Immune System and Production of monoclonal antibodies

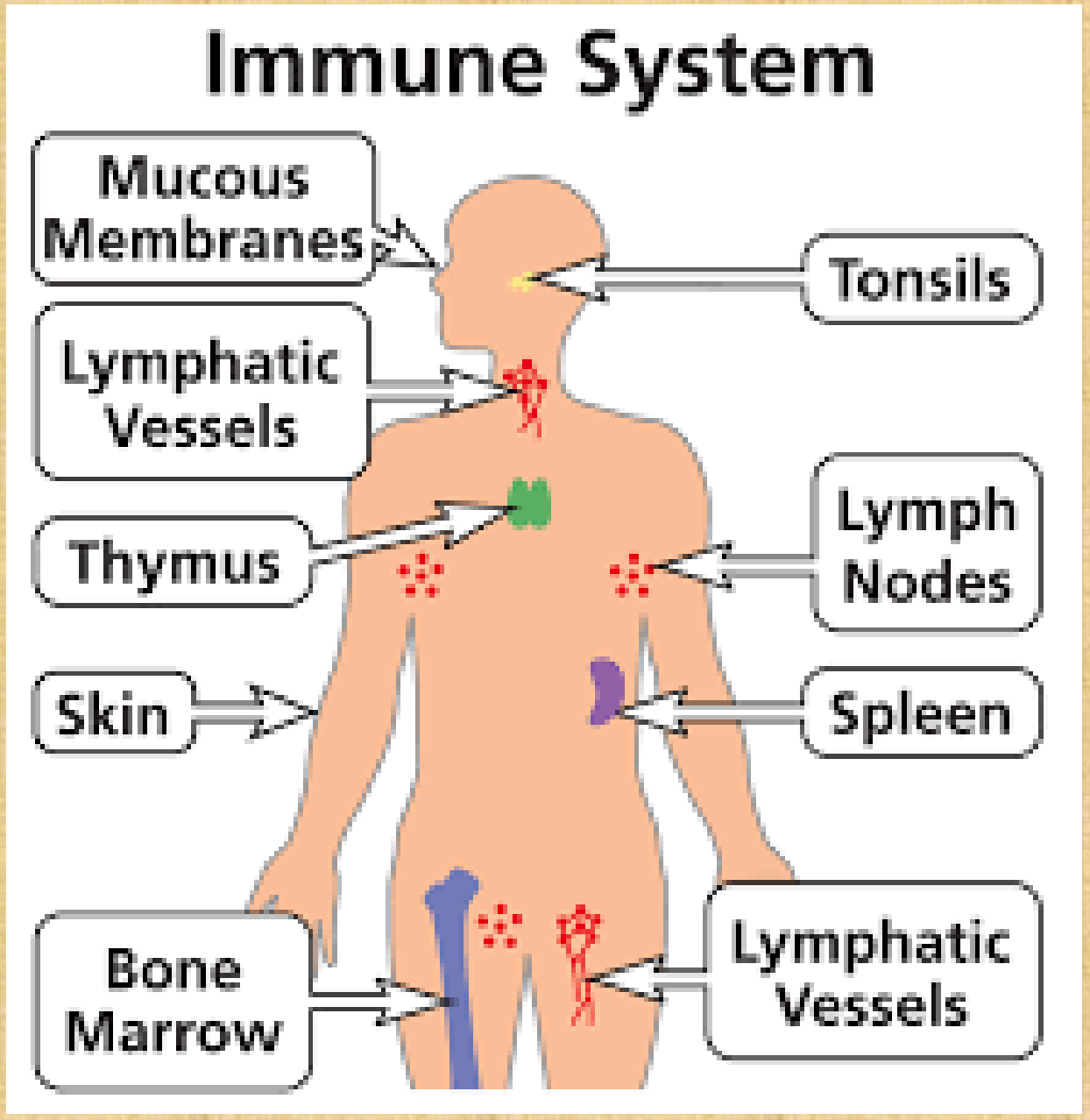
Immune system

- The immune system is spread throughout the body and involves many types of cells, organs, proteins, and tissues. Crucially, it can distinguish our tissue from foreign tissue — self from non-self. Dead and faulty cells are also recognized and cleared away by the immune system.
- If the immune system encounters a pathogen, for instance, a bacterium, virus, or parasite, it mounts a so-called immune response
- White blood cells are also called leukocytes. They circulate in the body in blood vessels and the lymphatic vessels that parallel the veins and arteries.
- White blood cells are on constant patrol and looking for pathogens. When they find a target, they begin to multiply and send signals out to other cell types to do the same.
- Our white blood cells are stored in different places in the body, which are referred to as lymphoid organs. These include the following:
- **Thymus** — a gland between the lungs and just below the neck.
- **Spleen** — an organ that filters the blood. It sits in the upper left of the abdomen.
- **Bone marrow** — found in the center of the bones, it also produces red blood cells.
- **Lymph nodes** —small glands positioned throughout the body, linked by lymphatic vessels.

There are two main types of leukocyte:

- **1. Phagocytes:** These cells surround and absorb pathogens and break them down, effectively eating them. There are several types, including:
- **Neutrophils** — these are the most common type of phagocyte and tend to attack bacteria.
- **Monocytes** — these are the largest type and have several roles.
- **Macrophages** — these patrol for pathogens and also remove dead and dying cells.
- **Mast cells** — they have many jobs, including helping to heal wounds and defend against pathogens.
- **2. Lymphocytes:** Lymphocytes help the body to remember previous invaders and recognize them if they come back to attack again.
- Lymphocytes begin their life in bone marrow. Some stay in the marrow and develop into B lymphocytes (B cells), others head to the thymus and become T lymphocytes (T cells). These two cell types have different roles:
- **B lymphocytes** — they produce antibodies and help alert the T lymphocytes.
- **T lymphocytes** — they destroy compromised cells in the body and help alert other leukocytes.

