

Veterinary Immunology

By Dr. Betelihem Tegegne

The basics of Immunology

Brief history of immunology

- The discipline of immunology grew out of the observation that individuals who had recovered from certain infectious diseases were thereafter protected from the disease.
- The Latin term *immunis*, meaning “exempt” is the source of the English word *immunity*, meaning the state of protection from infectious disease. The scientific study of the immunity/ body defense is immunology.

Con't..

- When infections such as smallpox or plague spread through early human societies, many people died, but some individuals recovered. It was rarely noticed that these recovered individuals remained healthy during subsequent outbreaks—a sign that they had developed immunity.

Con't..

- The earliest recorded is about Thucydides, the great historian of the Peloponnesian War. In describing a plague in Athens, he wrote in 430 BC that only those who had recovered from the plague could nurse the sick because they would not contract the disease a second time.

Con't..

- By the 12th century, the Chinese had observed that persons who recovered from smallpox were resistant to further attacks of this disease. So, they deliberately infected infants with smallpox by inserting scabs from infected individuals into small cuts in their skin. Those infants who survived the resulting disease were protected from smallpox in later life.

- In 1718, Lady Mary Wortley Montagu observed the positive effects of variolation (inserting lesions to the skin of healthy individuals) on the native population and had the technique performed on her own children. The method was significantly improved by the English physician Edward Jenner, in 1798.

Con't..

- Outbreaks of rinderpest (cattle plague) had been a common occurrence throughout Western Europe since the 9th century and inevitably killed huge numbers of cattle. Since none of the traditional remedies appeared to work and the skin lesions in affected animals vaguely resembled those seen in smallpox, it was suggested in 1754 that inoculation might help.

Con't..

- In 1798, Edward Jenner demonstrated that material from cowpox lesions could be substituted for smallpox in variolation. Since cowpox does not cause severe disease in humans, its use reduced the risks caused by variolation to insignificant levels.
- The effectiveness of this procedure, called vaccination (vacca is Latin for

- In the year 1879, Louis Pasteur in France investigated fowl cholera, a disease caused by the bacterium *Pasteurella multocida*. Pasteur had a culture of this organism that was accidentally allowed to age on a laboratory bench while his assistant was on vacation. When the assistant returned and tried to infect

Con't..

- Pasteur used these chicken for a second experiment in which they were challenged again, this time with a fresh culture of *P. multocida* known to be capable of killing chickens. Surprisingly, birds were resistant to the infection and did not die. Then Pasteur immediately recognized that this phenomenon was similar in principle to Jenner's use of cowpox for vaccination

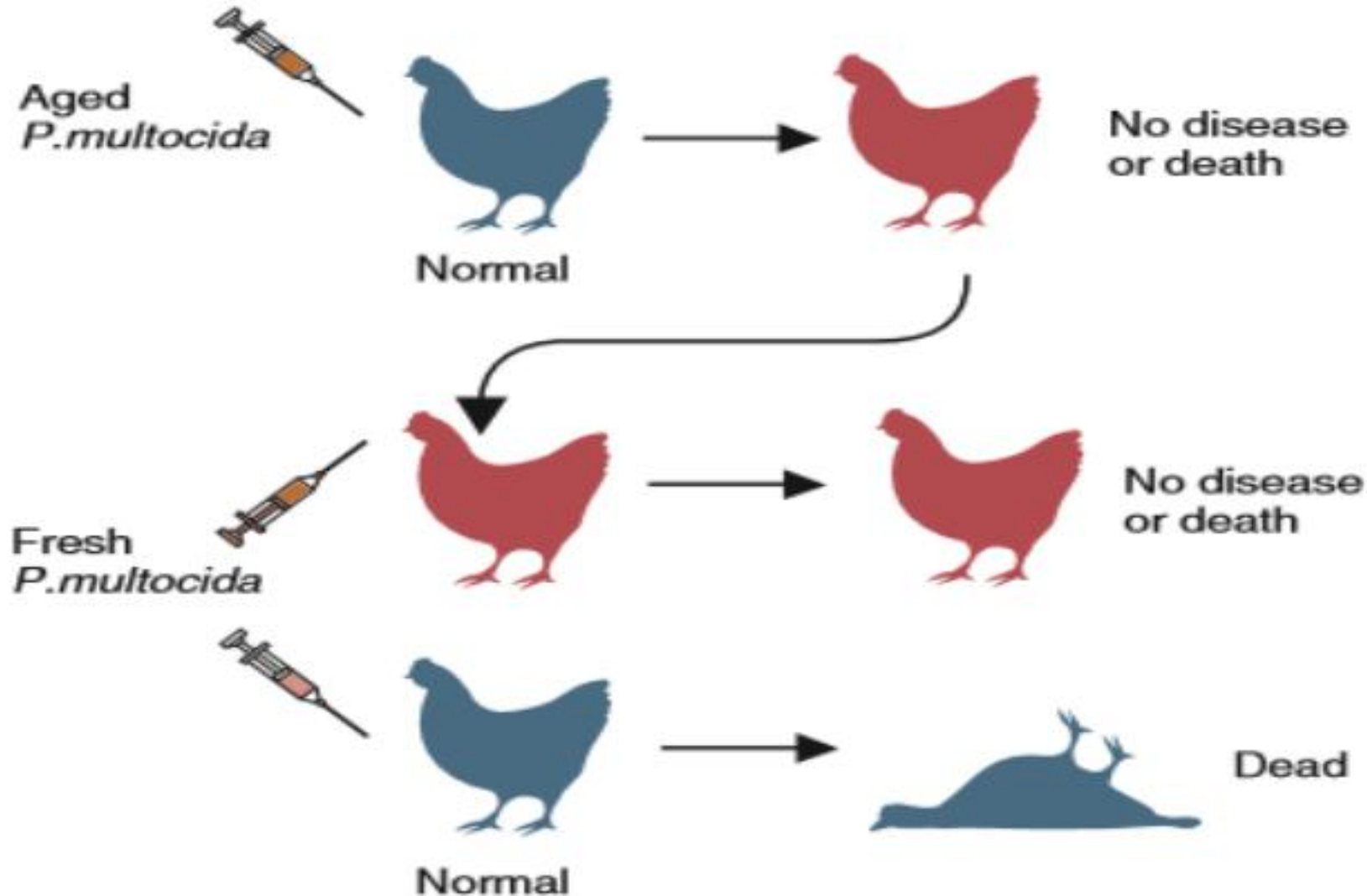


FIGURE 1-2 Pasteur's fowl cholera experiment. Birds inoculated with an aged culture of *Pasteurella multocida* did not die. However, when subsequently inoculated with a fresh culture of virulent *P. multocida*, the birds were found to be protected. It was this experiment that launched the science of immunology.

Con't..

- By 1900 many vaccines had been developed, and the development of immunity to infectious diseases of animals was a well-recognized phenomenon. Since then, immunologists have determined the molecular and cellular basis of this antimicrobial immunity.
- This understanding has brought the ability to use immune mechanisms to enhance resistance to infectious diseases. The role

Immunity (immune response)

- The defenses of the body, collectively called the immune system, consist of complex, interacting networks of biochemical and cellular reactions.
- Immunity refers to the state of protection from infectious diseases. Understanding immunity requires an understanding of dynamic immunological networks. These networks possess redundancies and

Con't..

- Multiple mechanisms are needed to ensure freedom from invasion. These include physical barriers that exclude invaders, innate immunity that provides rapid initial protection, and adaptive immunity that provides prolonged effective immunity.

Physical Barriers

- The body employs multiple, overlapping layers of defense.
- The first and most obvious of these defenses are the physical barriers to invasion. Thus intact skin provides an effective barrier to microbial invasion. If skin is damaged, microbes may invade; however, wound healing ensures that this is repaired very rapidly.

- On other body surfaces, such as in the respiratory and gastrointestinal tracts, simple physical defenses include the “self-cleaning” processes: coughing, sneezing, and mucus flow in the respiratory tract; vomiting and diarrhea in the gastrointestinal tract; and urine flow in the urinary system.

Con't..

