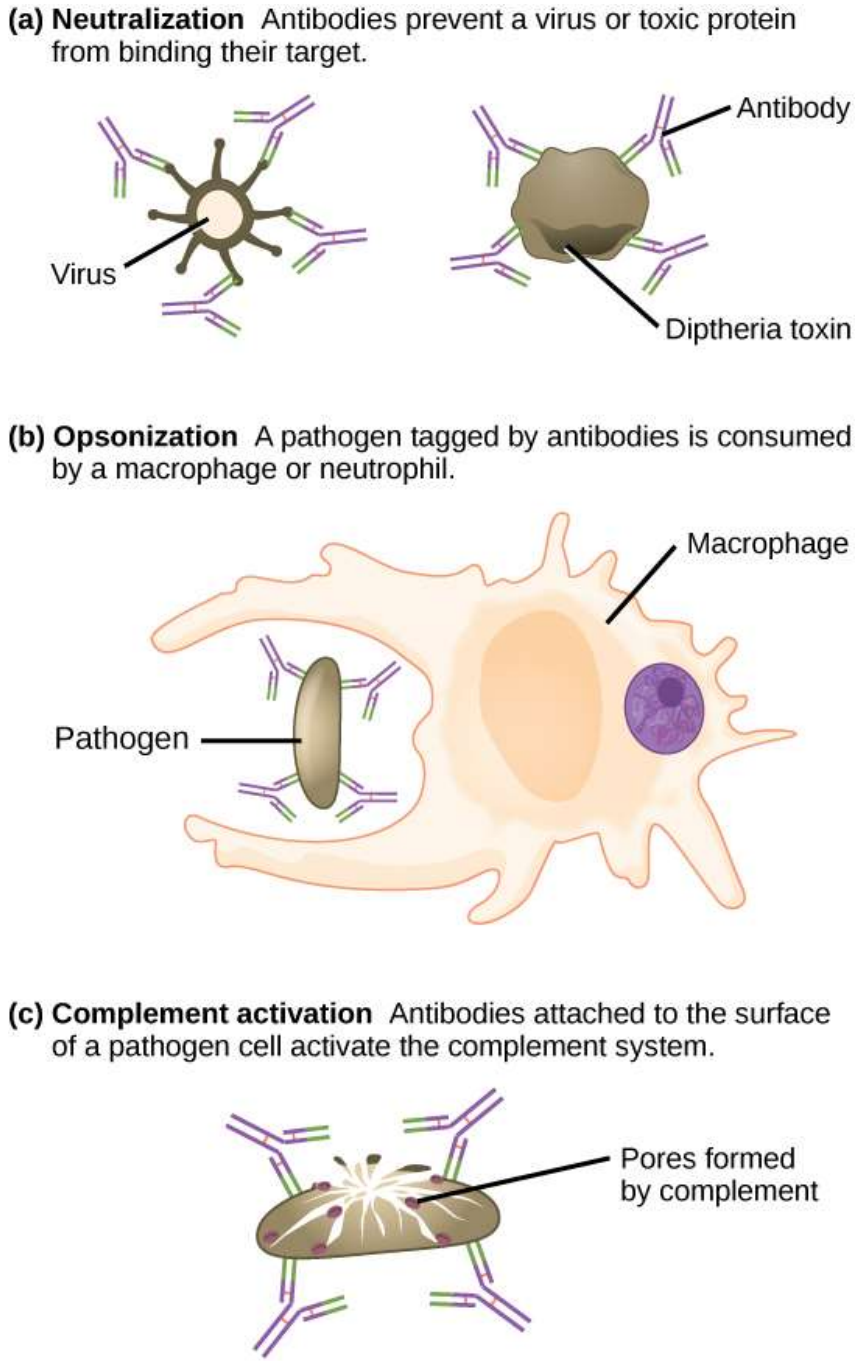
Antibody Structure



- ✓ **An antibody molecule is comprised of four polypeptides: two identical heavy chains (large peptide units) that are partially bound to each other in a "Y" formation, which are flanked by two identical light chains (small peptide units).**
- ✓ **Bonds between the cysteine amino acids in the antibody molecule attach the polypeptides to each other (Disulfide bridge).**
- ✓ **The areas where the antigen is recognized on the antibody are variable domains and the antibody base is composed of constant domains.**

Antibody Functions

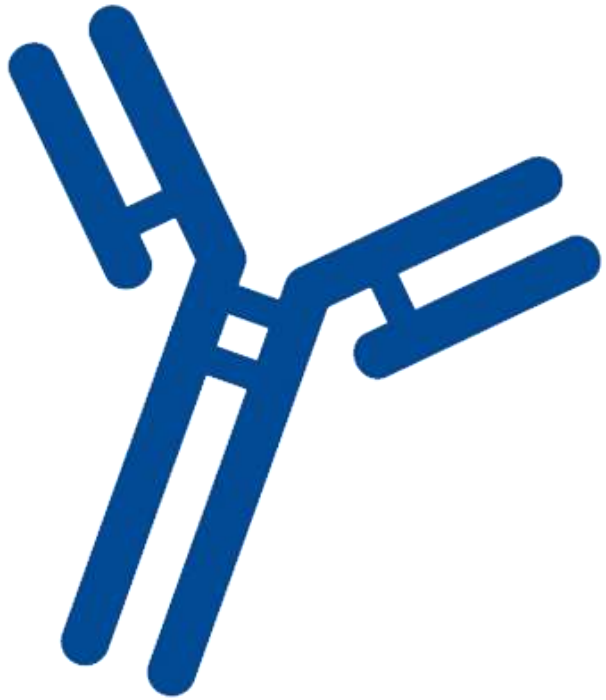
- ✓ **Differentiated plasma cells are crucial players in the humoral response, and the antibodies they secrete are particularly significant against extracellular pathogens and toxins.**
- ✓ **Antibodies circulate freely and act independently of plasma cells.**
- ✓ **Antibodies coat extracellular pathogens and neutralize them, by blocking key sites on the pathogen that enhance their.**
- ✓ **Antibody neutralization can prevent pathogens from entering and infecting host cells.**
- ✓ **Antibodies also mark pathogens for destruction by phagocytic cells.**



Antibody Classes

Antibodies can be divided into five classes based on their physiochemical, structural, and immunological properties:

Immunoglobulin-M (IgM)



Immunoglobulin-G (IgG)

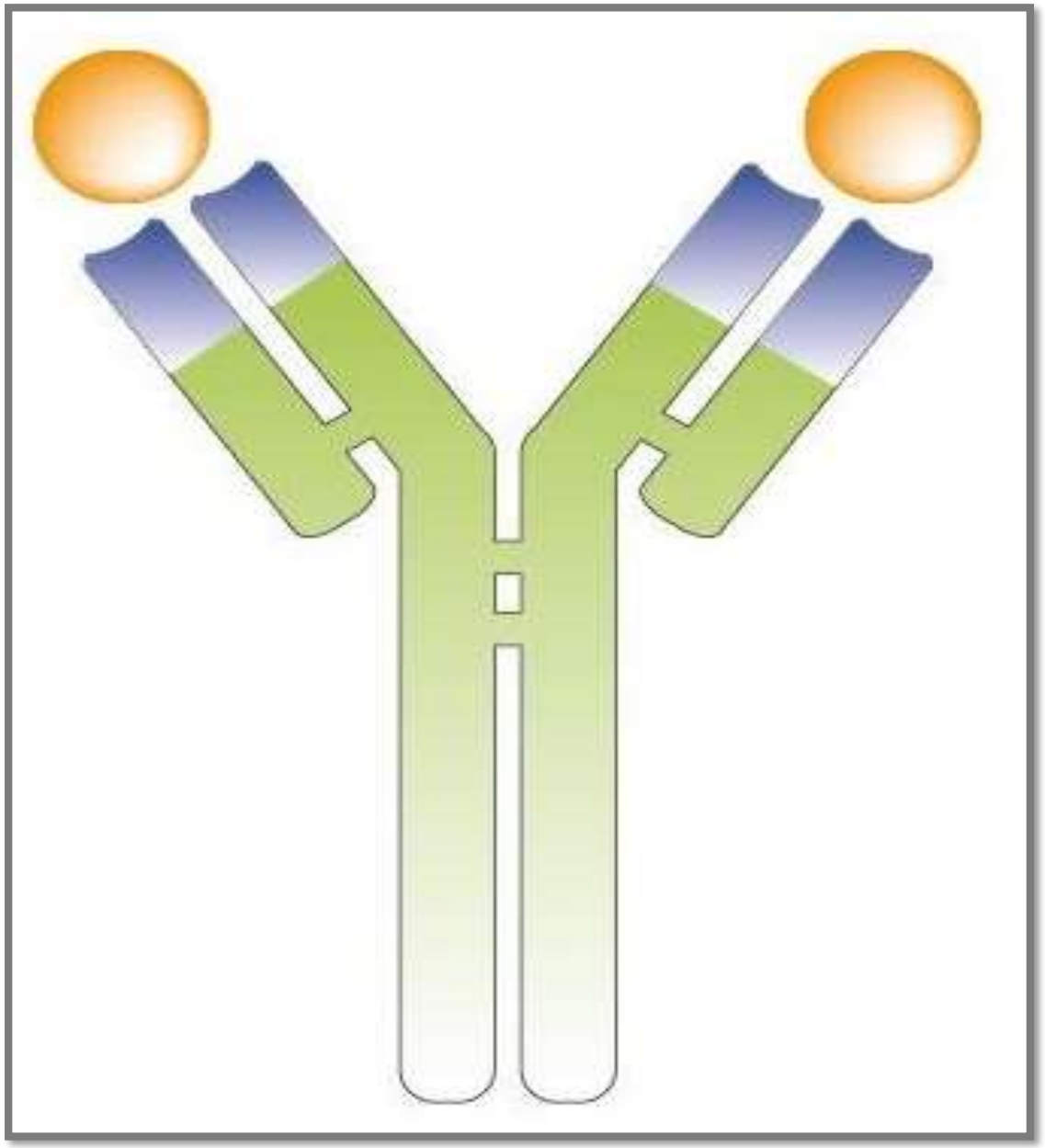
Immunoglobulin-A (IgA)

Immunoglobulin-D (IgD)

Immunoglobulin-E (IgE)

Immunoglobulin G

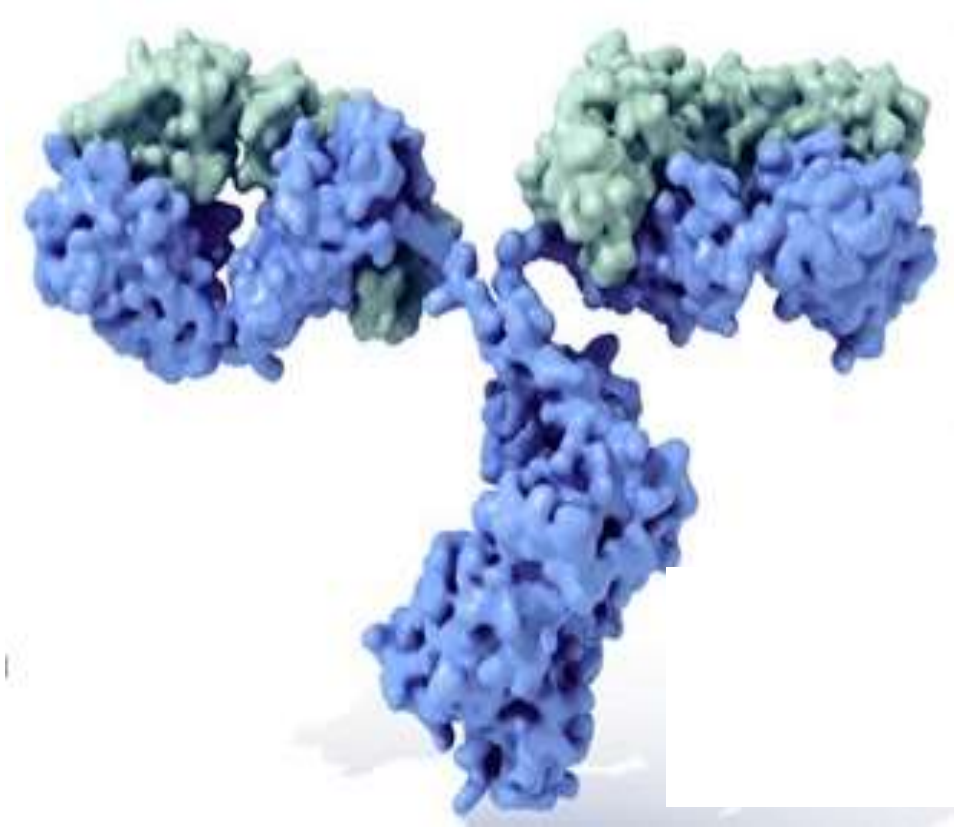
Immunoglobulin G (IgG) is a type of antibody. Each IgG has **two antigen binding sites**. Representing approximately 75% of serum antibodies in humans, IgG is the **most common type of antibody found in the circulation**. IgG molecules are created and released by plasma B cells.