Immune System



Different antigen types, examples, and their origins

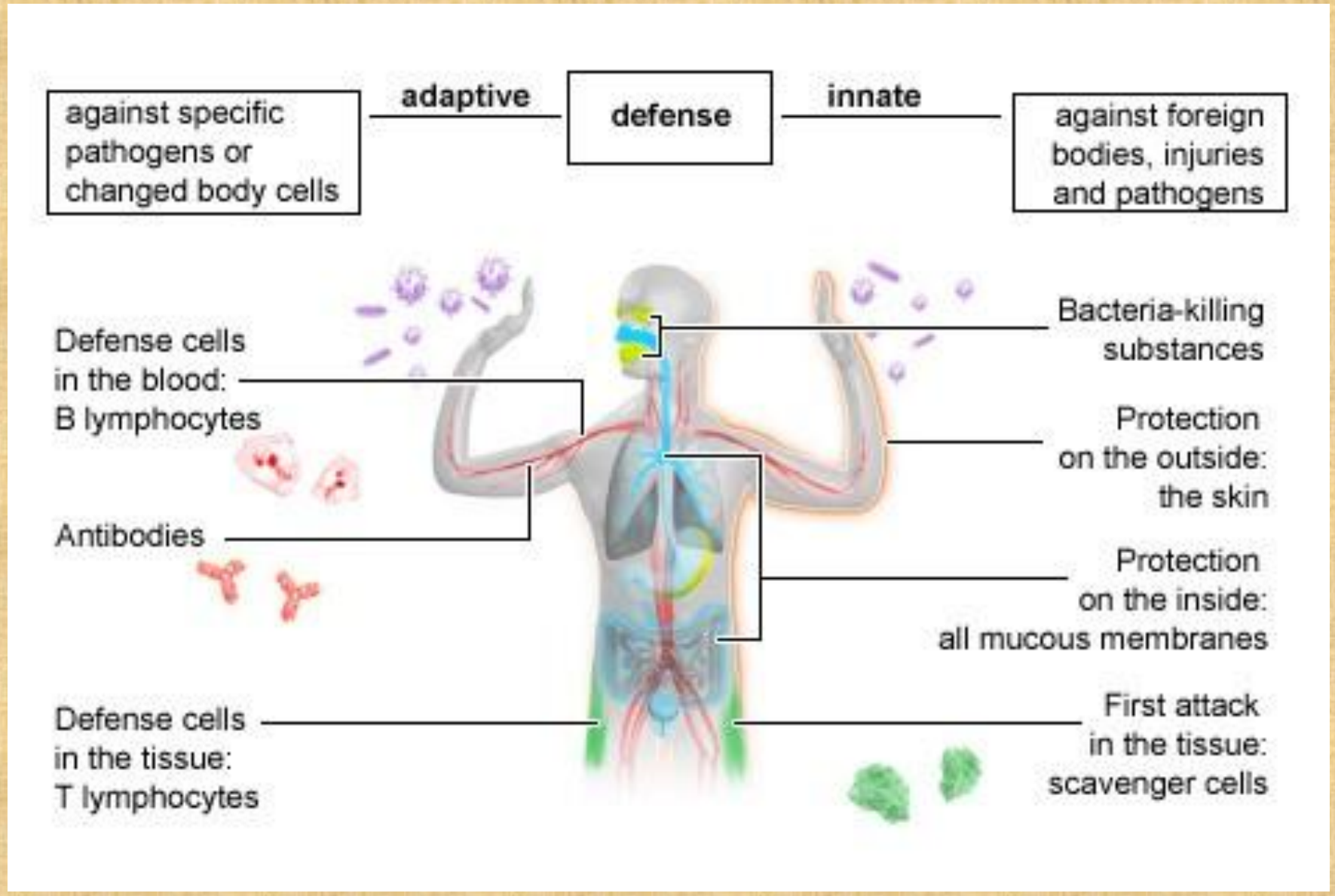
Class	Origin	Examples	
		Example Type	Specific Example
Natural	Plants, Bacteria, Animals	Particulate	Blood cells, bacteria, viruses
		Soluble	Toxins, toxoids, proteins, carbohydrates, glycoproteins. Liproproteins
Artificial	Chemically modified natural antigens	Iodinated proteins, protein-hapten conjugates	
Synthetic	Chemically synthesized molecules	Polypeptides, polyaminoacids, multichain aminoacid copolymers	

Innate Immunity

- The first line of defense against non-self pathogens is the innate, or non-specific, immune response. The innate immune response consists of physical, chemical and cellular defenses against pathogens. The main purpose of the innate immune response is to immediately prevent the spread and movement of foreign pathogens throughout the body.
- The innate immune system is the most evolutionarily conserved arm of the immune system and it generates rapid, non-specific inflammatory responses in response to signals from **Pattern Recognition Receptors (PRR).**
- **Pathogen Associated Molecular Patterns (PAMPs)** are conserved molecular structures of bacteria, viruses and other pathogens that bind to PRRs.
- The innate immune response has an important role in controlling infections during the first 7 days after an infection.
- Many of the cells in the innate immune system (such as **dendritic cells, macrophages, mast cells, neutrophils, basophils** and **eosinophils**) produce cytokines or interact with other cells directly in order to activate the adaptive immune system.
- There are other cell types, such as **gamma-delta T cells** and **Natural Killer (NK) cells** that are lymphocytes without antigen specificity, and therefore are considered to be innate cells with some similarities to effector lymphocytes.
- The effector mechanisms used to clear an infection depend on the type of pathogen that has initiated the immune response.