- The presence of a huge population of commensal bacteria on the skin and in the intestine also excludes many potential invaders. Well-adapted commensal organisms adapted to living on body surfaces can easily outcompete poorly adapted pathogenic organisms.

Innate Immunity

- Physical barriers cannot be totally effective in themselves. Invading microorganisms will eventually overcome physical obstacles. So, most microbial attempts at invasion should be blocked before they can result in disease. This is the task of the innate immune system. Many different innate defense

- The mammalian innate immune system is a collection of distinct subsystems that work through diverse mechanisms. Innate immunity is activated immediately when a pathogen penetrates the epithelial barriers, it ideally lasts for just a few hours, and is directed toward the rapid elimination of the pathogen.

- Innate immune mechanisms in general rely on the fact that microbes such as bacteria and viruses differ structurally and chemically from normal animal tissues.
- Animals make molecules that can kill invaders directly or promote their destruction by defensive cells.

- Phagocytic cells, such as macrophages^t and neutrophils and a variety of antimicrobial compounds synthesized are parts of innate immunity
- Other innate subsystems include the complement system, a set of complex enzyme pathways that are lethal to invaders.
- Some of the cells involved in inflammation may also help repair damaged tissues

- Innate immunity components are not specific to a particular pathogen and have no any memory regarding a specific pathogen.

Adaptive Immunity

- Inflammation and the other subsystems of the innate immune system are critical to the defense of the body. Animals that cannot develop effective innate responses may die from overwhelming infections.

- These innate mechanisms cannot offer the ultimate solution to the defense of the body. What is really needed is a defense system that can recognize and destroy invaders and then learn from the process, so that if they invade again, they will be destroyed even more effectively.

Con't..

- In this system, the more often an individual encounters an invader, the more effective will be its defenses against that organism. This type of response is the function of the adaptive immune system, so called since it adapts itself to the requirements of the animal (also called the acquired immune system)

Con't..

- The adaptive immune system takes several days or weeks to become effective. Although it develops slowly, when an animal eventually develops adaptive immunity to an invader, the chances of successful invasion by that organism decline and the animal is said to be immune.

- The adaptive immune system is a complex and sophisticated system that provides the ultimate defense of the body. The loss of adaptive immunity leads inevitably to uncontrolled infections and death.
- One form of adaptive immunity is mediated by antibodies. Antibodies are proteins that circulate in body fluids, especially in the bloodstream. They bind

Con't..

- Another form of adaptive immunity is mainly directed against viruses. It is called cell-mediated immunity. This employs cells that destroy abnormal cells such as those infected by viruses.
- The adaptive immune system can remember prior exposure

□ **Table 1-1** | **Comparison of Innate and Adaptive Immunity**

	INNATE IMMUNITY ALWAYS "ON"	ADAPTIVE IMMUNITY TURNED ON BY ANTIGENS
Cells engaged	Macrophages, dendritic cells neutrophils, natural killer cells	T and B cells
Evolutionary history	Ancient	Recent
Onset	Rapid (minutes to hours)	Slow (days to weeks)
Specificity	Common microbial structures	Unique antigens
Potency	May be overwhelmed	Rarely overwhelmed
Memory	None	Significant memory
Effectiveness	Does not improve	Improves with exposure

Innate immunity

- Even if some of immunologists deal physical barriers as separate systems of body defense, innate immunity can be seen to comprise four types of defensive barriers: anatomic, physiologic, phagocytic, and inflammatory.

The Skin and the Mucosal Surfaces

- They prevent the entry of pathogens and they are an organism's first line of defense against infection

Con't..

- The skin and the surface of mucous membranes are included in this category because they are effective barriers to the entry of most microorganisms.
- Breaks in the skin resulting from scratches, wounds, or abrasion are obvious routes of infection. The skin may also be penetrated by biting insects (e.g., mosquitoes, mites, ticks, fleas, and sandflies); if these harbor pathogenic organisms, they can

Con't..

- The conjunctivae and the alimentary, respiratory, and urogenital tracts are lined by mucous membranes.
- Although many pathogens enter the body by binding to and penetrating mucous membranes, a number of nonspecific defense mechanisms tend to prevent this entry. For example, saliva, tears, and mucous secretions act to wash away potential invaders and also contain antibacterial or antiviral substances

Physiologic Barriers to Infection

- The physiologic barriers that contribute to innate immunity include temperature, pH, and various soluble and cell associated molecules. Many species are not susceptible to certain diseases simply because their normal body temperature inhibits growth of the pathogens. Chickens, for example, have innate immunity to anthrax because their high body

Con't..

- Gastric acidity is an innate physiologic barrier to infection because very few ingested microorganisms can survive the low pH of the stomach contents. One reason newborns are susceptible to some diseases that do not afflict adults is that their stomach contents are less acid than those of adults.

Con't..

- A variety of soluble factors contribute to innate immunity, among them the soluble proteins lysozyme, interferon, and complement. Lysozyme, a hydrolytic enzyme found in mucous secretions and in tears is able to cleave the peptidoglycan layer of the bacterial cell wall.

Con't..

- Interferon comprises a group of proteins produced by virus-infected cells. Among the many functions of the interferons is the ability to bind to nearby cells and induce a generalized antiviral state. Complement is a group of serum proteins that circulate in an inactive state

Pathogen recognition in innate immunity

- Many of the molecules involved in innate immunity have the property of pattern recognition, the ability to recognize a given class of molecules. Because there are certain types of molecules that are unique to microbes and never found in multicellular organisms, the ability to immediately recognize and combat invaders displaying such molecules is a

Con't..

- Molecules with pattern recognition ability may be soluble, like lysozyme and the complement components described above, or they may be cell-associated receptors . Among the class of receptors designated the toll-like receptors (TLRs), TLR2 recognizes the lipopolysaccharide (LPS) found on Gram-negative bacteria.

Pathogen-Associated Molecular Patterns

- Microbes not only grow very fast but also are highly diverse and can mutate and change many of their surface molecules very rapidly. For this reason, the innate immune system does not attempt to recognize all possible microbial molecules. Rather, the body uses receptors that can bind and respond to abundant, essential molecules that are common to many different microorganisms but are absent from normal animal tissues. Because they tend not to change rapidly, they are essential for microbial survival.

Con't..

- They are widely distributed molecular patterns.
- For example, the walls of Gram-positive bacteria are largely composed of peptidoglycans (chains of alternating N-acetylglucosamine and N-acetylmuramic acid cross-linked by short peptide side chains). Gram-positive bacterial cell walls also contain lipoteichoic acids. The cell walls of Gram-negative bacteria consist of peptidoglycans covered by a layer of lipopolysaccharide (LPS). Acid-fast bacteria are covered in glycolipids. Yeasts have a mannan- or β -glucan-rich cell wall. Viruses, in contrast, grow within infected host cells, so the

Con't..

Phagocytic Barriers to Infection (cells of innate immunity that ingest and destroy pathogens)

- Another important innate defense mechanism is the ingestion of extracellular particulate material by phagocytosis.
- Phagocytosis is one type of endocytosis, the general term for the uptake by a cell of material from its environment.

Con't..

- In phagocytosis, a cell's plasma membrane expands around the particulate material, which may include whole pathogenic microorganisms, to form large vesicles called phagosomes. Most phagocytosis is conducted by specialized cells, such as blood monocytes, neutrophils, and tissue macrophages.

Lymphocytes and Lymphoid organs

- Lymphocytes are the central cells of the immune system, responsible for adaptive immunity and the immunologic attributes of diversity, specificity, memory, and self/nonself recognition. Lymphoid Cells (Lymphocytes): constitute 20%–40% of the body's white blood cells and 99% of the cells in the lymph.

Con't..

- These lymphocytes continually circulate in the blood and lymph and are capable of migrating into the tissue spaces and lymphoid organs, thereby integrating the immune system to a high degree.
- The lymphocytes can be broadly subdivided into three populations—B cells, T cells and natural killer cells—on the basis of function and cell-membrane components. Natural killer cells (NK cells)

Con't..

- The T and B lymphocytes (T and B Cells) are involved in the acquired or antigen-specific immune response given that they are the only cells in the organism able to recognize and respond specifically to each antigenic epitope.
- The B Cells have the ability to transform into plasmocytes and are responsible for producing antibodies (Abs). Thus, humoral immunity depends on the B Cells while

Con't..