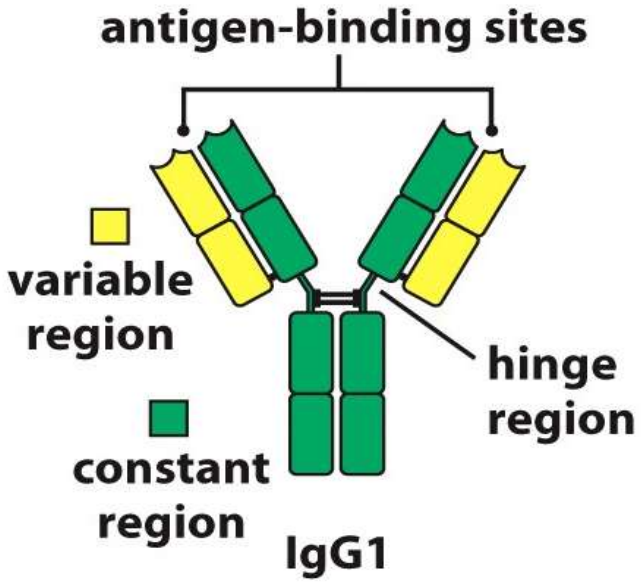


- **Fundamental roles:** Key player in the humoral immune response. Can activate the complement system. Phagocytosis of microorganisms.
- **Where found:** the major immunoglobulin in blood, lymph fluid, cerebrospinal fluid, and peritoneal fluid.

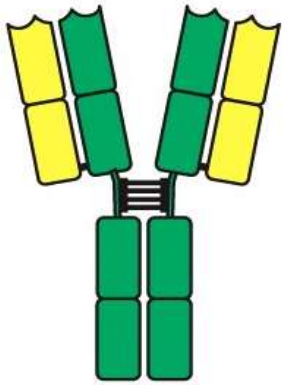
- **Reacts with:** macrophages, neutrophils, natural killer (NK) cells.

- **Presence in serum:** makes up approximately 15% of total proteins in healthy humans.

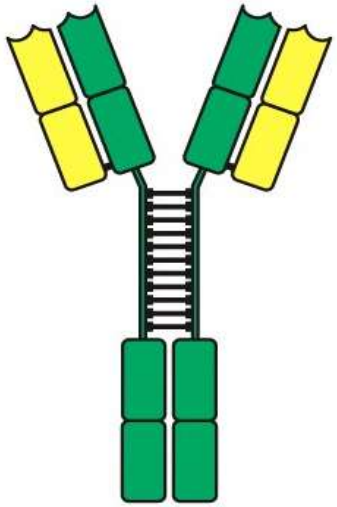




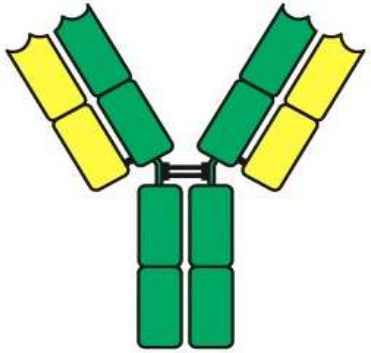
IgG1



IgG2



IgG3



IgG4

IgG1 comprises 60-65% of the total main subclass IgG, and is predominantly responsible for the thymus mediated immune response against proteins and polypeptide antigens. IgG1 immune response can already be measured in new borns and reaches its typical concentration in infancy. A deficiency in IgG1 isotype typically is a sign of a Hypogamma globulinemia.

IgG2, the second largest part of IgG isotypes, comprises 20-25% of the main subclass and is the prevalent immune response against carbohydrate antigens. “Adult” concentrations are reached around the age of 6-7. A deficiency in IgG2 is the most common one and associated with recurring airway/respiratory infections in infants.

IgG3 comprises around 5 - 10% of total igG and plays a major role in the immune responses against protein or polypeptide antigens. The affinity of IgG3 can be higher than that of IgG1.

Testing for IgG4 has been associated with food allergies in the past and recent studies have shown that elevated serum levels of IgG4 are found in patients suffering from sclerosing pancreatitis, cholangitis and interstitial pneumonia caused by infiltrating IgG4 positive plasma cells.

Immunoglobulin-A

IgA is an antibody that plays a crucial role in the immune function of mucous membranes. The amount of IgA produced in association with mucosal membranes is greater than all other types of antibody combined.

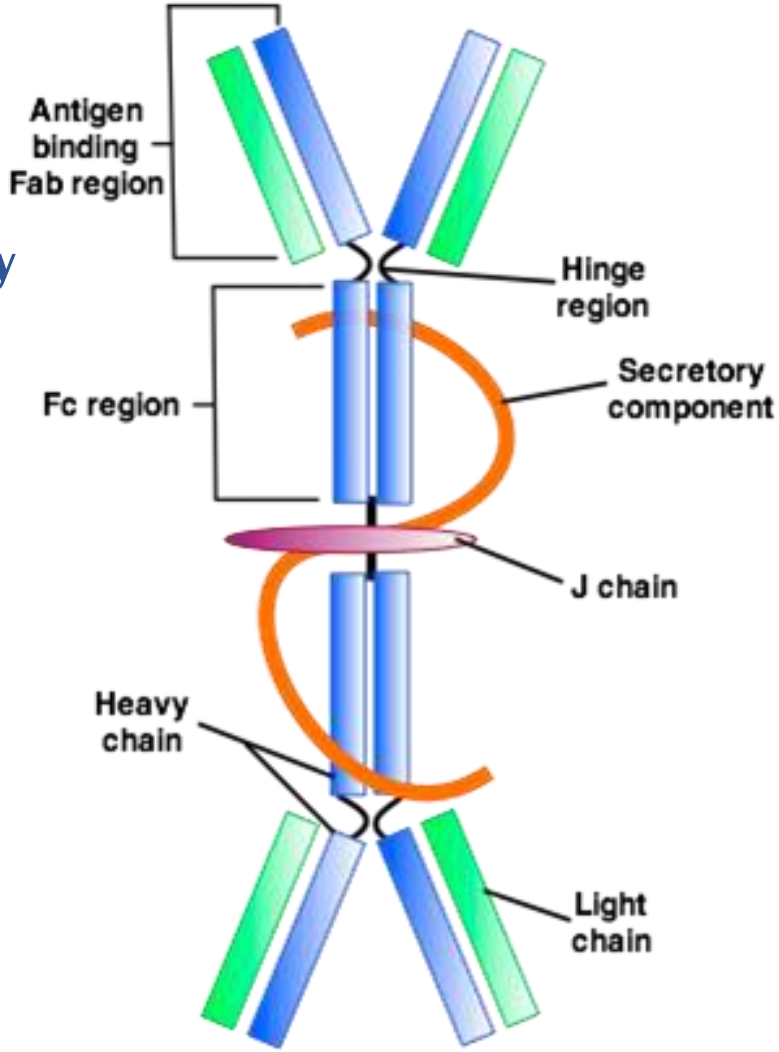
This represents up to 15% of total immunoglobulins produced throughout the body.

The IgA dimeric form is the most prevalent and is also called secretory IgA (sIgA).

The secretory component of sIgA protects the immunoglobulin from being degraded by proteolytic enzymes.

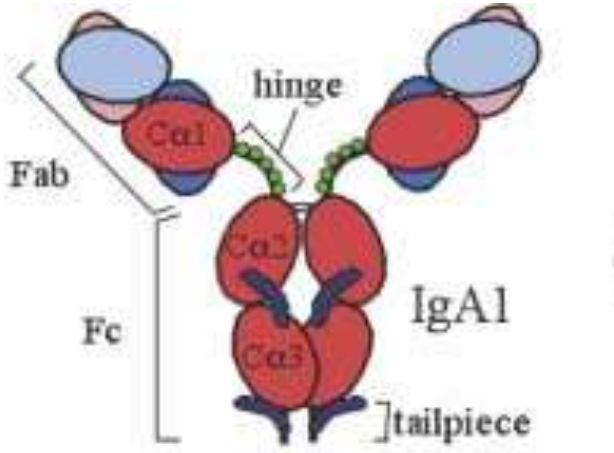
Immunoglobulin-A can be found in mucous secretions:

- tears
- saliva
- sweat
- colostrum
- gastrointestinal tract
- prostate
- respiratory epithelium



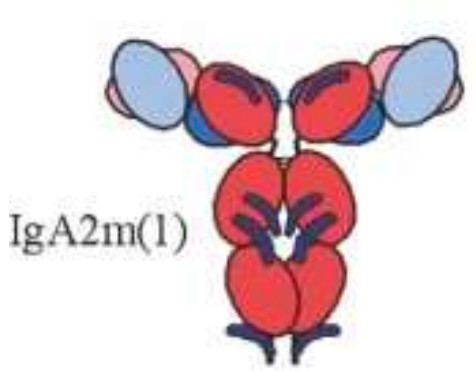
IgA1

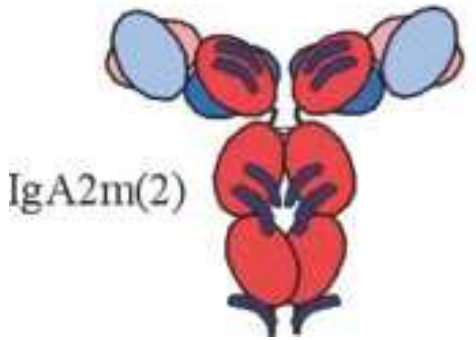
IgA1 is the predominant IgA subclass found in serum. Most lymphoid tissues have a predominance of IgA1-producing cells.



IgA2

In IgA2, the heavy and light chains are not linked with disulfide, but with noncovalent bonds. In secretory lymphoid tissues (e.g., gut-associated lymphoid tissue), the share of IgA2 production is larger than in the non-secretory lymphoid organs (e.g. spleen, peripheral lymph nodes).





Both IgA1 and IgA2 have been found in external secretions like colostrum, maternal milk, tears and saliva, where IgA2 is more prominent than in the blood. Polysaccharide antigens tend to induce more IgA2 than protein antigens.

Both IgA1 and IgA2 can be in membrane-bound form.