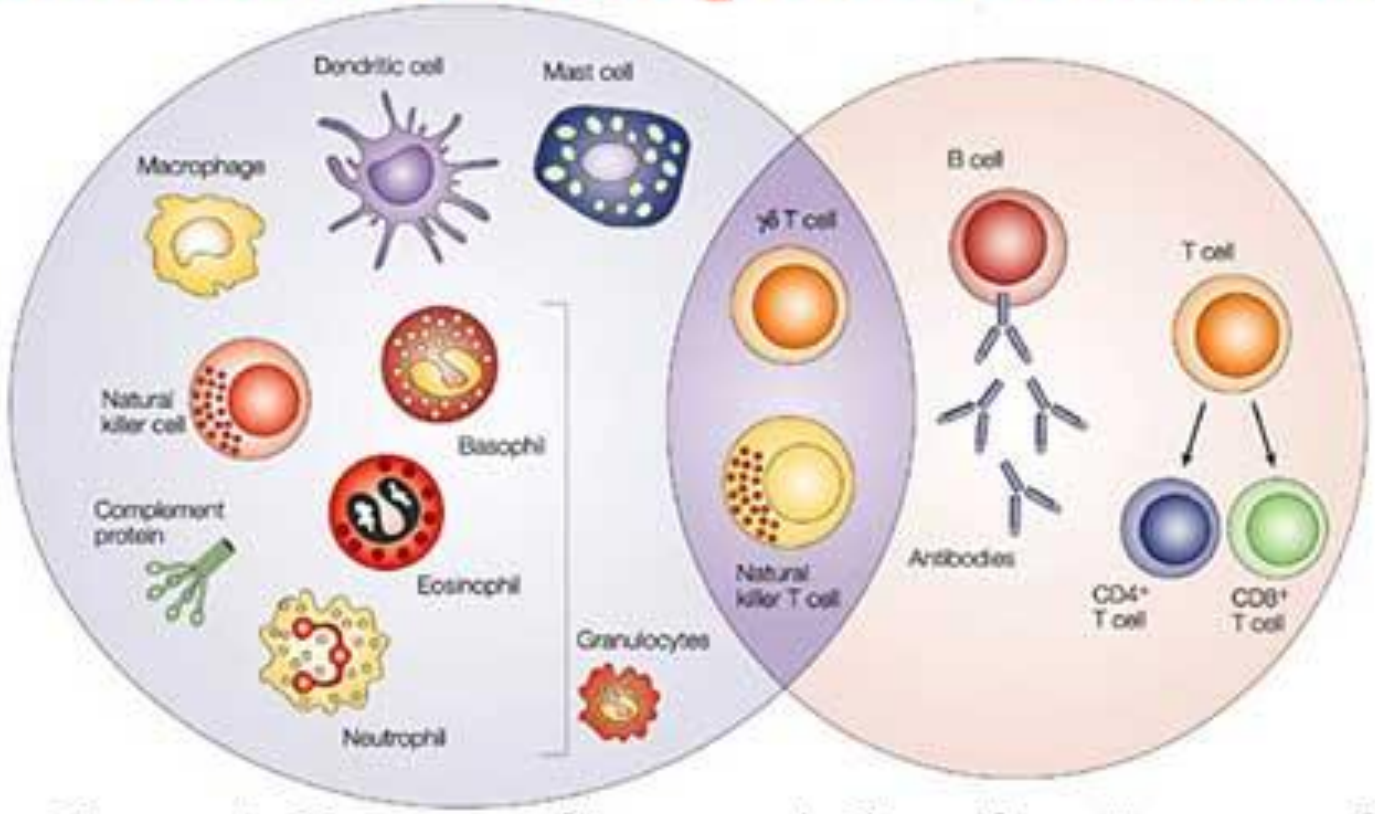
Adaptive Immunity

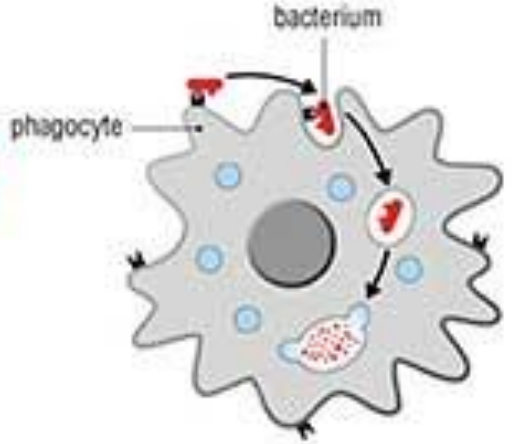
- The second line of defense against non-self pathogens is called adaptive immune response. Adaptive immunity is also referred to as acquired immunity or specific immunity and is only found in vertebrates. The adaptive immune response is specific to the pathogen presented. The adaptive immune response is meant to attack non-self pathogens but can sometimes make errors and attack itself. When this happens, autoimmune diseases can develop (e.g., lupus, rheumatoid arthritis).
- The adaptive immune system is based on **clonal selection** of lymphocytes with **antigen receptors** (B cell receptors and T cell receptors).
- Antigen receptors are genetically rearranged clonal receptors that bind to antigen displayed in **Major Histocompatibility Complex (MHC)** molecules on antigen-presenting cells.
- During the course of an adaptive immune response, memory T and B cells are generated which allow for more rapid and effective response to reinfection.
- **Immunologic memory** is the hallmark of adaptive immunity because it allows vertebrates to survive in a world where they are re-exposed to pathogens throughout their lifetimes.


against specific pathogens or changed body cells
adaptive
defense
innate
against foreign bodies, injuries and pathogens
Defense cells in the blood: B lymphocytes
Antibodies
Defense cells in the tissue: T lymphocytes
Bacteria-killing substances
Protection on the outside: the skin
Protection on the inside: all mucous membranes
First attack in the tissue: scavenger cells

Difference between

Innate and Adaptive Immunity





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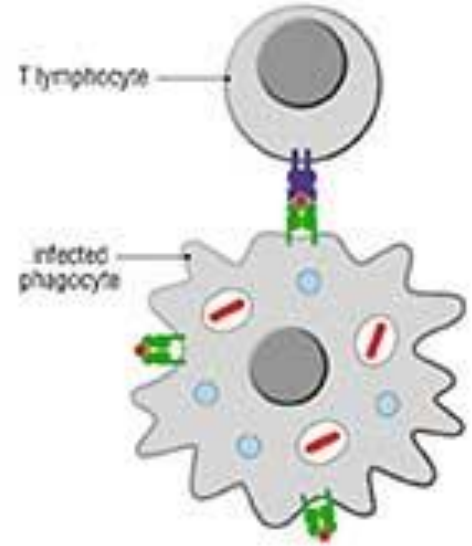