- **B lymphocytes:** The B lymphocyte derived its letter designation from its site of maturation, in the bursa of Fabricius in birds and the bone marrow is its major site of maturation in a number of mammalian species, including humans and mice.
- Mature B cells are definitively distinguished from other lymphocytes by their synthesis and display of membrane-bound immunoglobulin (antibody)

- **T lymphocytes:** T lymphocytes derive their name from their site of maturation in the thymus. Like B lymphocytes, these cells have membrane receptors for antigen. Although the antigen binding T-cell receptor is structurally distinct from immunoglobulin, the T-cell receptor (TCR) does not recognize free antigen. Instead the TCR recognizes only antigen that is bound to particular classes of self-molecules.
- Most T cells recognize antigen only when it is bound to a self-molecule encoded by genes within the major histocompatibility

Con't..

Natural killer cells: The natural killer cells are a small population of large, granular lymphocytes that display cytotoxic activity against a wide range of tumor cells in the absence of any previous immunization with the tumor. NK cells play an important role in host defense both against tumor cells and against cells infected with some viruses. They do not express the membrane molecules and receptors that distinguish T

Lymphoid Organs

- A number of morphologically and functionally diverse organs and tissues have various functions in the development of immune responses. These can be distinguished by function as the primary and secondary lymphoid organs.

Con't..

Primary Lymphoid Organs

- The organs that regulate the development of lymphocytes are called primary lymphoid organs. and rodents, and the intestinal lymphoid tissues in rabbits, ruminants, and pigs.
- The primary lymphoid organs all develop early in fetal life.

Con't..

- As animals develop, newly produced, immature lymphocytes migrate from the bone marrow to the primary lymphoid organs, where they mature.
- The primary lymphoid organs are not sites where lymphocytes encounter foreign antigens, and they do not enlarge in response to antigenic stimulation.

Thymus

- The thymus is located in the thoracic cavity in front of and below the heart.
- In horses, cattle, sheep, pigs, and chickens, it also extends up the neck as far as the thyroid gland.
- The size of the thymus varies, its relative size being greatest in the newborn animal and its absolute size being great

Con't..

- The thymus is the site of T-cell development and maturation. It is a flat, bilobed organ situated above the heart. Each lobe is surrounded by a capsule and is divided into lobules, which are separated from each other by strands of connective tissue called trabeculae.
- Each lobule is organized into two compartments: the outer compartment, or cortex, is densely packed with immature T cells, called thymocytes, whereas the inner compartment, or medulla, is sparsely populated with thymocytes.

- The function of the thymus is to generate and select a repertoire of T cells that will protect the body from infection.
- As thymocytes develop, an enormous diversity of T-cell receptors is generated by a random process that produces some T cells with receptors capable of recognizing antigen-MHC complexes.

Bone marrow

- In humans and other animals, bone marrow is the site of B-cell origin and development. Arising from lymphoid progenitors, immature B cells proliferate and differentiate within the bone marrow, and stromal cells within the bone marrow interact directly with the B cells and secrete various cytokines that are required for development

- Like thymic selection during Tcell maturation, a selection process within the bone marrow eliminates B cells with self-reactive antibody receptors. Bone marrow is not the site of B-cell development in all species. In birds, a lymphoid organ called the bursa of Fabricius,

Bursa of Fabricius

- The bursa of Fabricius is found only in birds. It is a round sac located just above the cloaca. The bursa reaches its greatest size in the chick about 1 to 2 weeks after hatching and then shrinks as the bird ages.
- It is very difficult to identify in older birds.

Peyer's Patches

- Peyer's patches (PPs) are primary lymphoid organs located in the walls of the small intestine. Their structure and functions vary among species.
- Thus in ruminants, pigs, horses, dogs, and humans (group I), 80% to 90% of the PPs are found in the ileum, where they form a single continuous structure that extends forward from the ileocecal junction.

Con't..

- In young ruminants and pigs, the ileal PPs may be as long as 2m. Ileal PPs consist of densely packed lymphoid follicles, each separated by a connective tissue sheath, and contain only B cells

Secondary lymphoid organs

- Mature lymphocytes leave the primary lymphoid organs to reside in secondary lymphoid organs, where their role is to encounter and respond to foreign antigens.
- In contrast to the primary lymphoid organs, the secondary lymphoid organs arise late in fetal life and persist in adults. Unlike primary lymphoid organs, they enlarge in response to antigenic stimulation.

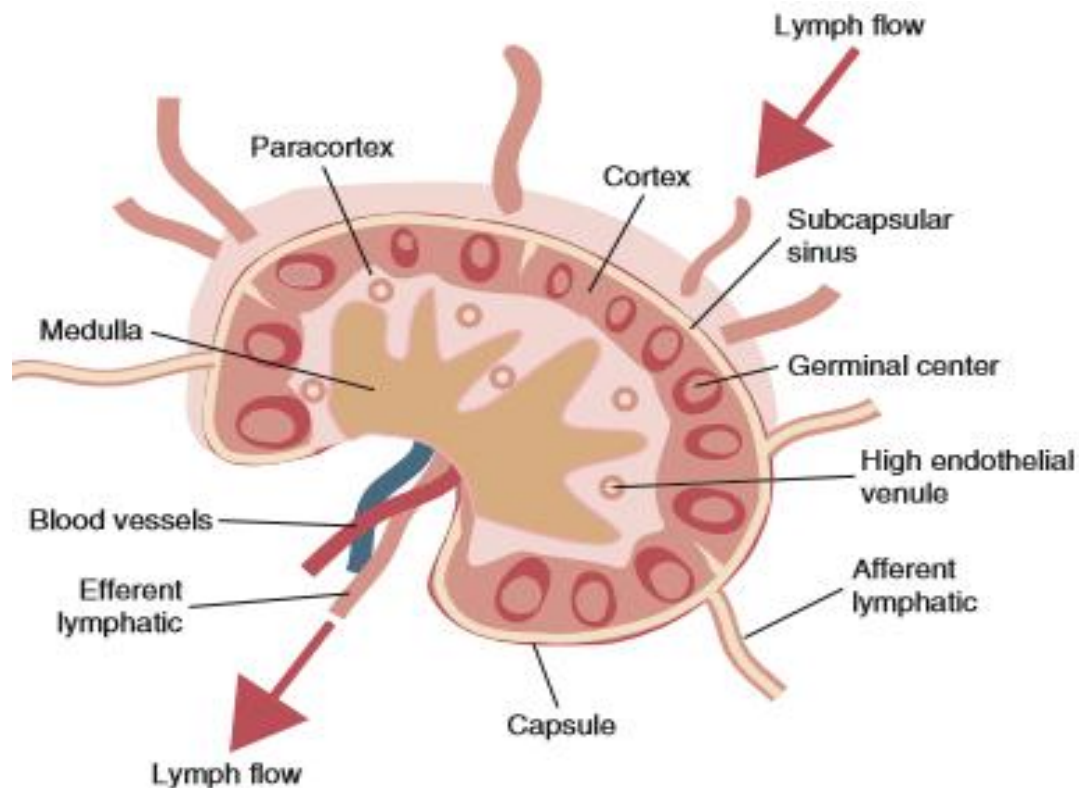
Con't..

- The major secondary lymphoid organs include lymph nodes, spleen, bone marrow, and some Peyer's patches within the intestine.

Lymph Nodes

- Lymph nodes are round or bean-shaped filters strategically placed on lymphatic vessels in such a way that they can sample antigens carried in the lymph

- Lymph nodes consist of a capsule beneath which is a reticular network filled with lymphocytes, macrophages, and dendritic cells and through which lymphatic sinuses penetrate. There is a filter f



Spleen

- The spleen plays a major role in mounting immune responses to antigens in the blood stream. It is a large, ovoid secondary lymphoid organ situated high in the left abdominal cavity. While lymph nodes are specialized for trapping antigen from local tissues, the spleen specializes in filtering blood and trapping blood-borne antigens; thus, it can respond to systemic

Con't..