Pathology

Genetic:

Decreased or absent IgA due to an inherited inability to produce IgA is termed selective IgA deficiency and can produce a clinically significant immunodeficiency.

Microbial:

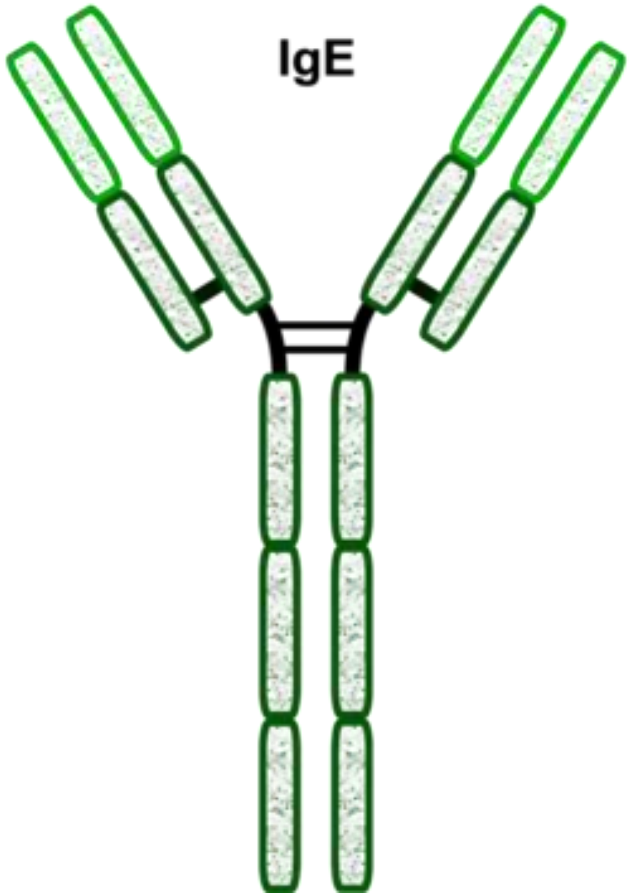
Neisseria species including *Neisseria gonorrhœae* (which causes gonorrhea), *Streptococcus pneumoniae*, and *Haemophilus influenzae* type B all release a protease that destroys IgA. Additionally, Blastocystis spp. has been shown to have several subtypes (ST) that generate cysteine and aspartic protease enzymes which degrade human IgA

Autoimmune and immune-mediated:

IgA nephropathy is caused by IgA deposits in the kidneys. It is not yet known why IgA deposits occur in this chronic disease.

Henoch–Schönlein purpura (HSP) is a systemic disorder caused by deposits of IgA in the small vessels.

Immunoglobulin-E

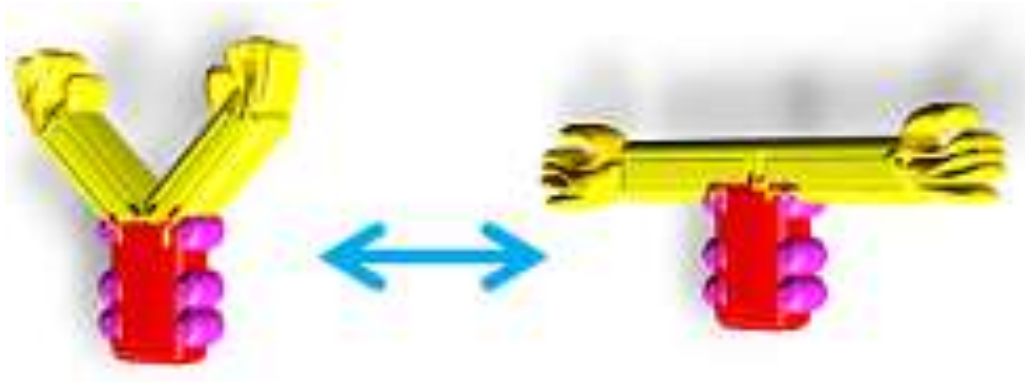


- **Immunoglobulin E (IgE) is a type of antibody that has only been found in mammals.**
- **IgE's main function is immunity to parasites such as helminths like Schistosoma mansoni, Trichinella spiralis, and Fasciola hepatica.**
- **IgE is utilized during immune defense against certain protozoan parasites such as Plasmodium falciparum.**

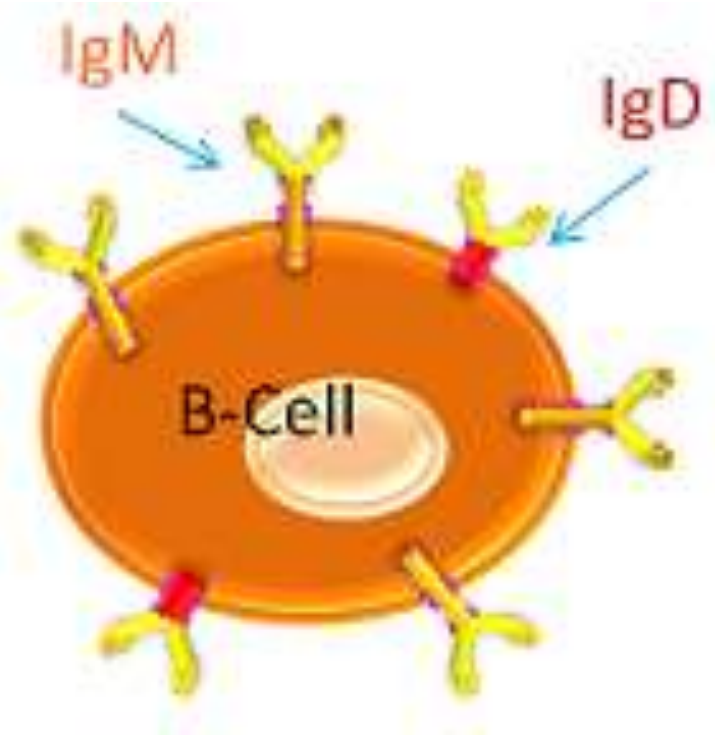
The observed higher serum IgE concentration in patients with IHD may serve as evidence contribution to atherogenesis and myocardial ischemia.

Immunoglobulin-D

- IgD named 'D' because it was distinct from the other three Igs known at that time. It turns out that D was a good name for this antibody because IgD is also very diverse. IgD is a very mysterious antibody.



- At first, scientists thought it had a Y-shaped structure, like all other, but experiments indicate that IgD can also adapt a T-shaped structure.



- When B-cells mature, they not only have IgM, but also exhibit IgD on their surfaces; this is called co-expression.

- In healthy people, very little IgD is secreted.

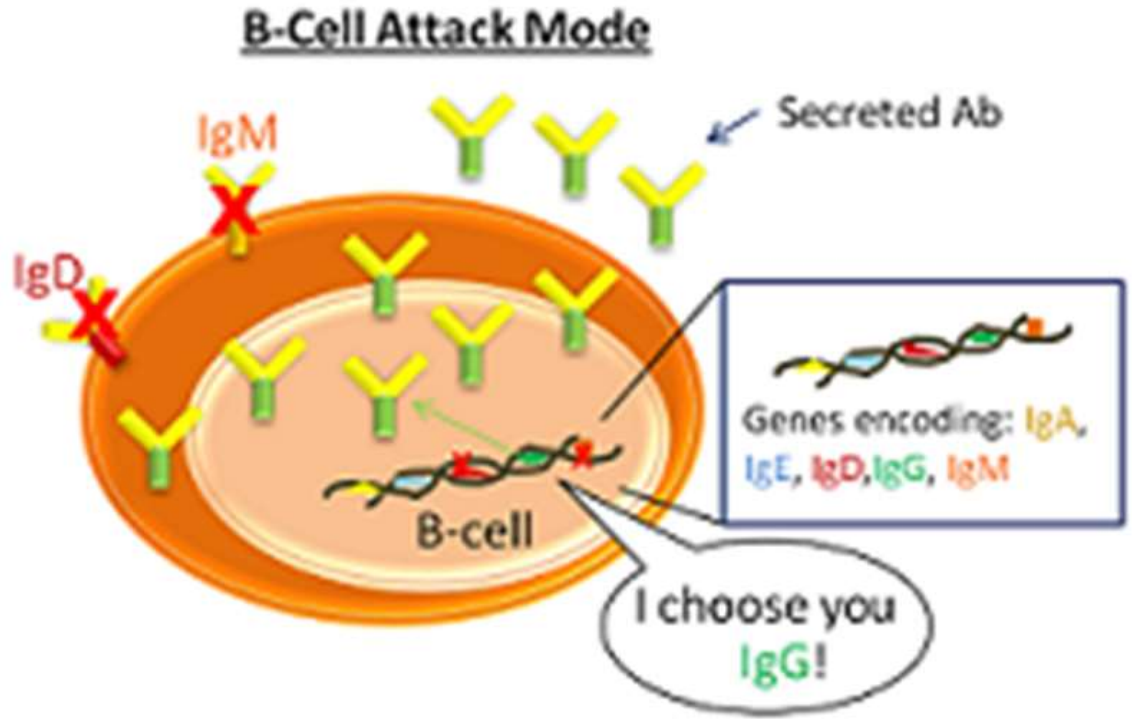
Function of IgD

- Only rarely do B-cells choose to make, express or secrete only IgD when in battle mode. Why is this? Good question because the exact functions of IgD are still being uncovered.

- In B cells, IgD's function is to signal the B cells to be activated. To be ready to defense the body in the immune system.

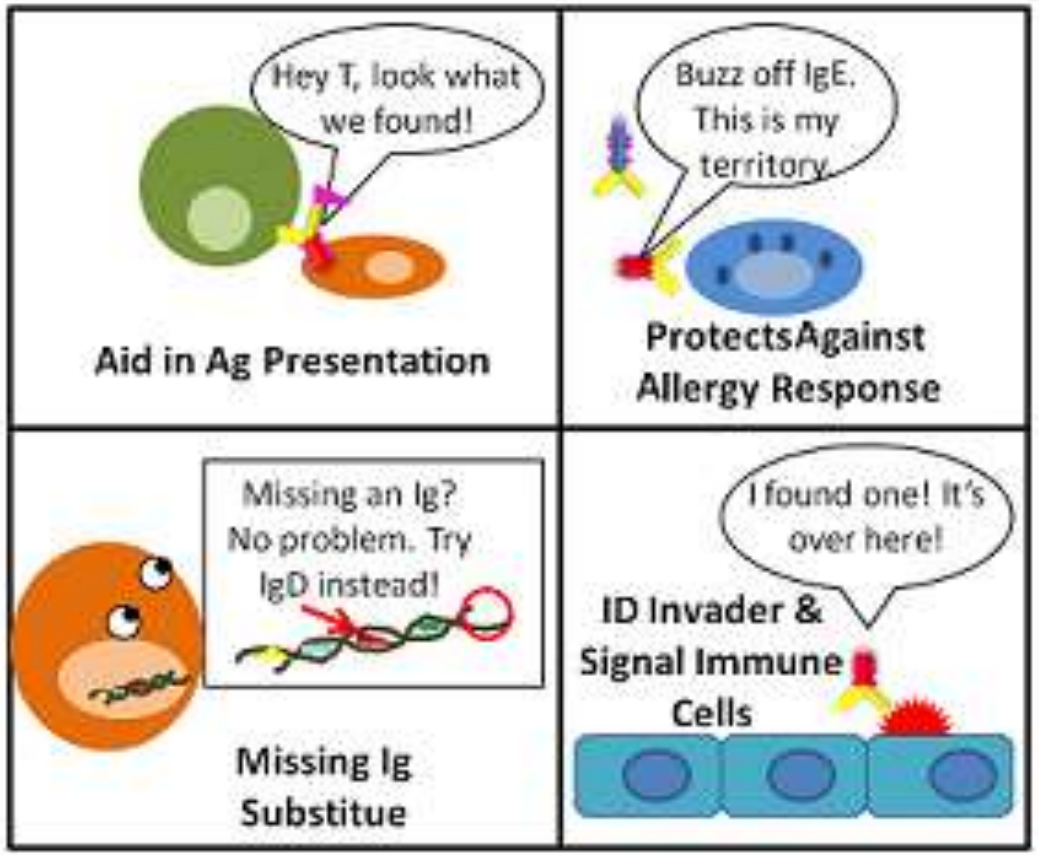
- Recently, IgD was found to bind to basophils/mast cells and activate these cells to produce antimicrobial factors to participate in respiratory immune defense in humans.