Innate Immunity **Adaptive Immunity**

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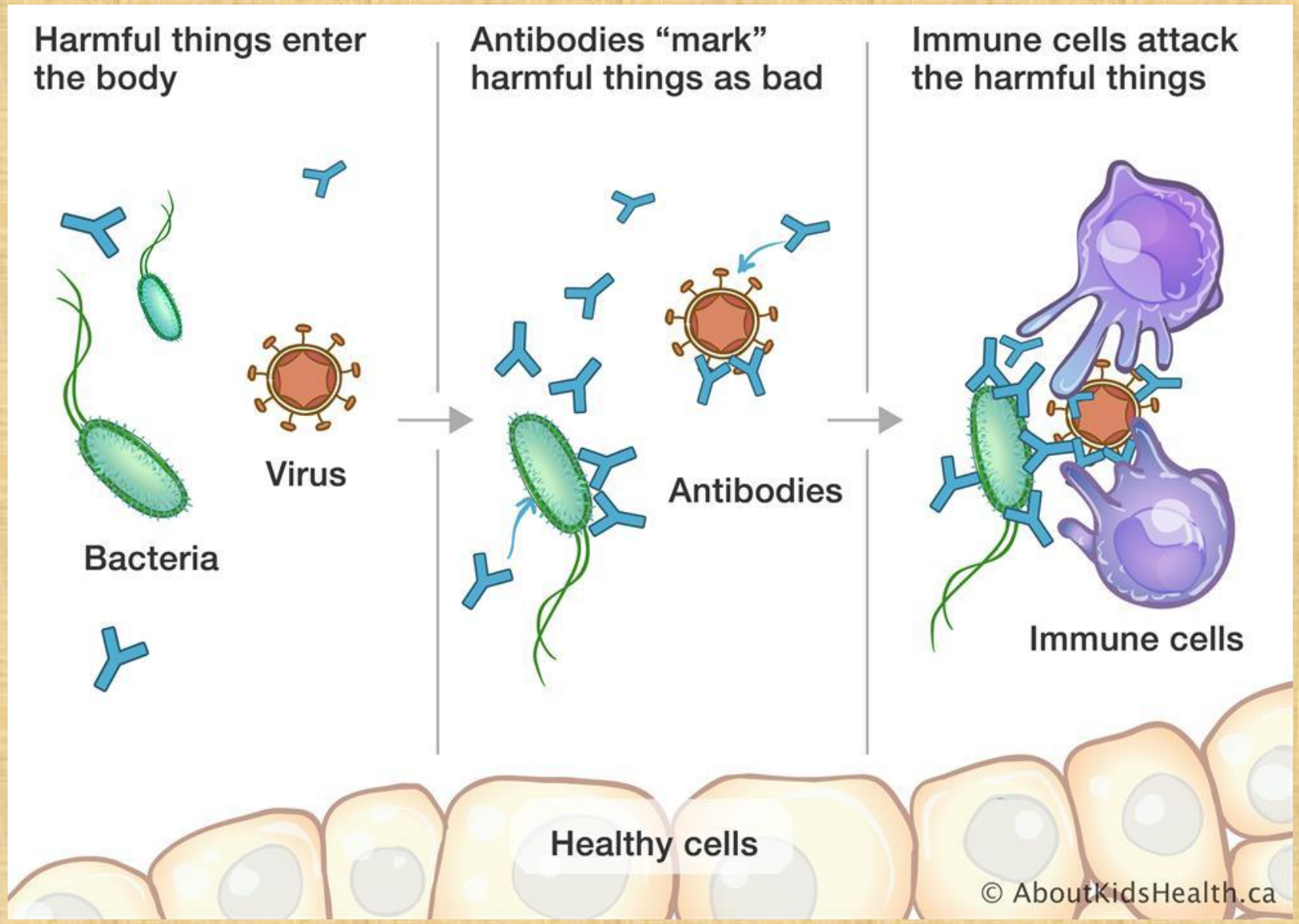
	Line of Defense	Timeline	Cells	Antigen Dependency	Examples
Innate (non-specific)	First	Immediate response (0-96 hours)	Natural killer cells, macrophages, neutrophils, dendritic cells, mast cells, basophils, eosinophils	Independent	Skin, hair, cough, mucous membranes, phagocytes, granulocytes
Adaptive (specific)	Second	Long term (>96 hours)	T and B lymphocytes	Dependent	Pus, swelling, redness, pain, T and B lymphocyte response