- Unlike the lymph nodes, the spleen is not supplied by lymphatic vessels. Instead, bloodborne antigens and lymphocytes are carried into the spleen through the splenic artery.

Humoral (antibody-mediated) Immunity

Antibodies

- Antibodies are antigen binding proteins present on the B-cell membrane and secreted by plasma cells.
- Membrane-bound antibody confers antigenic specificity on B cells; antigen-specific proliferation of B-cell clones is elicited by the interaction of membrane antibody with antigen.

Con't..

- Secreted antibodies circulate in the blood, where they serve as the effectors of humoral immunity by searching out and neutralizing antigens or marking them for elimination.
- All antibodies share structural features, bind to antigen, and participate in a limited number of effector functions

Con't..

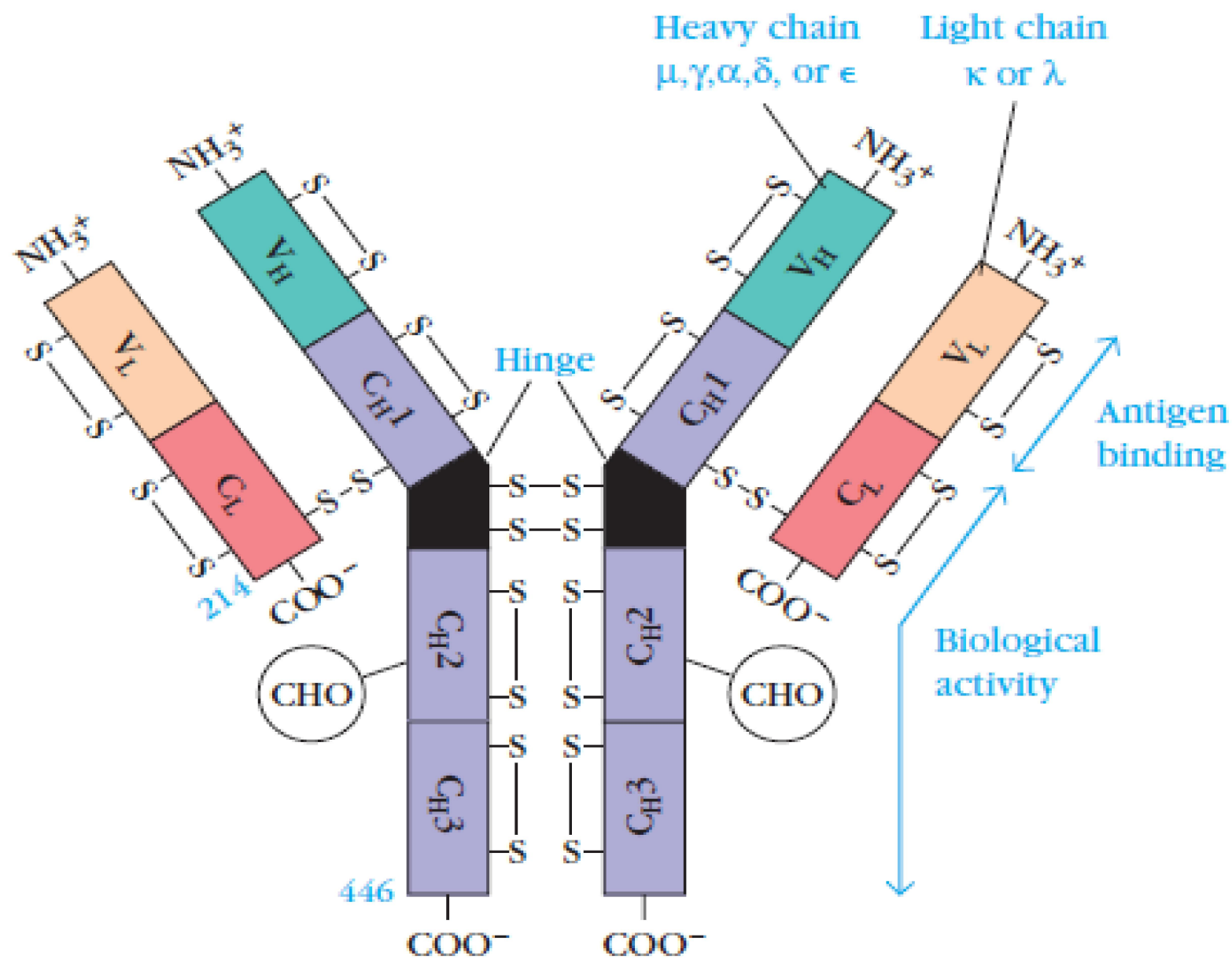
- Antibody molecules are also glycoproteins called immunoglobulins (abbreviated as Ig). The term immunoglobulin is used to describe all soluble B Cell Receptors.
- Blood can be separated in a centrifuge into a fluid and a cellular fraction. The fluid fraction is the plasma and the cellular fraction contains red blood cells, leukocytes, and platelets.

Con't..

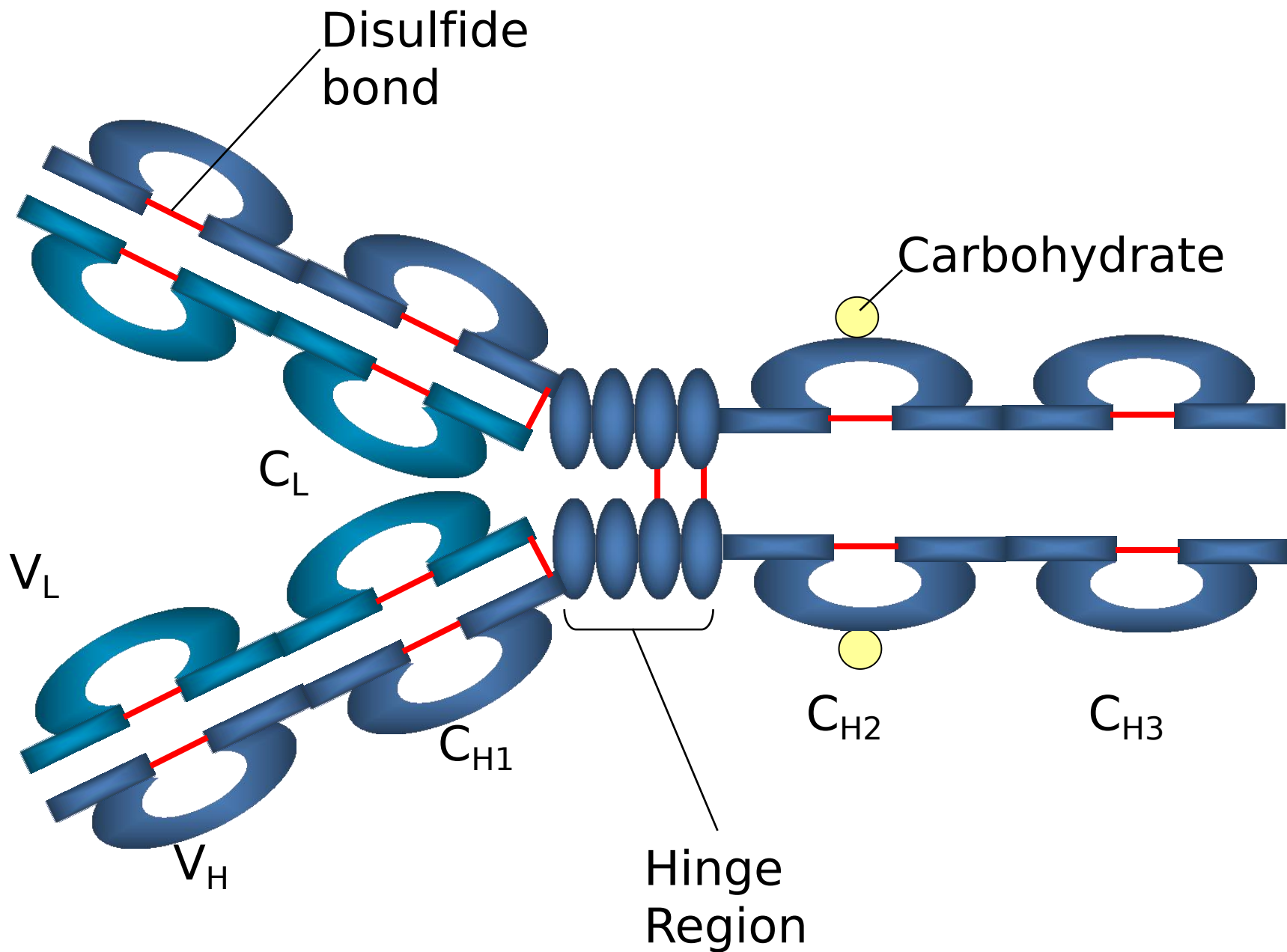
- Plasma contains all of the soluble small molecules and macromolecules of blood, including fibrin and other proteins required for the formation of blood clots. If the blood or plasma is allowed to clot, the fluid phase that remains is called serum and antibodies reside in the serum.