- In some studies, patients with human immunodeficiency virus (HIV) and little or no symptoms were found to have high levels of IgD.



IgD	
Heavy chain	δ
Light chain	κ or λ
Molecular formula	$\delta_2\kappa_2$ or $\delta_2\lambda_2$
Valency	2
Structure	Monomer
Abundance in serum (in relation to total immunoglobulins present)	0,2 %
Carbohydrate content	13 %
Concentration in serum	0 - 0.4 mg/ml
IgE subclasses	None
Heavy chain MW (kDa)	62
Light chain MW (kDa)	23
Total MW (kDa)	180
Distribution	Lymphocyte surface
Function	Unclear

Other Suspected Functions of IgD:



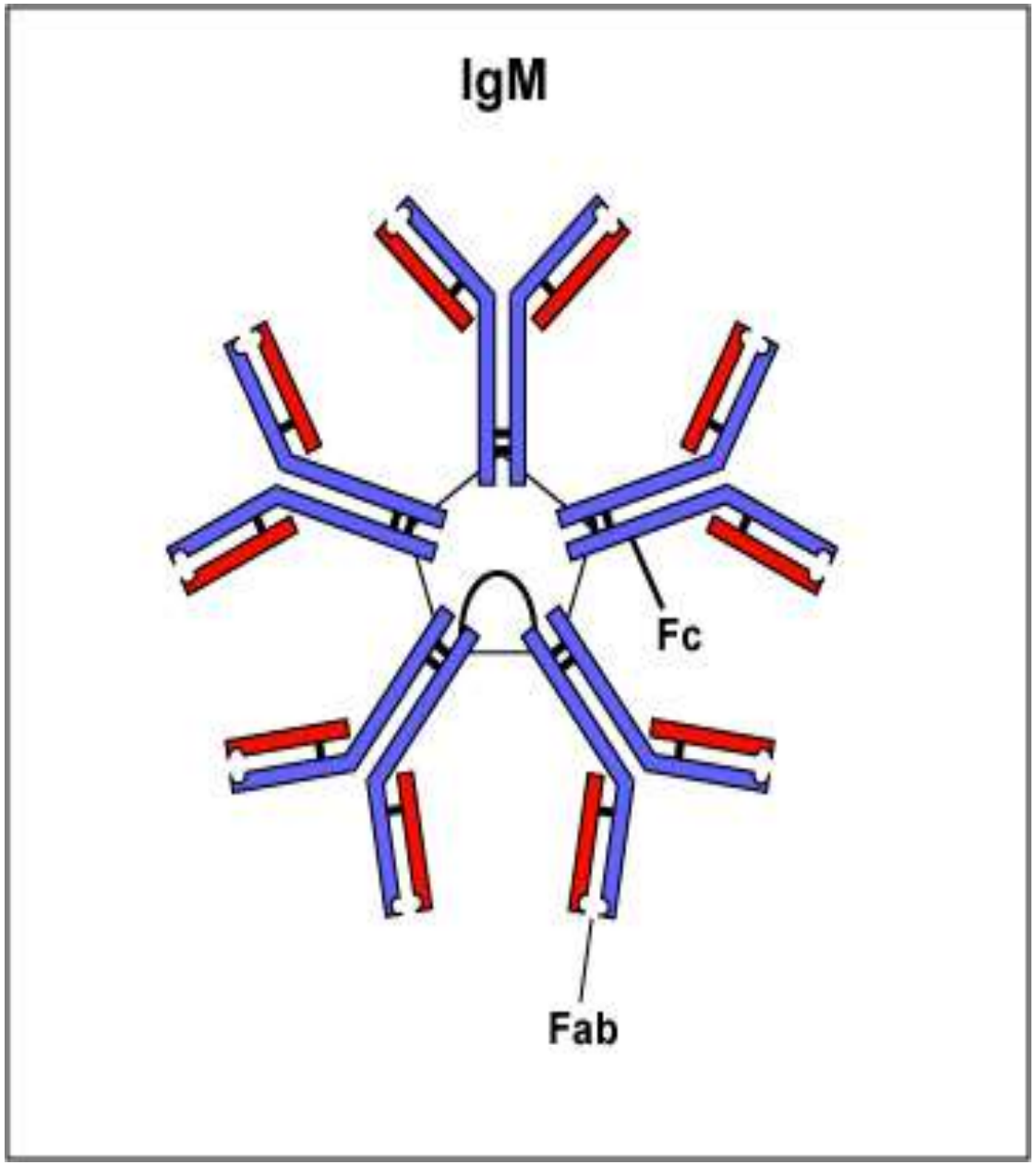
Immunoglobulin-M

Feature	IgM Antibody
Size	970 kDa
Immunoglobulin Structure	Pentameric (monomeric as B-cell receptor)
Location	Lymph and Blood
Binding Sites	10
Immune Function	• Innate and Adaptive • Agglutinates • Fixes complement C3b

- **IgM is a polymer, where multiple immunoglobulins are linked together by strong covalent bonds known as disulfide bonds. This occurs mostly to produce pentamers (5 linked immunoglobulins) but also as a hexamer (six linked immunoglobulins).**

- **IgM has a molecular mass of approximately 970 kDa (in its pentamer form). Because each immunoglobulin monomer has two antigen binding sites, a pentameric IgM has 10 binding sites.**

- **Typically, however, IgM cannot bind 10 antigens at the same time because the large size of most antigens hinders binding to nearby sites.**



- **During infection, innate or “natural immunity” is provided by poly-reactive IgM antibody made by (B1a) B cells. IgM antibody acts to quickly recognize and initiate an immune response by directly neutralizing pathogens or clearing novel antigens.**

- **The three components of the IgM antibody-mediated immune response are activation of complement (C1qR and Fcα/μR), recruitment of phagocytic cells, and opsonization. Current research suggests that B1b B cells which make IgM antibodies may provide memory to certain pathogens and support T-cell independent immune responses.**

- **IgM antibody also acts as an educator of the immune system by transporting antigens to lymph tissues where memory is induced.**

Applications

- The IgM class of antibodies recognizes a large variety of pathogenic antigens and is highly active in cytotoxic and cytolytic reactions due to its superior activation of the complement system. IgM antibody has applications in Antibody Drug Discovery.

- IgM antibody can make a good biologic against cancer owing to its strong avidity as well as complement fixation property.

- For certain targets, particularly those such as glycol-epitopes on cancer cells, an IgM antibody might be the best approach.

- An IgM antibody might also be useful as a vaccine adjuvant, acting as soluble toll-like receptors through the formation of immune complexes with antigen.
- It has been suggested to use IgMs as preventive vaccine.

- The innate ability of IgM to mediate autoimmunity can be utilized to ameliorate graft rejections during transplantation procedures.

- The inflammatory control IgM antibody provides systemic exposure to self antigens could be harnessed to treat arthritis.

Immunoglobulin analysis

A test for quantitative immunoglobulins (Igs) is used to detect an excess or deficiency in the three major classes of immunoglobulins (IgG, IgA, and IgM). It gives important information about the health of an individual's immune system and is used to help diagnose various conditions and diseases that affect the levels of one or more of these Ig classes.

In general, immunoglobulin disorders can be classified as:

Immunoglobulin excess:

- Polyclonal: Excess is the sum of immunoglobulins from many different immune (plasma) cells
- Monoclonal: Excess immunoglobulins are from the clones of one plasma cell

Immunoglobulin deficiency:

- Secondary (acquired)—the most common are caused by an underlying condition or contributing factor
- Primary (inherited)—rare disorders in which the body is not able to produce one or more classes of immunoglobulins

Examples of conditions that may cause increased immunoglobulins:

Immunoglobulin Result	Associated Conditions
Polyclonal increase in any or all of the three classes (IgG, IgA and/or IgM)	•Infections, acute and chronic •Autoimmune disorders (rheumatoid arthritis, systemic lupus erythematosus, scleroderma) •Cirrhosis •Chronic inflammation, inflammatory disorders •Hyperimmunization reactions •Wiskott-Aldrich syndrome •In a newborn, infection during pregnancy (congenital— syphilis, toxoplasmosis, rubella, CMV)
Monoclonal increase in one class with or without decrease in other two classes	•Multiple myeloma •Chronic lymphocytic leukemia (CLL) •MGUS (monoclonal gammopathy of undetermined significance) •Lymphoma •Waldenstrom's macroglobulinemia (IgM)

Some of the common causes of low levels of immunoglobulins:

Conditions/factors that affect immunoglobulin production	**•Drugs such as phenytoin, carbamazepine, immunosuppressant drugs** **•Complications from conditions such as kidney failure or diabetes** **•Transient delay in production in newborns, particularly premature infants**
Conditions that cause an abnormal loss of protein	**•Nephrotic syndrome—kidney disease in which protein is lost in the urine** **•Burns** **•Protein-losing enteropathy—any condition of the gastrointestinal tract that affects the digestion or absorption of protein**

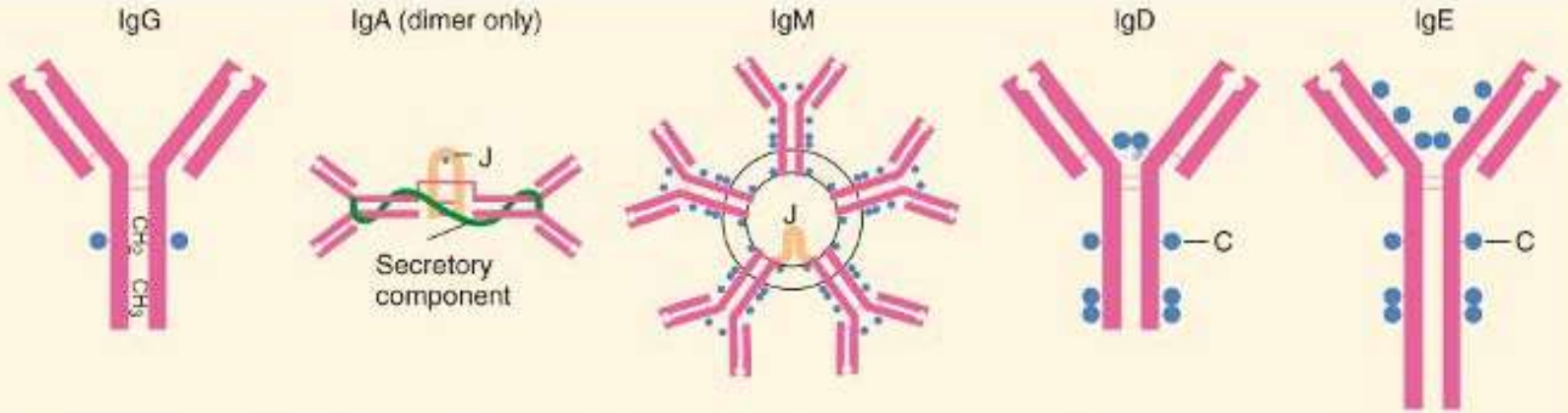
Immunoglobulin therapy

Immunoglobulin therapy, also known as normal human immunoglobulin (NHIG), is the use of a mixture of antibodies (immunoglobulins) to treat a number of health conditions.