Harmful things enter the body
Antibodies "mark" harmful things as bad
Immune cells attack the harmful things
Virus
Bacteria
Antibodies
Immune cells
Healthy cells
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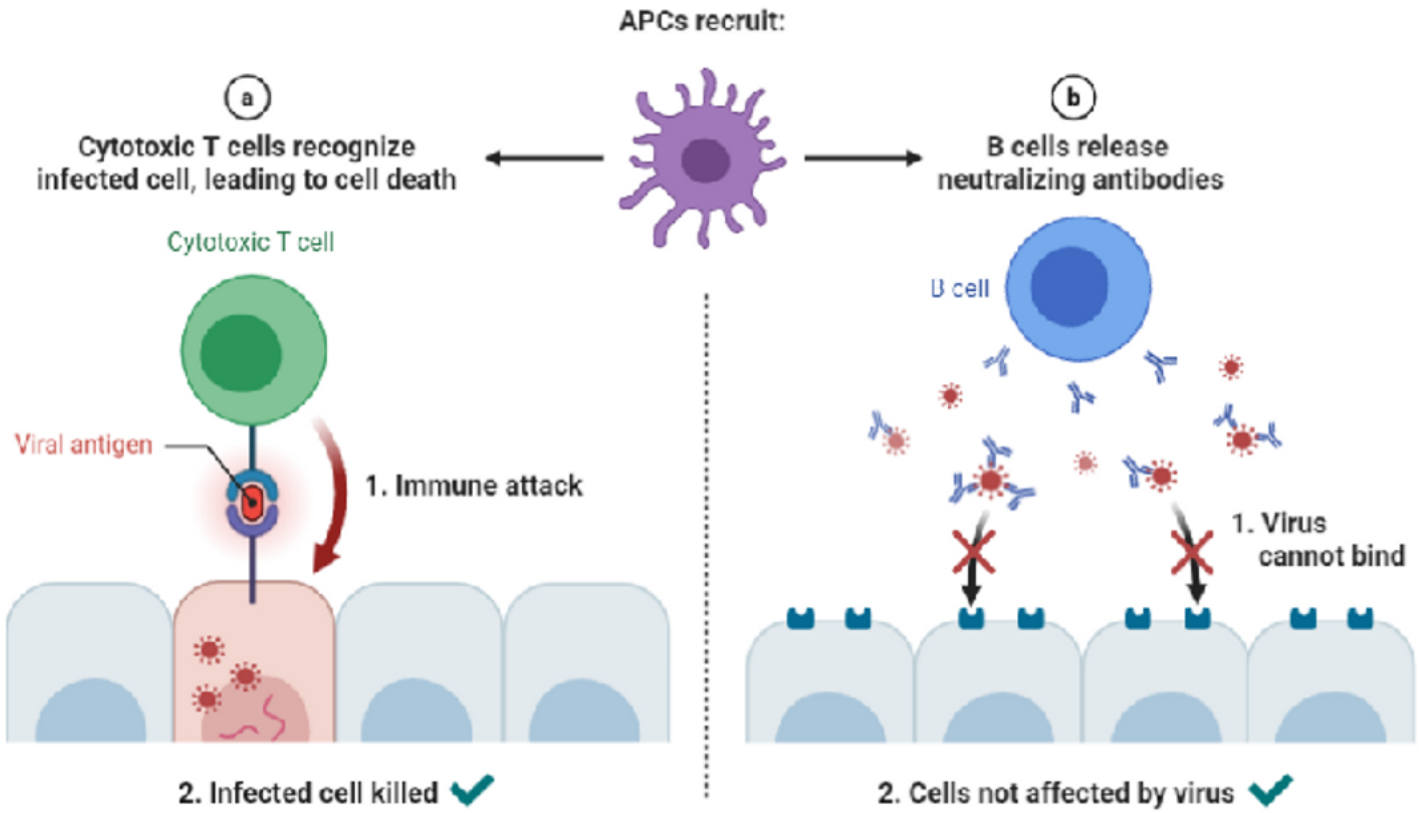
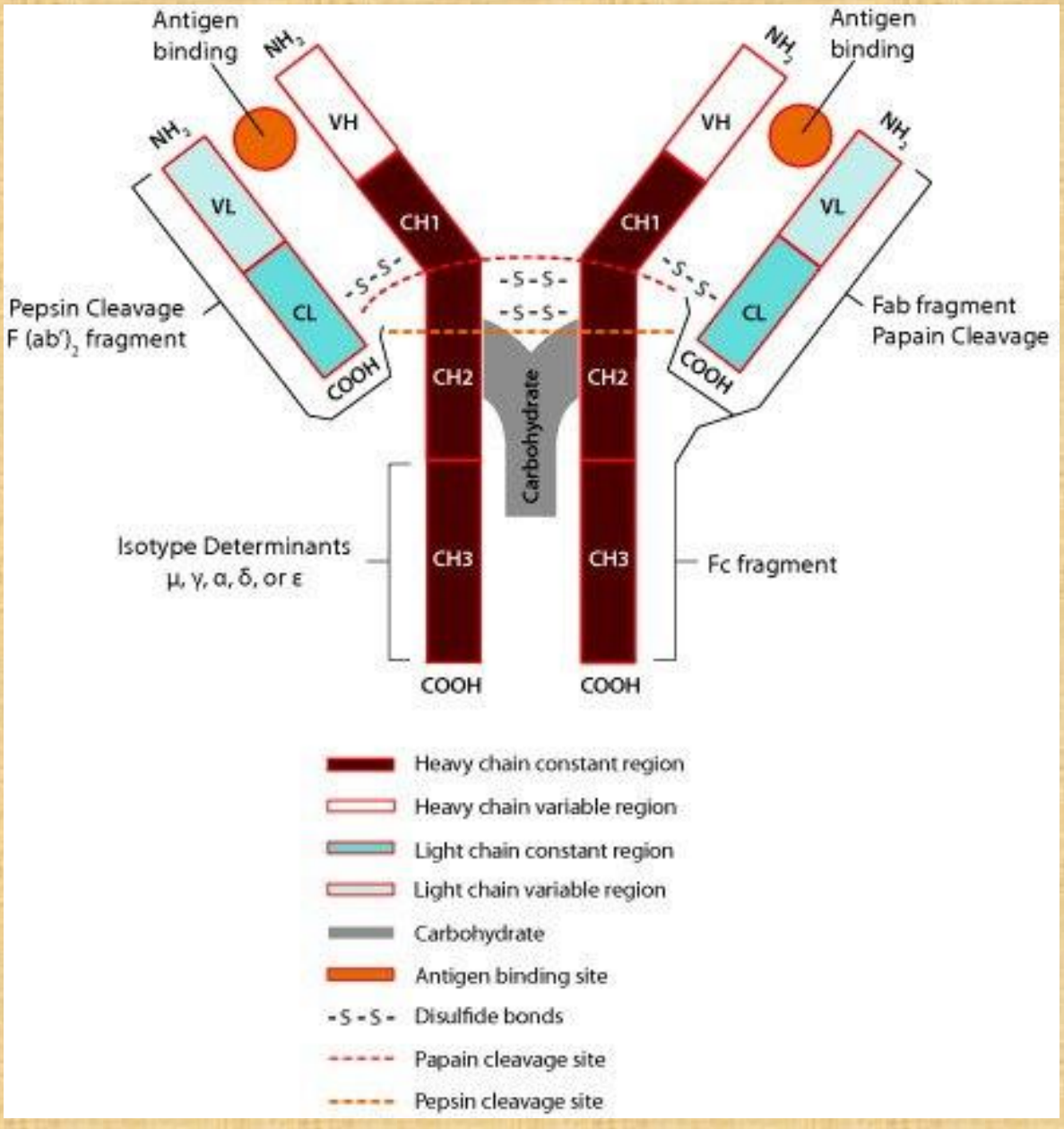

APCs recruit:
a
Cytotoxic T cells recognize infected cell, leading to cell death
b
B cells release neutralizing antibodies
Cytotoxic T cell
Viral antigen
1. Immune attack
2. Infected cell killed
B cell
1. Virus cannot bind
2. Cells not affected by virus