Structure of Antibodies

- Antibody molecules have a common structure of four peptide chains.
- This structure consists of two identical light (L) chains, polypeptides of about 25,000 molecular weight, and two identical heavy (H) chains, larger.
- Each light chain is bound to a heavy chain by a disulfide bond, and by such non covalent interactions as salt linkages,



Con't..



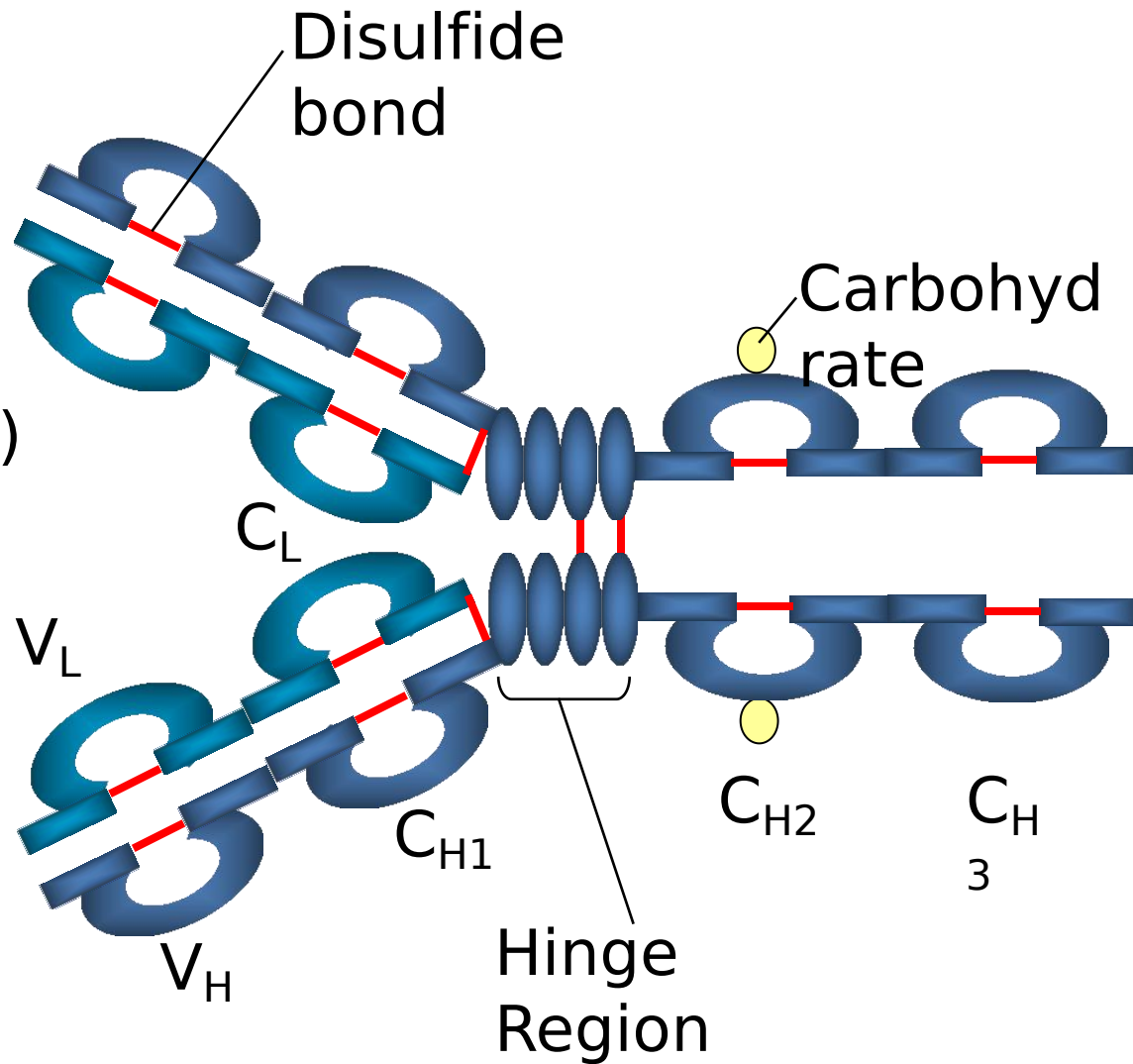
- The first 110 or so amino acids of the amino-terminal region of a light or heavy chain varies greatly among antibodies of different specificity.
- These segments of highly variable sequence are called *V regions*: V_L in light chains and V_H in heavy.
- All of the differences in specificity displayed by different antibodies can be

- In fact, most of the differences among antibodies fall within areas of the V regions called *complementarity-determining regions (CDRs)*, and it is these *CDRs*, on both light and heavy chains, that constitute the antigen binding site of the antibody molecule.

- The regions of relatively constant sequence beyond the variable regions have been dubbed C regions, C_L on the light chain and C_H on the heavy chain.
- Antibodies are glycoproteins; with few exceptions, the sites of attachment for carbohydrates are restricted to the constant region.
- We do not completely understand the role played by glycosylation of antibodies, but it

...Contd

- Domains
 - V_L & C_L
 - V_H & $C_{H1} - C_{H3}$ (or C_{H4})
- Oligosaccharides



Antigen-Binding Component

- The antigen-binding component of the BCR (or immunoglobulin) is a glycoprotein of 160 to 180 kDa consisting of four linked peptide chains.
- These chains consist of two identical pairs of heavy chains, each 60 kDa in size, and a pair of light chains, about 25 kDa each. The light chains are linked by disulfide bonds to

Con't..

- The tail of the Y (called the Fc region) is formed from paired heavy chains and is attached to the B cell surface. The arms of the Y (called the Fab regions) are formed by paired light and heavy chains, and they bind antigens.
- The antigen-binding sites are formed by the grooves between the light and heavy chains. Thus each BCR has two identical antigen-binding sites.

Immunoglobulin Classes

- IgG - Gamma (γ) heavy chains
- IgM - Mu (μ) heavy chains
- IgA - Alpha (α) heavy chains
- IgD - Delta (δ) heavy chains
- IgE - Epsilon (ϵ) heavy chains

Immunoglobulin Subclasses

IgG Subclasses(IgG1, IgG2, IgG3, IgG4 in mice and humans)

- IgG1,IgG2,IgG3 -in bovines
- IgA subclasses, most species
 - IgA1 - Alpha 1 (α 1) heavy chains
 - IgA2 - Alpha 2 (α 2) heavy chains
 - Rabbits have as many as 13 IgA subclasses.