These conditions include:
primary immunodeficiency, idiopathic thrombocytopenic purpura, chronic inflammatory demyelinating polyneuropathy, Kawasaki disease, certain cases of HIV/AIDS.

Depending on the formulation it can be given by injection into muscle, a vein, or under the skin. The effects last a few weeks.

Common side effects include pain at the site of injection, muscle pain, and allergic reactions.



	Monomer	Dimer, Monomer	Pentamer	Monomer	Monomer
Number of Antigen Binding Sites	2	4 2	10	2	2
Molecular Weight	150,000	170,000–385,000	900,000	180,000	200,000
Percentage of Total Antibody in Serum	80%	13%	6%	1%	0.002%
Average Half-Life in Serum (Days)	23	6	5	3	2.5
Crosses Placenta?	Yes	No	No	No	No
Fixes Complement?	Yes	No	Yes	No	No
Fc Binds To	Phagocytes				Mast cells and basophils
Biological Function	Long-term immunity; memory antibodies; neutralizes toxins, opsonizes, fixes complement	Secretory antibody; on mucous membranes	Produced at first response to antigen; can serve as B-cell receptor	Receptor on B cells	Antibody of allergy; worm infections

C = carbohydrate. J = J chain.

Summary

Antibodies

Polyclonal vs. Monoclonal

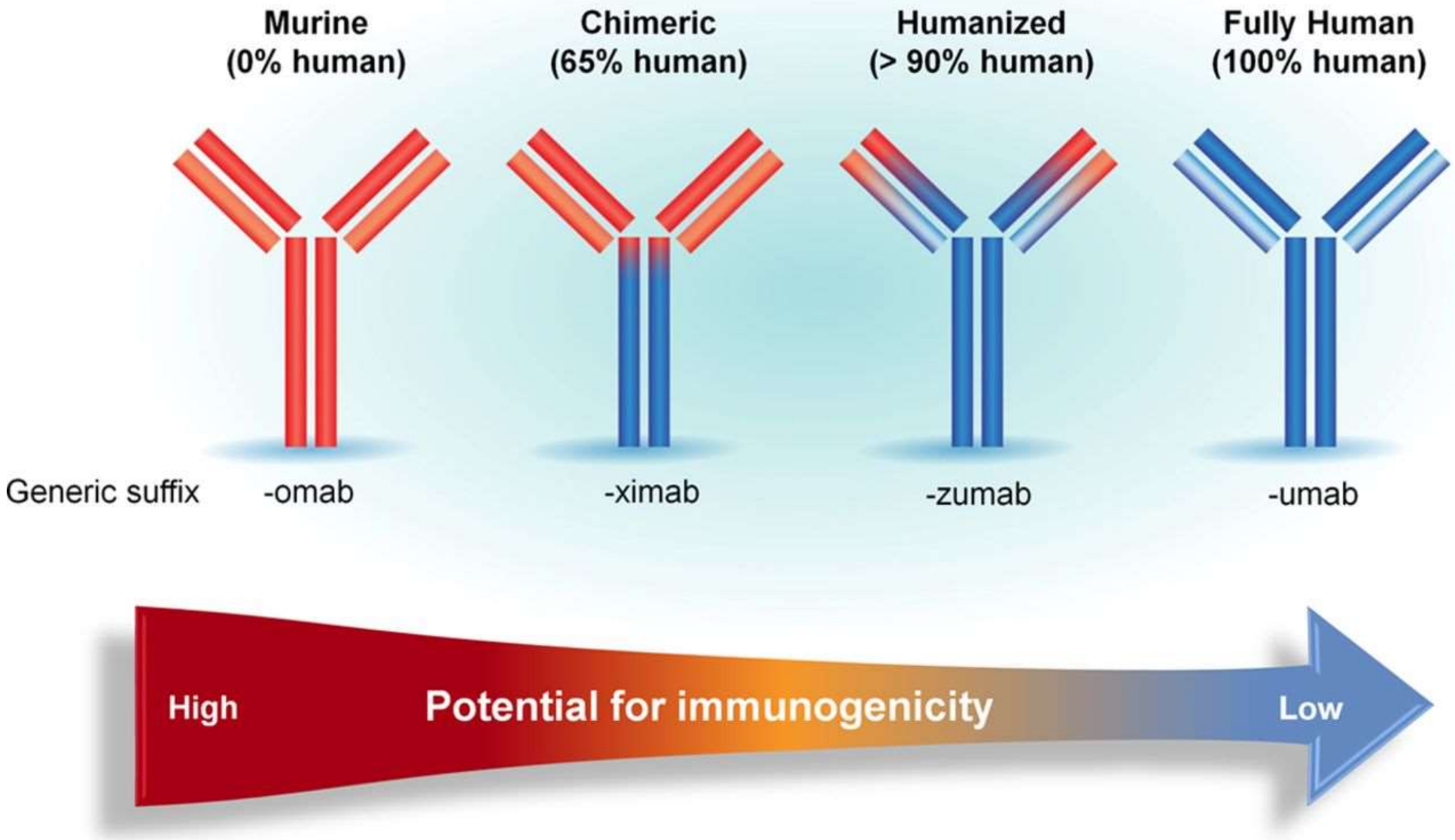
Polyclonal:

- Cheap to produce
- Mixed population of antibodies
- May bind to different areas of target molecule
- Tolerant of small changes in protein structure (denaturation, dimerisation, phosphorylation)

Monoclonal:

- Expensive to produce
- Single antibody species
- Will only bind single specific site
- May only recognise a particular protein form (phosphorylation, dimersied)
- Infinitely renewable Polyclonal antibodies Monoclonal antibodies

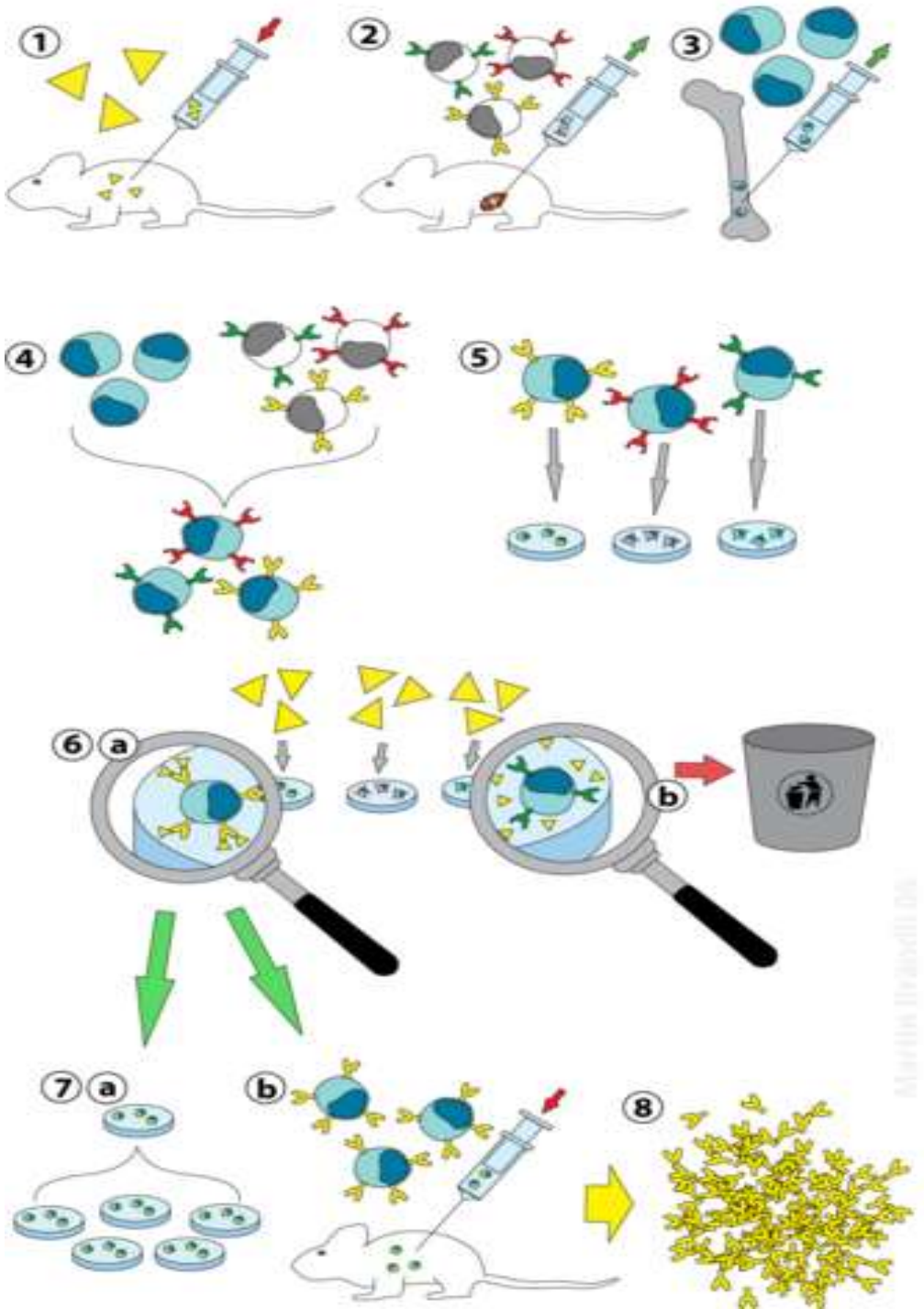
Types of monoclonal antibodies and their efficiency



Murine monoclonal Antibodies

Hybridomas are immortalized cells derived from the fusion of B lymphoblasts with a myeloma fusion partner.

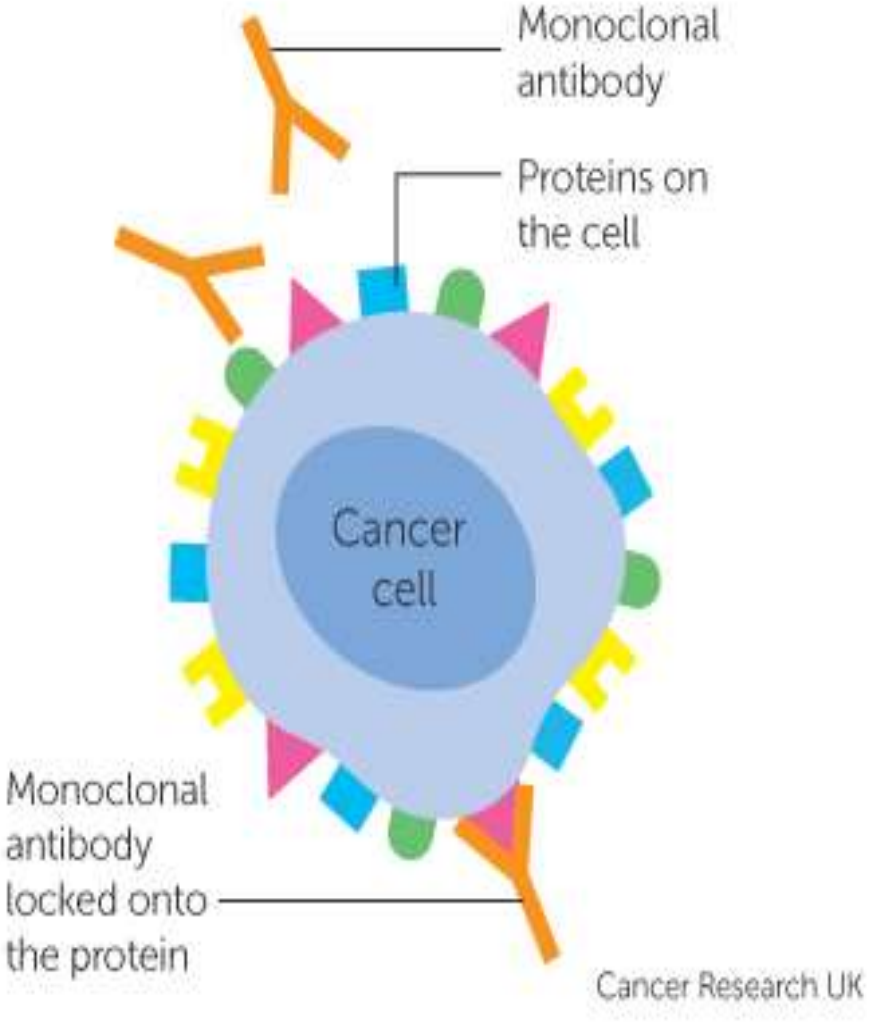
1. **Immunisation of a mouse**
2. **Isolation of B cells from the spleen**
3. **Cultivation of myeloma cells**
4. **Fusion of myeloma and B cells**
5. **Separation of cell lines**
6. **Screening of suitable cell lines**
7. **in vitro (a) or in vivo (b) multiplication**
8. **Harvesting**