Structure of antibody

- Antibodies are immune system-related proteins called immunoglobulins. Each antibody consists of four polypeptides– two heavy chains and two light chains joined to form a "Y" shaped molecule.
- The amino acid sequence in the tips of the "Y" varies greatly among different antibodies. This variable region, composed of 110-130 amino acids, give the antibody its specificity for binding antigen. The variable region includes the ends of the light and heavy chains. Treating the antibody with a protease can cleave this region, producing Fab or fragment antigen binding that include the variable ends of an antibody. Material used for the studies shown below originated from Fab.
- The constant region determines the mechanism used to destroy antigen. Antibodies are divided into five major classes, IgM, IgG, Iga, IgD, and IgE, based on their constant region structure and immune function.
- The variable region is further subdivided into hypervariable (HV) and framework (FR) regions. Hypervariable regions have a high ratio of different amino acids in a given position, relative to the most common amino acid in that position. Within light and heavy chains, three hypervariable regions exist – HV 1, 2 and 3. Four FR regions which have more stable amino acids sequences separate the HV regions.
- The HV regions directly contact a portion of the antigen's surface. For this reason, HV regions are also sometimes referred to as complementarity determining regions, or CDRs. The FR regions form a beta-sheet structure which serves as a scaffold to hold the HV regions in position to contact antigen.

Structure of antibody



Antibodies are immune system-related glycoproteins called immunoglobulins, and produced by differentiated B-cells called plasma cells. They are present in bodily fluids, secretions and on the surface of B-cells. Antibodies recognise and bind to unique epitopes, which are molecular structures on the surface of their cognate antigens. Each antibody consists of four polypeptides– two heavy chains and two light chains joined to form a "Y" shaped molecule.

Types of antibodies

- **IgG antibody structure and function:** Immunoglobulin G (IgG) antibodies are large globular proteins with a molecular weight of about 150 kDa made of four peptide chains. It contains two identical γ (gamma) heavy chains of about 50 kDa and two identical light chains of about 25 kDa, thus a tetrameric quaternary structure. IgG provides long term protection because it persists for months and years after the presence of the antigen that has triggered their production. IgG protects against bacteria, viruses, neutralises bacterial toxins, triggers complement protein systems and binds antigens to enhance the effectiveness of phagocytosis.
- **IgM antibody structure and function:** Immunoglobulin M (IgM) antibodies are constructed of five or six units (i.e. mostly as pentamers but also hexamers occur) which are each comprised of two heavy-chains (μ-chains) and two light chains, bound together by disulfide bonds and a so-called J-chain. IgM is involved in the ABO blood group antigens on the surface of RBCs. IgM enhances ingestions of cells by phagocytosis.
- **IgA antibody structure and function:** Immunoglobulin A (IgA) antibodies consist of heavy (H) and light (L) chains. Each H chain is comprised of the constant region (Cα1, Cα2, Cα3), hinge region and the Variable (V) region. Light chains consist of the CL and Vκ or Vλ elements. The main function of IgA is to bind antigens on microbes before they invade tissues. It aggregates the antigens and keeps them in the secretions so when the secretion is expelled, so is the antigen. IgA is also first defense for mucosal surfaces such as the intestines, nose, and lungs.
- **IgE antibody structure and function:** Immunoglobulin E (IgE) antibodies have only been found in mammals. IgE is synthesised by plasma cells. Monomers of IgE consist of two heavy chains (ε chain) and two light chains, with the ε chain containing 4 Ig-like constant domains (Cε1-Cε4). IgE bind to mast cells and basophils which participate in the immune response. Some scientists think that IgE's purpose is to stop parasites.
- **IgD antibody structure and function:** Immunoglobulin D (IgD) antibodies are expressed in the plasma membranes of immature B-lymphocytes. IgD is also produced in a secreted form that is found in small amounts in blood serum. IgD plays a role in the induction of antibody production.

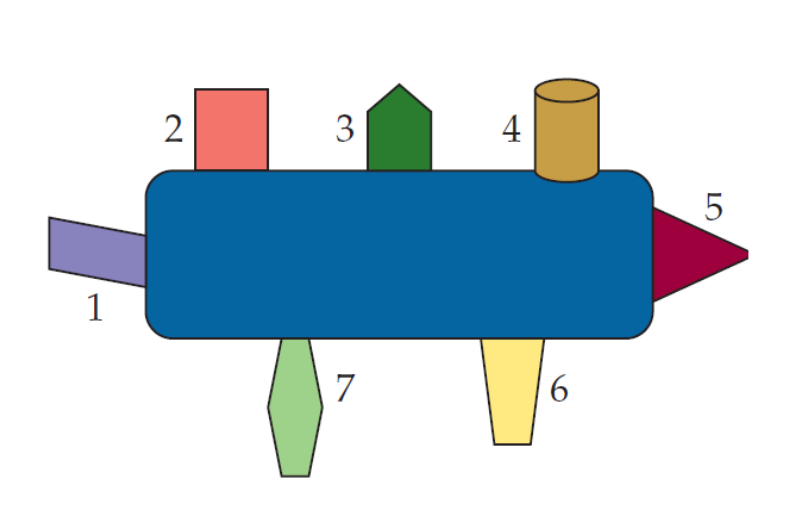
Human immunoglobulin types (isotypes)

Class	Additional Info	Functional Description
IgG	IgG1-IgG4	Predominant antibody found in blood and lymph. Predominant antibody involved in material immunity.
IgA	IgA1 (serum), IgA2 (secretory)	Predominant antibody found in saliva, tears, sweat, milk, intestinal secretions, and colostrum
IgM	Macroglobulin	First antibody type produced during a clonal response. Bound to lymphocytes and in serum
IgD	Surface bound	Bound to the surface of lymphocytes, very low concentrations in serum
IgE	Parasite Protection	Has a role in the protection from parasites. Low levels in serum

Monoclonal antibodies

- In mammals, a complex set of cellular systems has evolved to protect the body from toxic substances and invasion by infectious agents.
- As part of the defensive response, cells of the lymphatic system can be induced to produce specific proteins (antibodies) that bind to foreign substances (antigens) and—with the help of other immune system proteins, including the complement system—neutralize their biological impact.
- In response to an immunological challenge, each antibody-producing cell synthesizes and secretes a single antibody that recognizes with high affinity a discrete region (epitope, or antigenic determinant) of the immunizing antigen.
- Because an antigen generally has several different epitopes, normally several cells of the immune system each produce a different antibody against one of the many epitopes of the antigen. Such a set of antibodies, all of which react with the same antigen, is designated a polyclonal antibody (Figure).