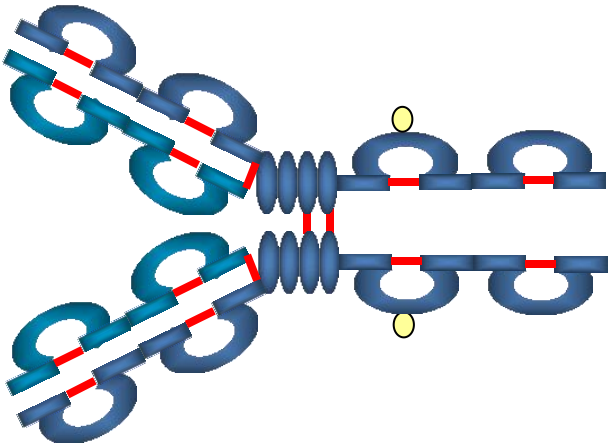
Immunoglobulin Light Chain Types

- Kappa (κ)
- Lambda (λ)

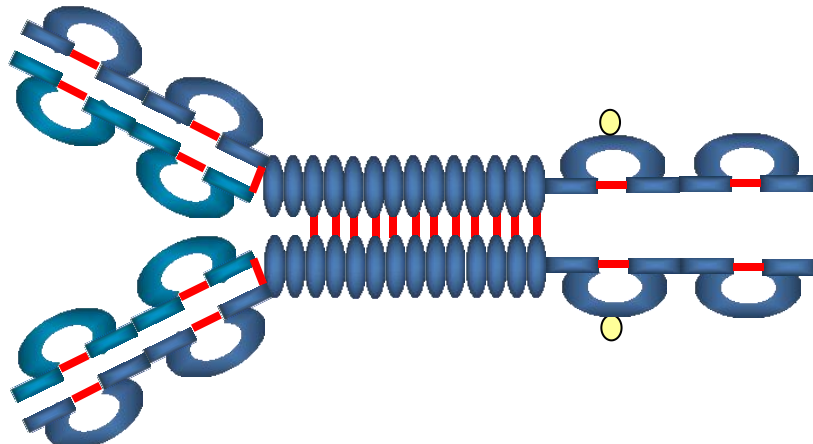
IgG

→ Structure

- Monomer (7S)



IgG1, IgG2 and
IgG4



IgG3

Con't..

- Properties
 - Major serum Immunoglobulin (Ig) (systemic immunity)
 - Major Ig in extravascular spaces
 - Placental transfer to the fetus and play an important role in protecting the developing fetus.
 - Fixes complement (except IgG4)
 - Binds to Fc receptors (low affinity, IgG2), (intermediate affinity, IgG4)
 - Phagocytes - opsonization
 - ADCC

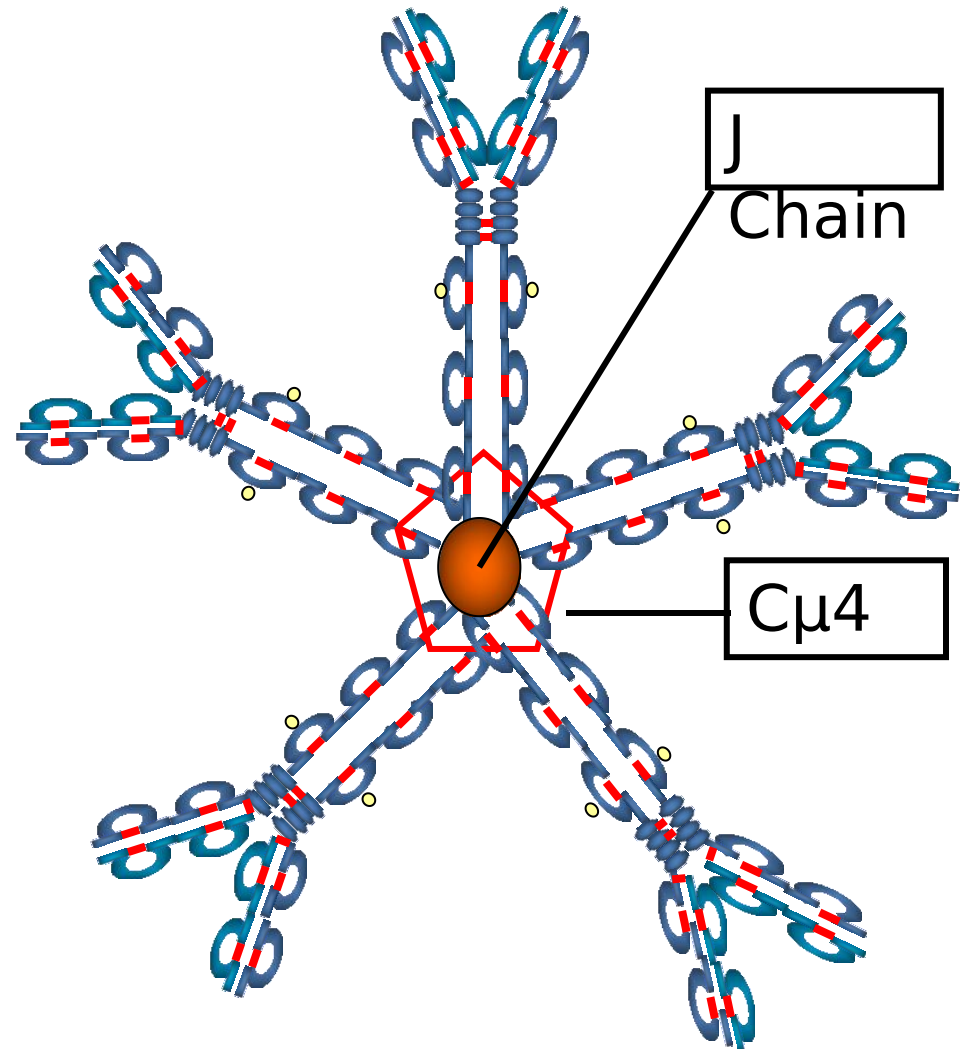
IgM

Structure

- Pentamer
- Extra domain
(C_{H4})

Properties

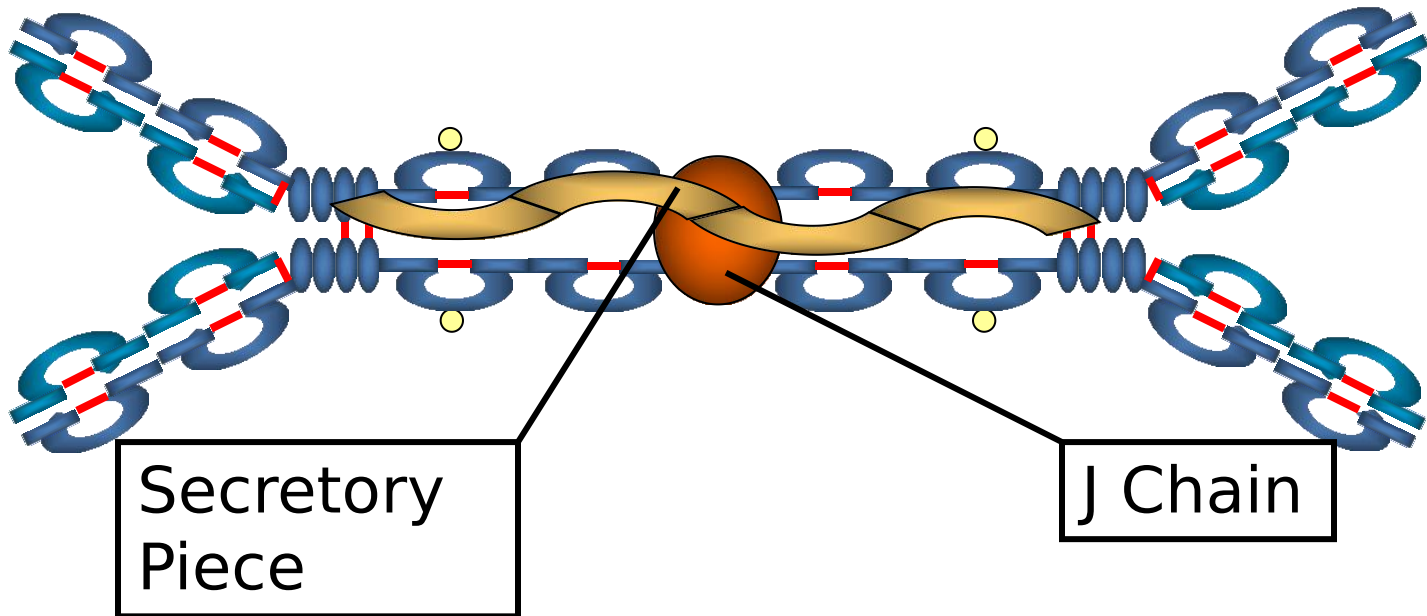
- 2nd highest serum Ig
- First Ig made by fetus and B cells
- Fixes complement
- Agglutinating Ig
- Binds to Fc receptors