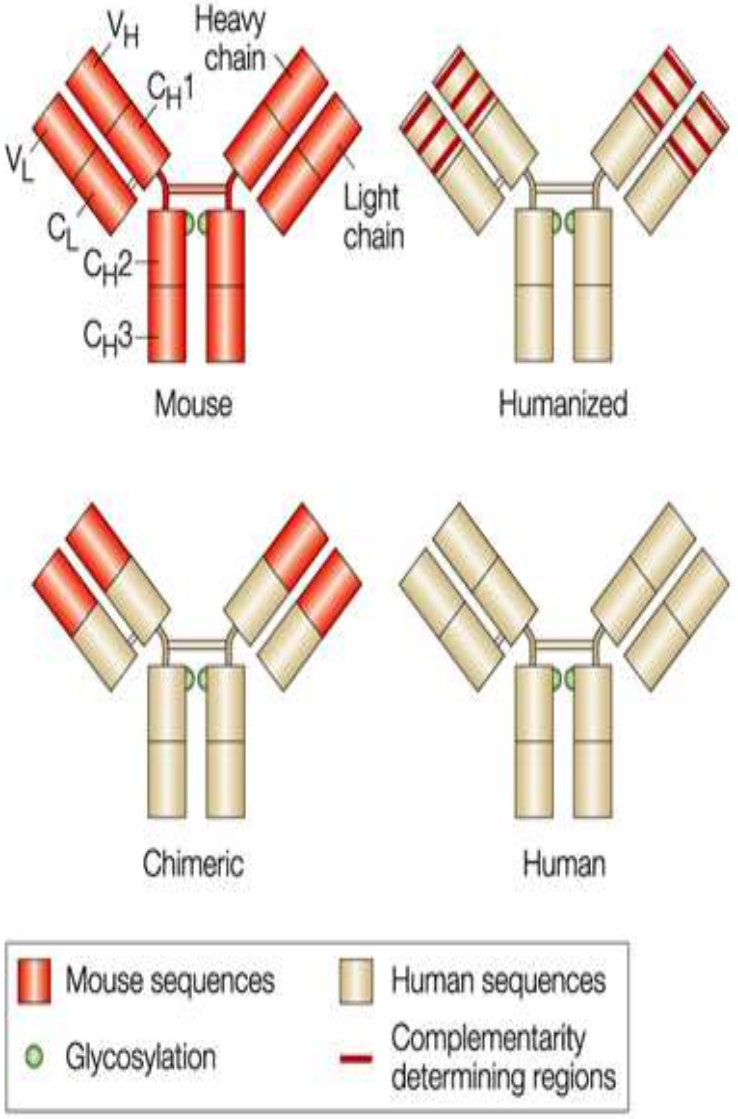
- These injections are typically followed by the use of in vivo electroporation, which significantly enhances the immune response.
- The fusion of the B cells with myeloma cells can be done using electrofusion.
- Electrofusion causes the B cells and myeloma cells to align and fuse with the application of an electric field.
- Fused cells are incubated in HAT medium (hypoxanthine-aminopterin-thymidine medium) for roughly 10 to 14 days.
- Only the B cell-myeloma hybrids survive, since the HGPRT gene coming from the B cells is functional.
- The next stage is a rapid primary screening process, which identifies and selects only those hybridomas that produce antibodies of appropriate specificity.
- The first screening technique used is called ELISA.

Type of monoclonal antibodies: triggering the immune system to attack cancer cells

- **Some monoclonal antibodies trigger the immune system to attack and kill cancer cells.**
- **Some monoclonal antibodies simply attach themselves to cancer cells, making them easier for the cells of the immune system to find them.**



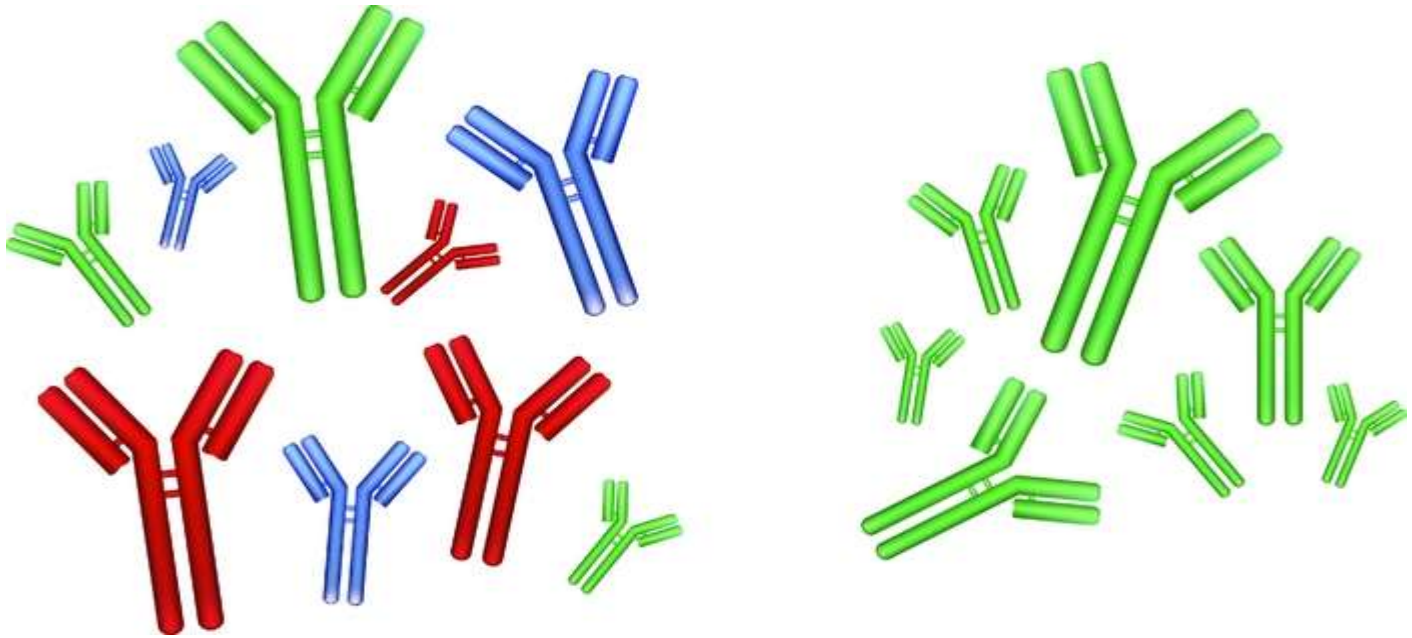
Chimeric monoclonal Antibodies



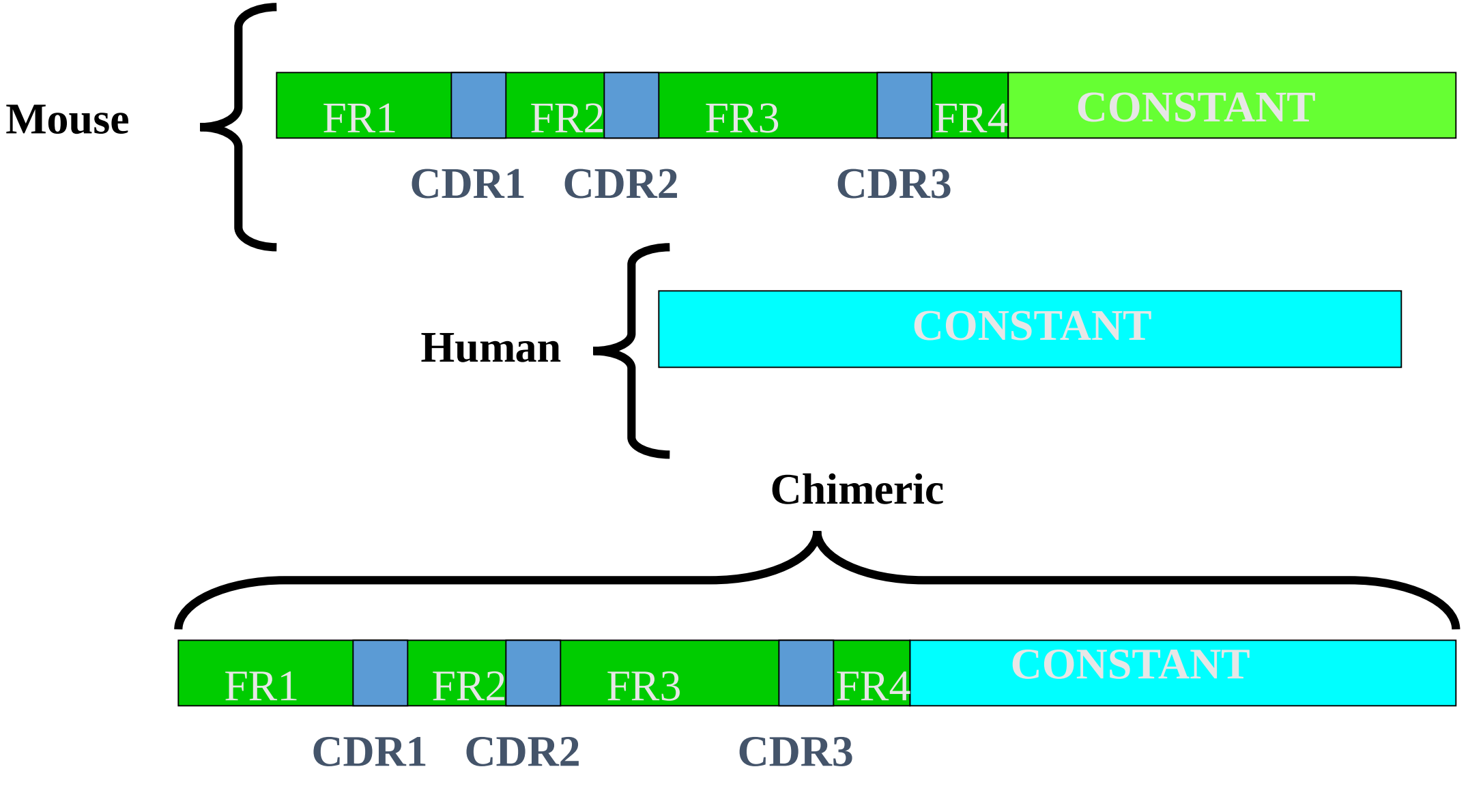
- **Chimeric antibodies are composed of murine variable regions fused onto human constant regions.**
- **Antibodies are approximately 65% human.**
- **This reduces immunogenicity and thus increases serum half-life.**