Schematic representation of a target antigen.



The surface of the antigen depicted has seven (numbered 1 to 7) different antigenic determinants (epitopes). When this antigen is used to immunize an animal, each antigenic determinant elicits the synthesis of a different antibody. Together, the different antibodies that interact with an antigen constitute a polyclonal antibody directed against that antigen.

Conti....

- Consequently, a fundamental objective for the applied use of antibodies, as diagnostic agents or as components of therapeutic agents, was to discover how to create a cell line that could be grown in culture and that would produce a single type of antibody molecule (monoclonal antibody) with a high affinity for a specific target antigen.
- Such a cell line would provide a consistent and continuous source of identical antibody molecules. Unfortunately, the B lymphocytes (B cells) that synthesize antibodies do not reproduce in culture. However, it was envisioned that a hybrid cell type could be created to solve this problem.
- This hybrid would have the B-cell genetic components for producing antibodies and the cell division functions of a compatible cell type to enable the cells to grow in culture. It was known that normal B lymphocytes sometimes become cancer cells (myelomas) that acquire the ability to grow in culture while retaining many of the attributes of B cells.
- Thus, myeloma cells, especially those that did not produce antibody molecules, became candidates for fusion with antibody-producing B cells. In the mid-1970s, these ideas became reality.

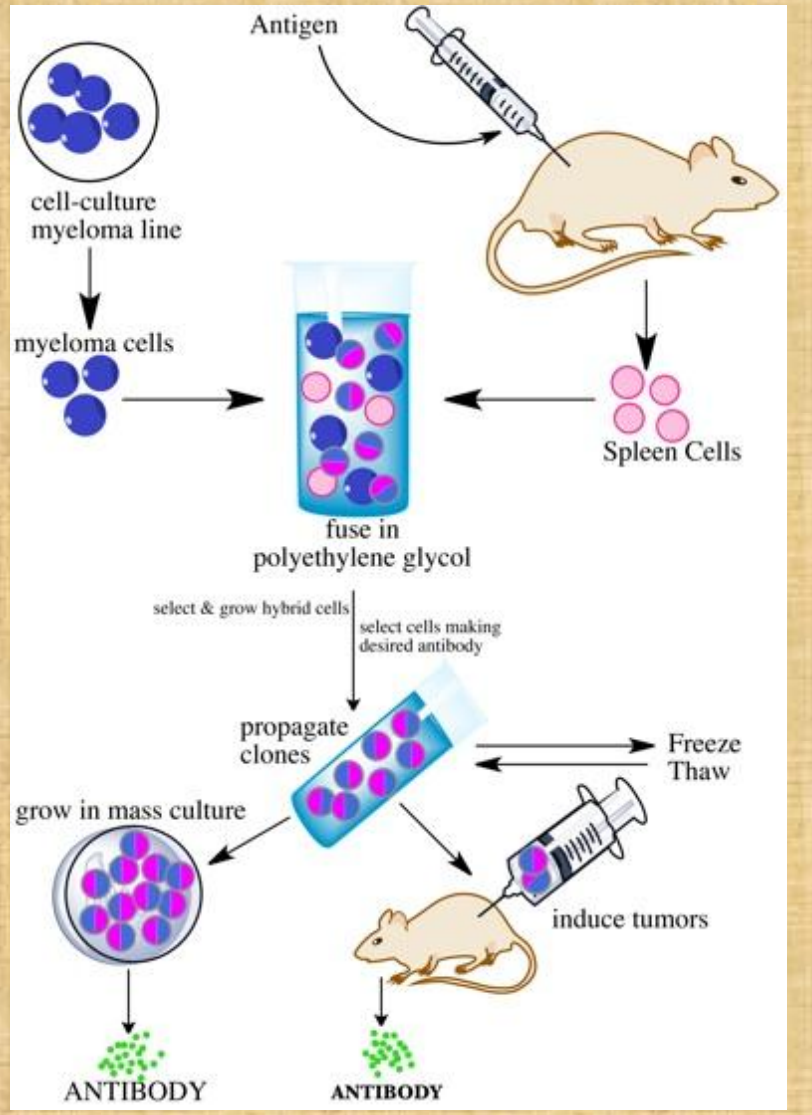
Monoclonal antibodies

- Monoclonal antibodies are a series of identical antibodies produced by a single clone of B cell. In 1975, monoclonal antibodies were first generated by Milstein and Köhler. This method for production of monoclonal antibodies is called hybridoma technology. Since then, monoclonal antibodies have been widely used as an essential tool of biomedical research and therapeutic applications.
- Another method of manufacturing monoclonal antibodies is by using phage display which was discovered by G. Smith in 1985. And it has become one of the most effective techniques for producing large amounts of peptides, proteins and antibodies.