IgA

Structure

- Serum - monomer
- Secretions (sIgA)
 - Dimer (11S)
 - J chain
 - Secretory component



...Contd

Properties

- Major secretory Ig (Mucosal or Local Immunity)
 - Tears, saliva, gastric and pulmonary secretions
- Does not fix complement (unless aggregated)
- Binds to Fc receptors on some cells

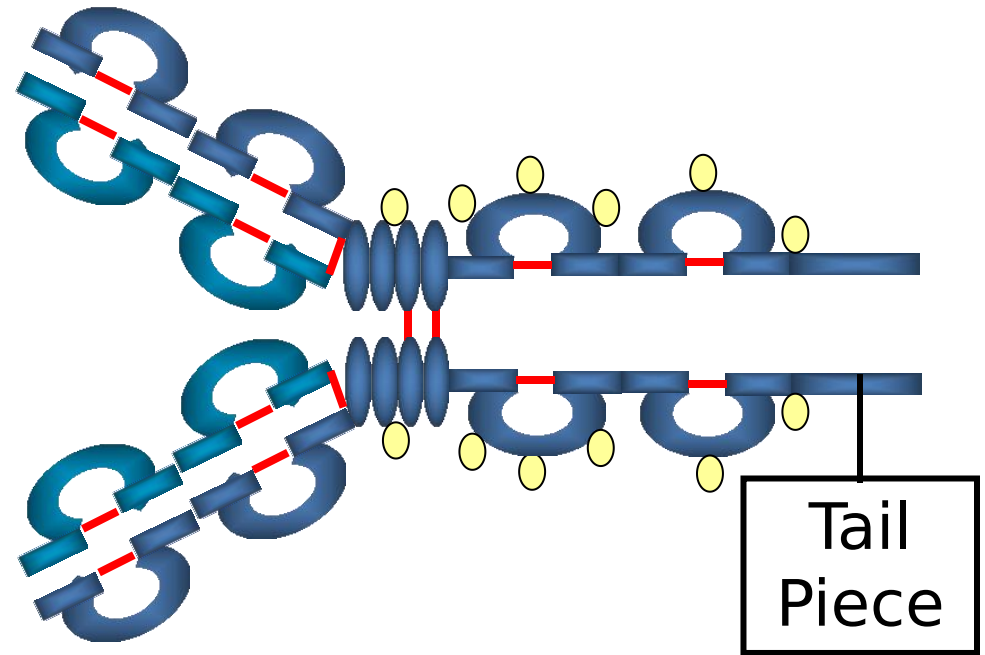
IgD

Structure

- Monomer, tail

Properties

- 4th highest serum Ig
- B cell surface Ig
- Does not bind complement
- Evolutionary labile and shows many variation in structure.



Not found in chickens

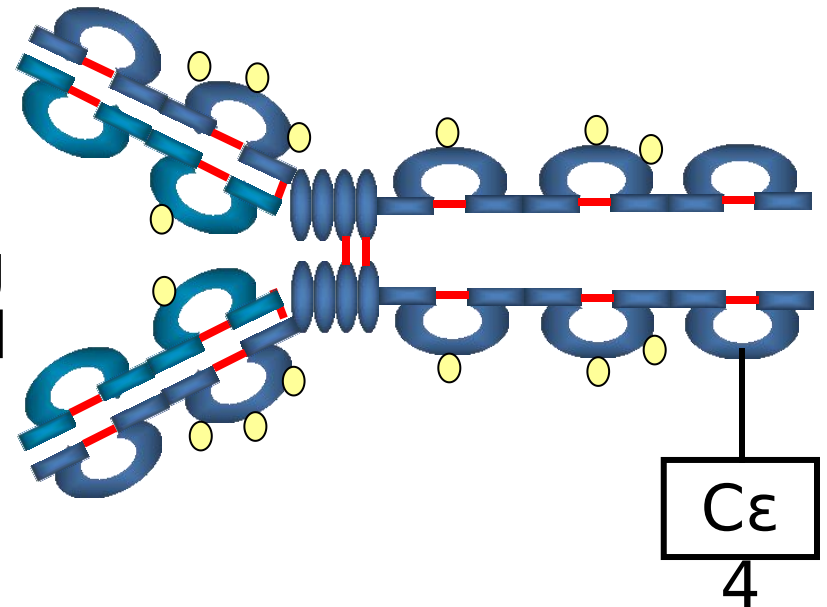
IgE

Structure

- Monomer
- Extra domain (C_{H4})

Properties

- Least common serum Ig
- Binds to basophils and mast cells (Does not require Ag binding)
- Allergic reactions
- Parasitic infections (Helminths)
- Binds to Fc receptor on eosinophils
- Does not fix complement
- Has shortest half life of all immunoglobulins



...Contd

Species	Immunoglobulin Levels (mg/dl)			
	IgG	IgM	IgA	IgE
Horse	1000-1500	100-200	60-350	8.4±9.09
Cattle*	1700-2700	250-400	10-50	
Sheep	1700-2000	150-250	10-50	
Pig	1700-2900	100-500	50-500	
Dog	1000-2000	70-270	20-150	2.3-4.2
Cat [†]	400-2000	30-150	30-150	
Chicken	300-700	120-250	30-60	
Human	800-1600	50-200	150-400	0.002-0.05

...Contd

Species	Immunoglobulin Classes				
	IgG	IgA	IgM	IgE	IgD
Horse	G1, G2, G3, G4, G5, G6	A	M	E	?
Cattle	G1, G2, G3	A	M	E	D
Sheep	G1, G2, G3	A1, A2	M	E	D
Pig	G1, G2a, G2b, G3, G4	A	M	E	D
Dog	G1, G2, G3, G4	A	M	E1, E2	D
Cat	G1, G2, G3, (G4?)	(A2?)	M	(E1, E2?)	?
Chimpanzee	G1, G2, G3,	A1, A2	M	E	D
Mouse	G1, G2a, G2b, G3	A1, A2	M	E	D
Human	G1, G2, G3, G4	A1, A2	M	E	D

Antibody-Mediated biological Functions

- In addition to binding antigen, antibodies participate in a broad range of other biological activities.
- In order to be effective against pathogens, antibodies must not only recognize antigen, but also activate responses, **effector functions**, that will result in removal of the antigen and death of the pathogen.

Con't..

- Because these effector functions result from interactions between heavy-chain constant regions and other serum proteins or cell-membrane receptors, not all classes of immunoglobulin have the same functional properties.

Opsonization is Promoted by Antibody

- Opsonization, the promotion of phagocytosis of antigens by macrophages and neutrophils, is an important factor in antibacterial defenses.
- Protein molecules called Fc receptors (FcR), which can bind the constant region of Ig molecules are present on the surfaces of macrophages and neutrophils.
- The binding of phagocyte Fc receptors with several antibody molecules complexed with the same target, such as a bacterial cell, produces an interaction that results in the binding of the pathogen to the phagocyte membrane.

...Contd

- This cross linking of the FcR by binding to an array of antibody Fc regions initiates a signal-transduction pathway that results in the phagocytosis of the antigen-antibody complex.
- Inside the phagocyte, the pathogen becomes the target of various destructive processes that include enzymatic digestion, oxidative damage, and the membrane-disrupting effects of antibacterial peptides.