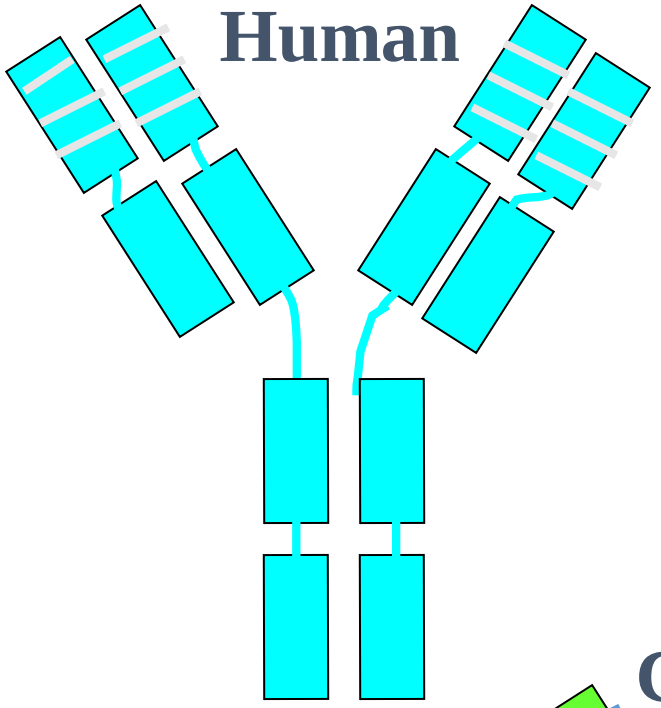
- **In one approach, mouse DNA encoding the binding portion of a monoclonal antibody was merged with human antibody-producing DNA in living cells, and the expression of this chimeric DNA through cell culture yielded partially mouse, partially human monoclonal antibody.**

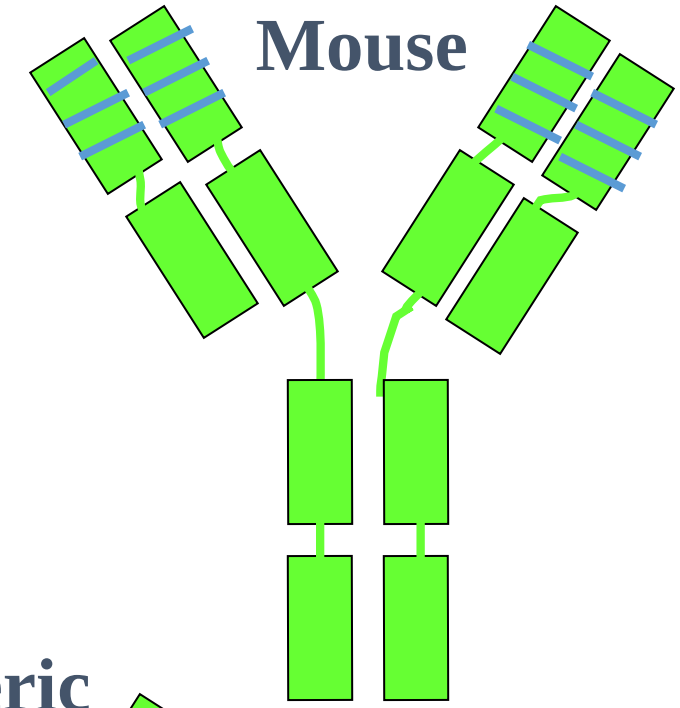
- **For this product, the descriptive terms "chimeric" and "humanised" monoclonal antibody have been used to reflect the combination of mouse and human DNA sources used in the recombinant process.**

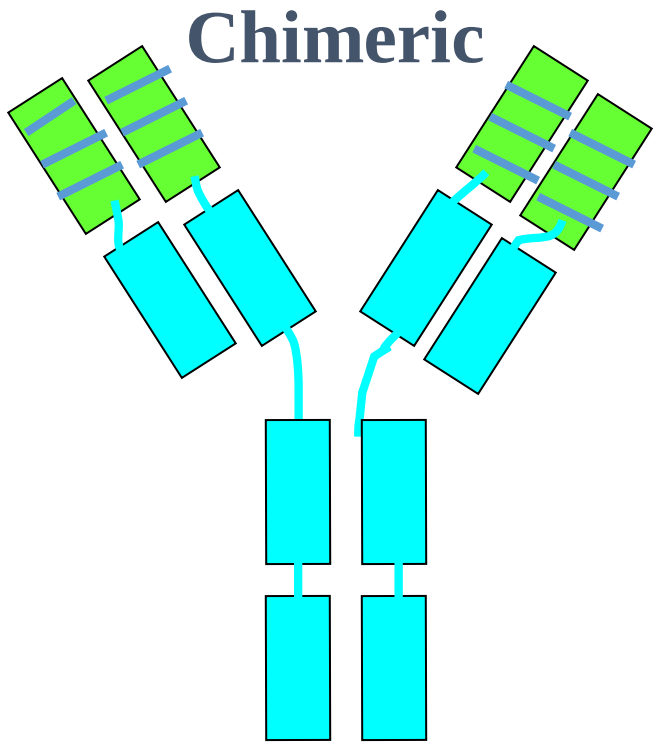


CHIMERIC HUMAN/MOUSE









Antigen binding parts (variable region) of mouse.

Ab with effector parts (constant region) of human.

Humanized monoclonal Antibodies

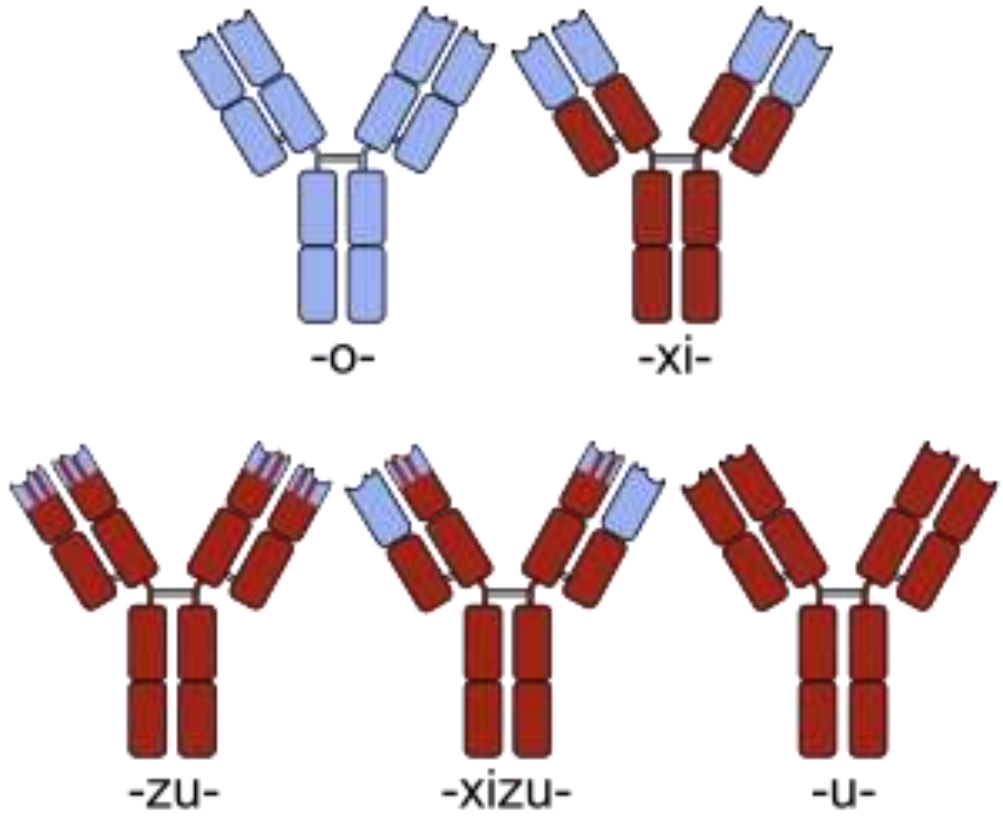
- **HA are antibodies from non-human species whose protein sequences have been modified to increase their similarity to antibody variants produced naturally in humans.**

- **The process of "humanization" is usually applied to monoclonal antibodies developed for administration to humans (for example, antibodies developed as anti-cancer drugs). Humanization can be necessary when the process of developing a specific antibody involves generation in a non-human immune system (such as that in mice).**

- **The creation of an antibody chimera is normally undertaken to achieve a more human-like antibody (by substituting the mouse Fc region of the antibody with that from human) simple chimeras of this type are not usually referred to as humanized.**

- **The humanization process may, however, include the creation of a mouse-human chimera in an initial step (mouse Fab spliced to human Fc). Thereafter the chimera might be further humanized by the selective alteration of the sequence of amino acids in the Fab portion of the molecule.Naming of humanized chimeras includes the stem for both designations (-xi- + -zu-)**

- **Chimeric antibody names contain a -xi- stem. Otelixizumab is an example of a humanized chimera currently in clinical trials for treatment of rheumatoid arthritis and diabetes mellitus**

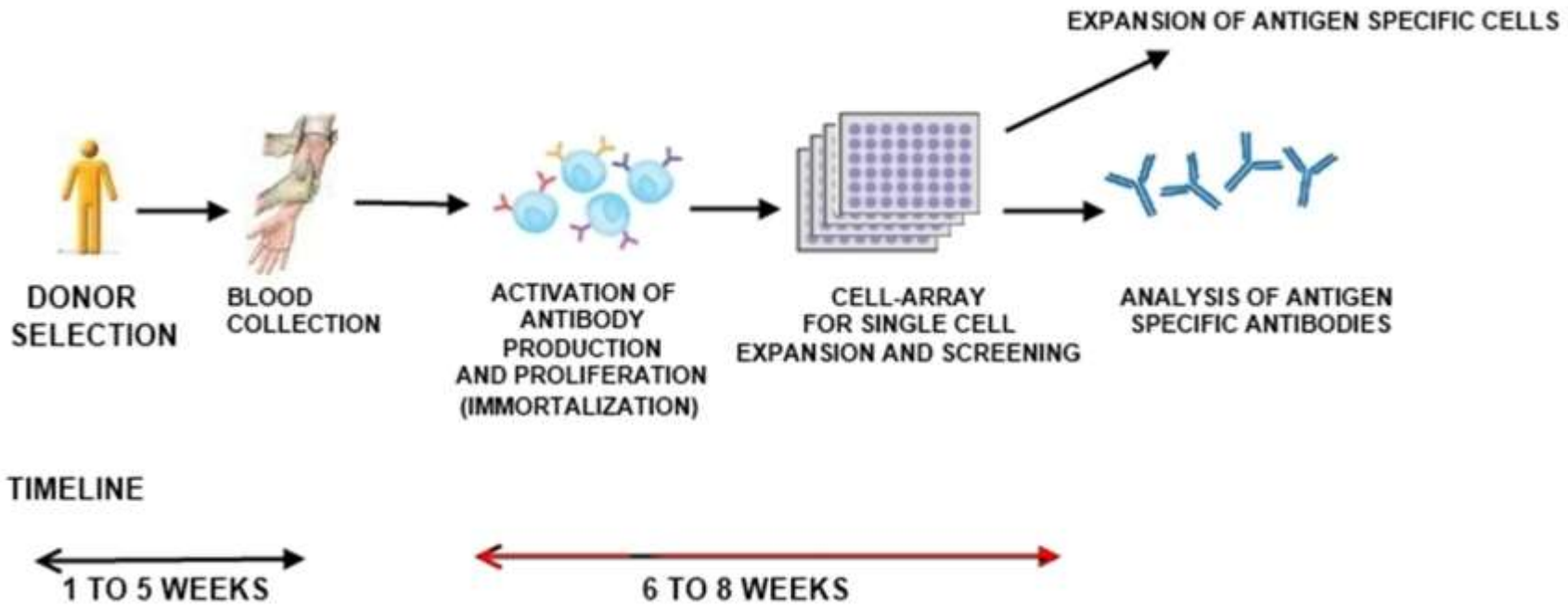


Humanization techniques

- **Capture and immortalize natural human antibody producing cells for in vitro production of antigen specific human antibodies.**