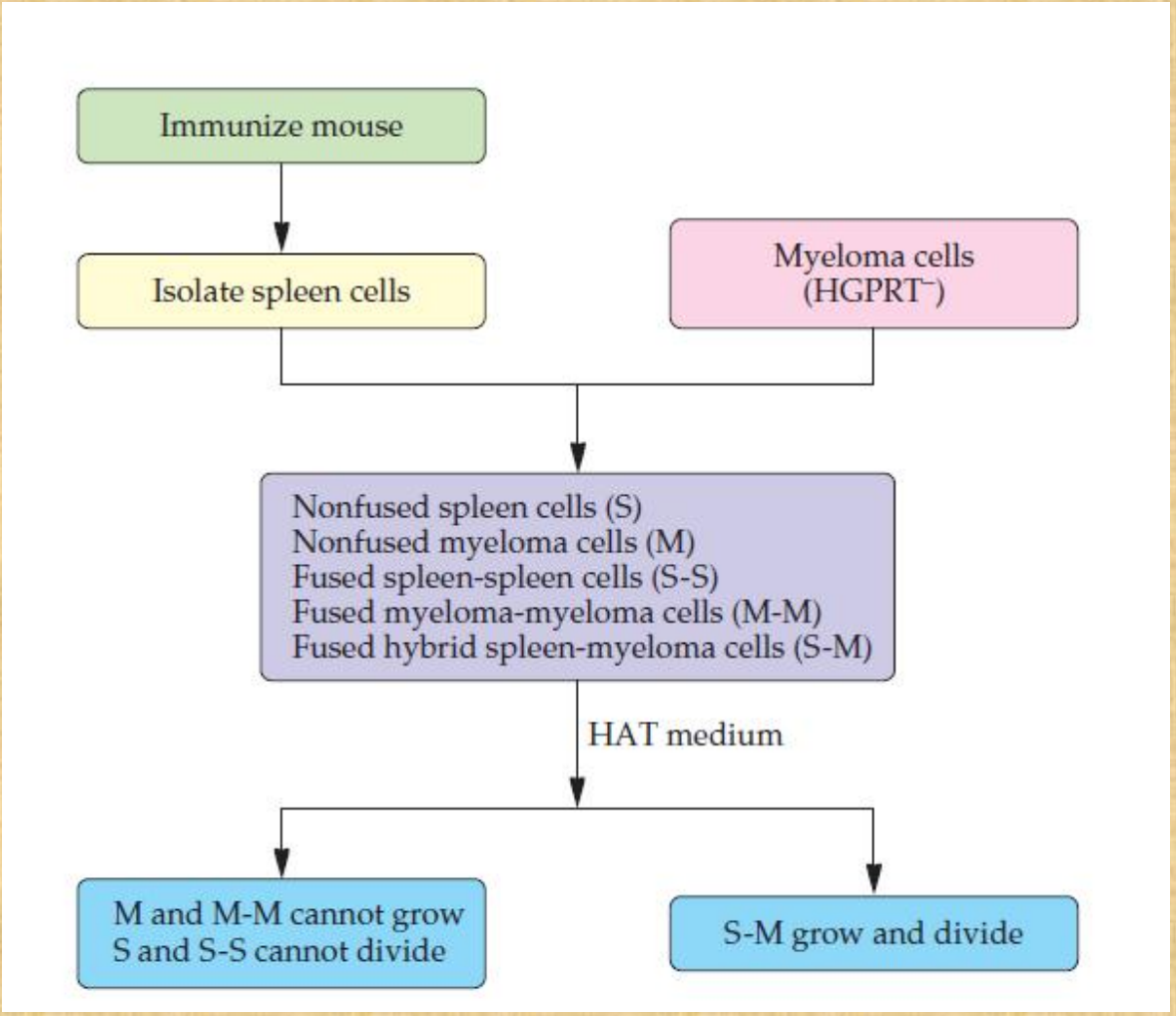
What are the advantages of monoclonal antibodies?

- Same quality of the antibody is maintained amongst the different production batches.
- Highly reproducible and scalable, unlimited production source.
- Speed and sensitivity and specificity of assays.
- Can produce antibodies when needed.
- No need to worry about maintaining the animals.
- Antigen or immunogen need not be pure.
- Selection helps to identify the right clones against the specific antigen.

Production of monoclonal antibodies



The HAT procedure for selecting hybrid spleen–myeloma (hybridoma) cells.



Procedure:

- The splenic cell suspension is mixed with a suspension of myeloma cells that are genetically defective for the enzyme hypoxanthine-guanine phosphor ribosyl transferase (HGPRT−).
- The combined cell suspensions are mixed with 35% polyethylene glycol for a few minutes and then transferred to a growth medium containing hypoxanthine, aminopterin, and thymidine (HAT medium).
- The polyethylene glycol treatment facilitates fusion between cells. Nevertheless, the fusion events are rare and random. There will be myeloma cells, spleen cells, myeloma–spleen fusion cells, myeloma–myeloma fusion cells, and spleen–spleen fusion cells in the mixture.
- The HAT medium, however, allows only the myeloma–spleen fusion cells to grow, because none of the other cell types can proliferate in this medium.
- Unfused spleen cells and spleen–spleen fusion cells cannot grow in any culture medium. The HGPRT− myeloma and the myeloma–myeloma fusion cells cannot use hypoxanthine as a precursor for the biosynthesis of the purines guanine and adenine, which are, of course, essential for nucleic acid synthesis. However, they have a second, naturally occurring pathway for purine biosynthesis that utilizes the enzyme dihydrofolate reductase. Therefore, aminopterin is included in the medium because it inhibits dihydrofolate reductase activity. Hence, HGPRT− myeloma and myeloma–myeloma fusion cells are unable to synthesize purines in HAT medium, so they die.
- The spleen–myeloma fusion cells survive in HAT medium because the spleen cell contributes a functional HGPRT, which can utilize the exogenous hypoxanthine in the medium even though purine production by means of dihydrofolate reductase is blocked by aminopterin, and because the cell division functions of the myeloma cell are active. Thymidine is provided to overcome the block in pyrimidine production that is caused by the inhibition of dihydrofolate reductase by aminopterin. About 10 to 14 days after the fusion treatment, only spleen–myeloma fusion cells have survived and grown in the HAT medium.

Identification of Specific Antibody-Producing Hybrid Cell Lines

- The next task is to identify those hybrid cells that produce antibody against the immunizing antigen. One common screening procedure uses the culture medium, which contains secreted antibodies.
- The medium is collected from the wells that have growing cells and is added to a well of another microtiter plate that has been precoated with the target antigen. If the culture medium contains an antibody (primary antibody) that recognizes an epitope of the antigen, it will bind to the antigen and not be washed away during a subsequent washing step.
- A second antibody (secondary antibody) that is specific for mouse antibodies is added to the wells of the test plate. It will bind to any primary antibody that is bound to the antigen. Before its use in the immunoassay, the secondary antibody is conjugated to an enzyme that can convert a colorless substrate to a colored compound.
- The presence of color in one of the test wells indicates that the original culture medium contained an antibody that was specific for the antigen (Figure). If the culture medium does not contain an antibody that binds to the antigen, then the first wash will remove the primary antibody. Therefore, when the secondary antibody is added, it has nothing to bind to and is removed by the second washing step. In a well where such a sequence of events occurs, the substrate solution remains colorless.