- **The cell lines produce antibodies into the medium, which can be purified or the immunoglobulin gene can be transferred to other cell lines (CHO or others) for large scale production.**

- **The best and most effective antibodies are generated by immunocompetent individuals who have been in the past naturally infected with infectious agents or developed natural antibodies against various antigens.**



Advantages of method

1. It does not require isolation and purification of B-cells therefore no loss of antigen specific B-cells occurs, which is associated with other methods.
2. It produces natural human antibodies, which has been innately produced by the immune system. It can be easily characterized and epitope mapped without recombinant technology for specific therapies.
3. Antibodies against multiple epitopes can be isolated, and combined as multiclonal or polyclonal mixture which might be more effective than a single monoclonal antibody for clinical use
4. Once the donor blood has been obtained, it will take only 6-8 weeks to obtain the monoclonal antibody producing clones compared to traditional methods which might take up to several years.
5. If blood donors with previous exposure to antigen of the interest cannot be found, in vitro immunization with antigens or peptides can be done to produce human monoclonal antibodies

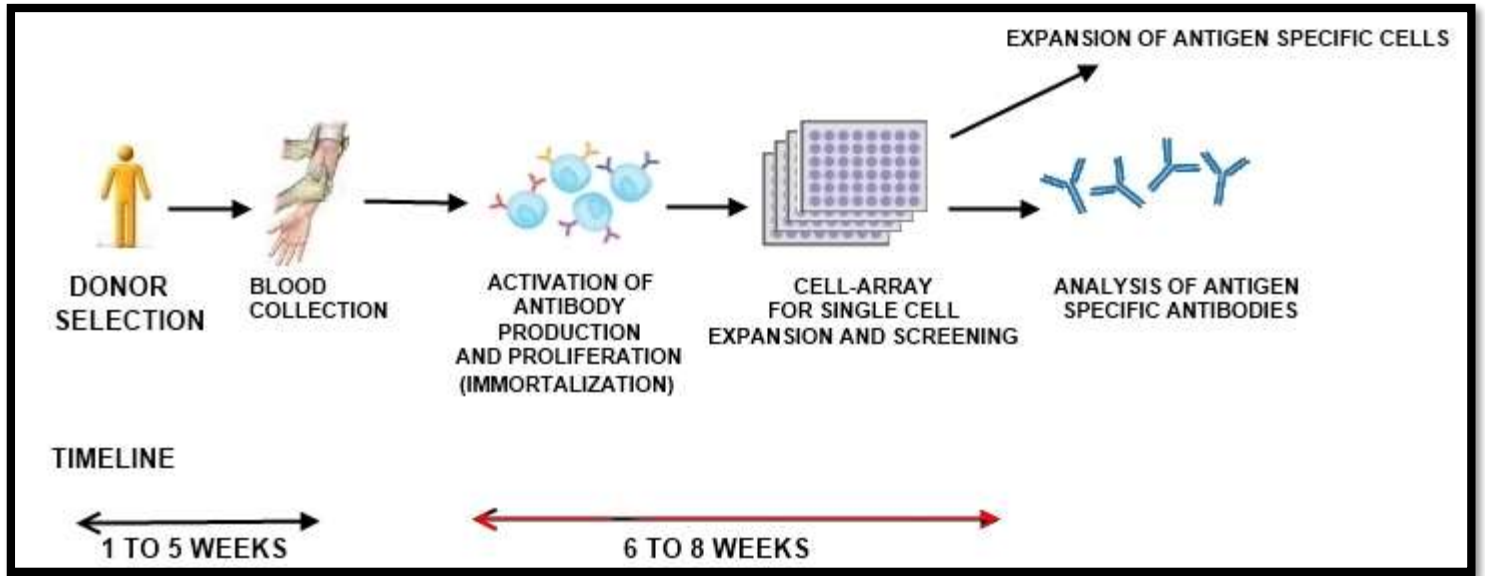
Human monoclonal antibodies

Ever since the discovery that monoclonal antibodies could be generated, scientists have targeted the creation of "fully" human products to reduce the side effects of humanized or chimeric antibodies. Two successful approaches have been identified: transgenic mice and phage display.

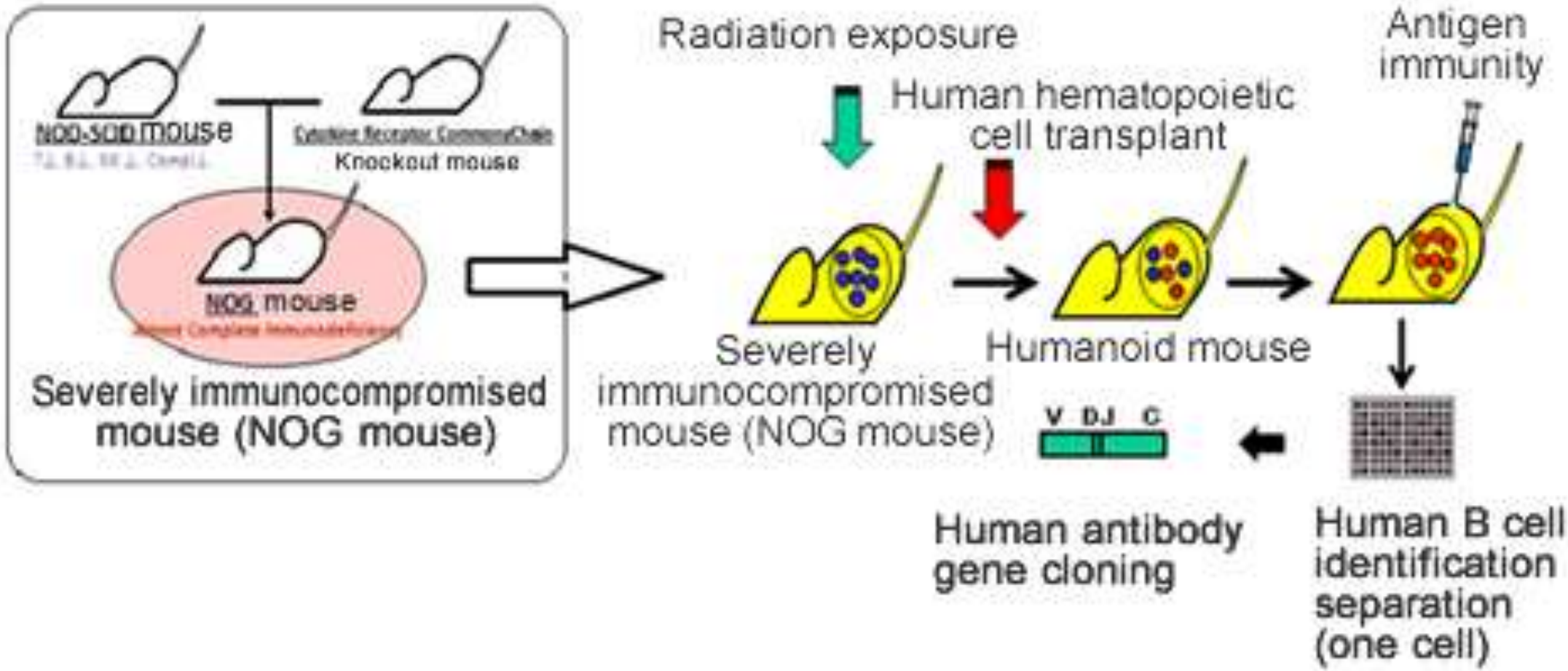
Fully human antibody that's lead to minimized immunogenicity, improved serum half- life

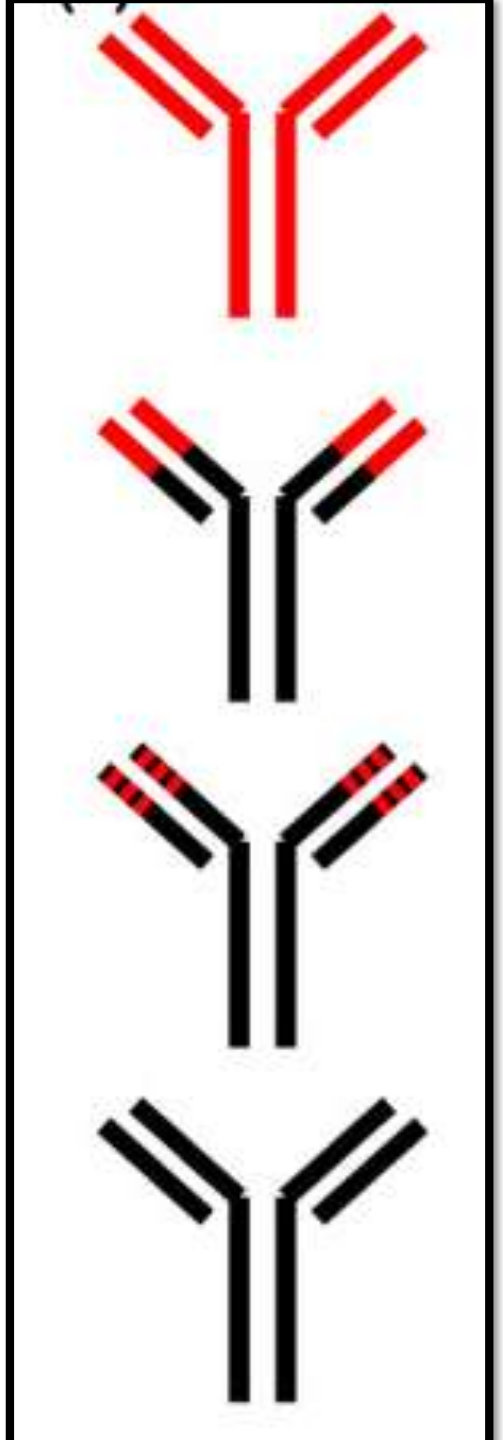
Fully human antibodies can be generated by the selection of human antibody fragments from in vitro libraries

Phage display can be used to express variable antibody domains on filamentous phage coat proteins (Phage major coat protein). These phage display antibodies can be used for various research applications.



Creation of fully human antibodies using humanoid mice





Unique proprietary method to capture and immortalize natural human antibody producing cells for in vitro production of antigen specific human antibodies.

it does not require isolation and purification of B-cells therefore no loss of antigen specific B-cells occurs, which is generally associated with other methods.

it produces natural human antibodies, which has been innately produced by the immune system. It can be easily characterized for specific therapies and epitope mapped without recombinant technology.

Monoclonal antibodies have been approved to treat cancer, cardiovascular disease, inflammatory diseases, macular degeneration, transplant rejection, multiple sclerosis and viral infection.

In **August 2006 the Pharmaceutical Research and Manufacturers of America reported that U.S. companies had 160 different monoclonal antibodies in clinical trials or awaiting approval by the Food and Drug Administration.**

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