Antibodies Activate Complement

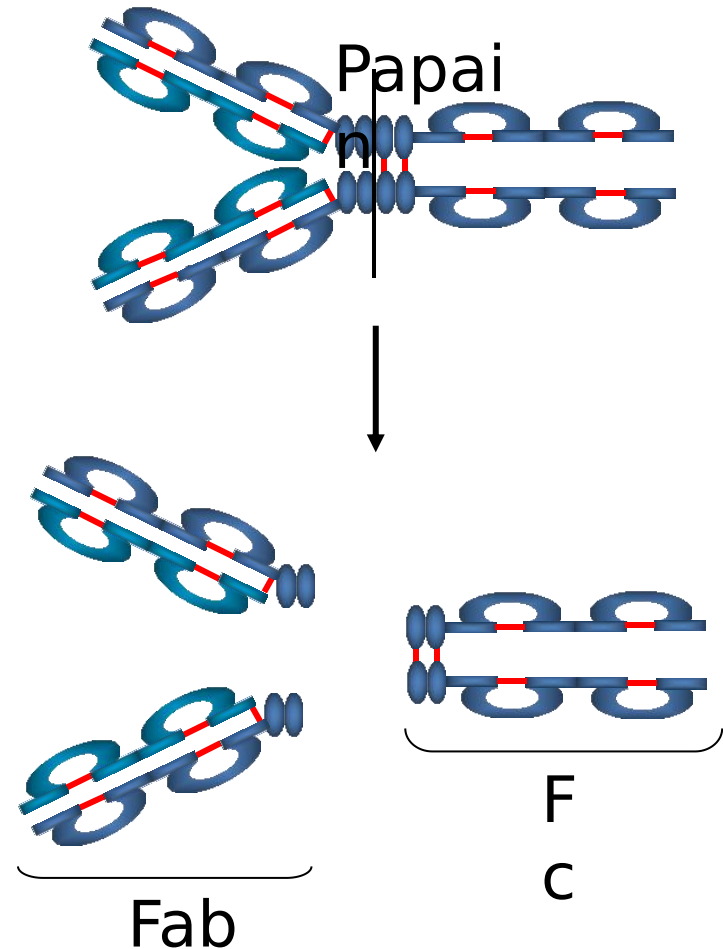
- IgM and most IgG subclasses can activate a collection of serum glycoproteins called the **complement system**.
- Complement includes a collection of proteins that can perforate cell membranes.
- An important by product of the complement activation pathway is a protein fragment called C3b, which binds non specifically to cell- and antigen-antibody complexes near the site at which complement was activated.
- Many cell types—for example, red blood cells and macrophages—have receptors for C3b and so bind cells or complexes to which C3b has adhered.
- Binding of adherent C3b by macrophages leads to phagocytosis of the cells or molecular complexes attached to C3b.

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) Kills Cells

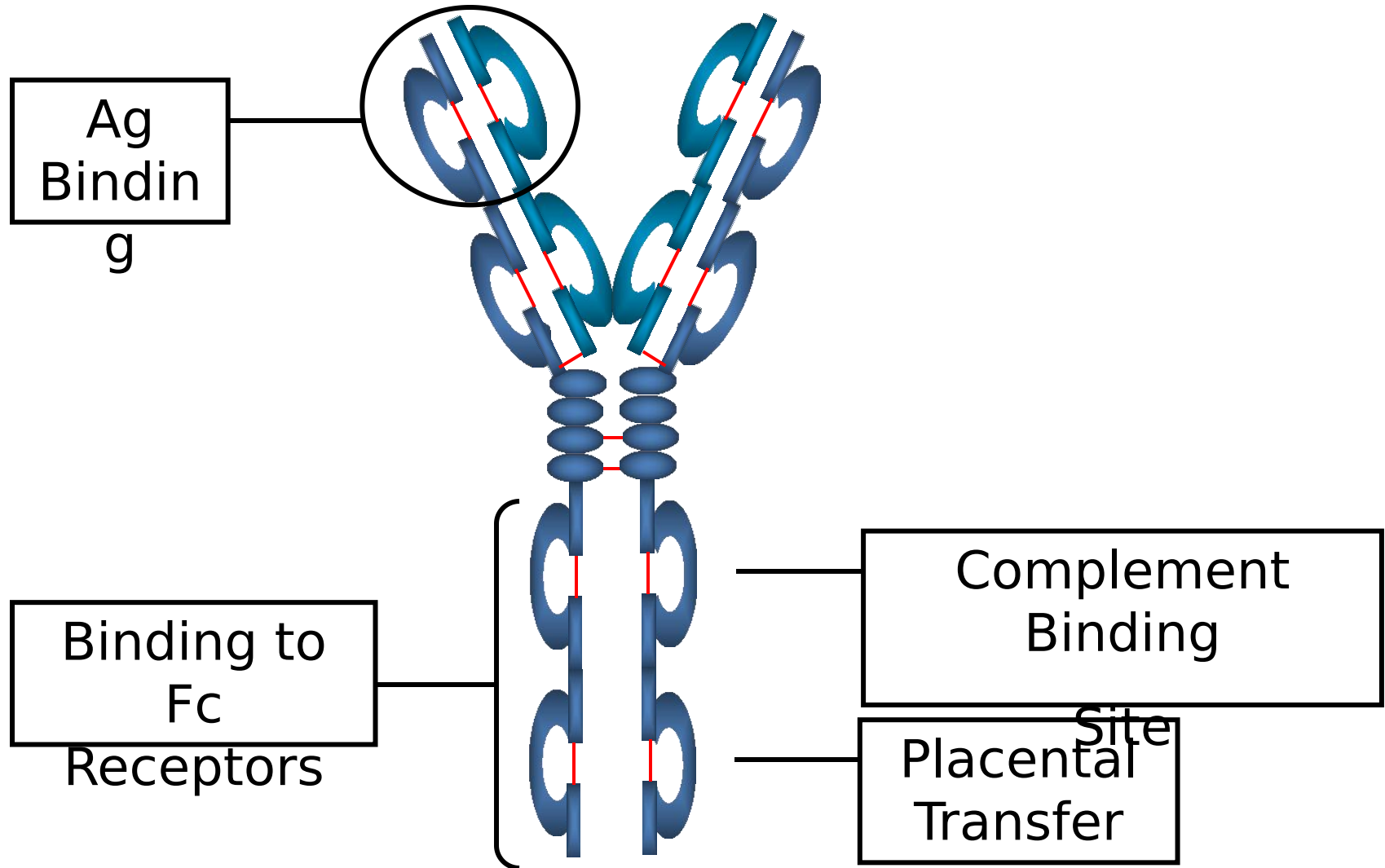
- 1 The linking of antibody bound to target cells (virus infected cells of the host) with the Fc receptors of a number of cell types, particularly natural killer (NK) cells, can direct the cytotoxic activities of the effector cell against the target cell.
- 2 In this process, called **antibody-dependent cell-mediated cytotoxicity (ADCC)**, the **antibody acts as a newly acquired receptor** enabling the attacking cell to recognize and kill the target cell.

Immunoglobulin Fragments: Structure/Function Relationships

- Fab
 - Ag binding
 - Valence = 1
 - Specificity determined by V_H and V_L
- Fc
 - Effector functions



Immunoglobulin Fragments: Structure/Function Relationships



Regulation of antibody production: overview

- B cells express multiple identical antigen binding receptors (BCRs) on their surface.
- When BCRs are shed into body fluids, they are called immunoglobulins or antibodies.
- B cells can recognize most antigens without prior processing. However, an optimal B cell response normally requires additional stimulation by helper T cells.

Con't..

- Helper T cells stimulate B cells through an immunologic synapse containing costimulatory molecules and interactive receptors. B cells also require co-stimulation by cytokines. Responding B cells may become either memory cells or antibody secreting plasma cells.
- Plasma cells are the progeny of B cells that have differentiated to secrete very large amounts of antibodies.

Antigenicity vs immunogenicity

- ⌘ Substances which can generate an immune response (humoral and/or cell mediated) are called immunogens and this ability of a substance is called immunogenicity.
- ⌘ antigens are the substances which react specifically with the products of an immune response, the effector molecules (Ab) / effector cells (CTLs) for elimination of foreign molecules. This property is known as antigenicity.

Con't..

- ⌘ All immunogens are antigens while the reverse is not true.
- ⌘ proteins, nucleoproteins, polysaccharides and glycolipids can act as antigens.
- ⌘ Proteins are known to be the most potent antigens and polysaccharides are second in the list.
- ⌘ To be antigenic a substance must first be recognized as foreign.

Factors Affecting Immunogenicity

Foreignness

- Immunocompetent lymphocytes during their maturation learn not to respond to self antigens, a property known as self tolerance.
- Any self molecule not exposed to maturing lymphocytes later is recognized as foreign/ non self by the immune system .
- e.g., the antigens sequestered from the immune system as sperm and corneal antigens.
- the degree of immunogenicity depends on the degree of its foreignness as Bovine Serum Albumin (BSA) is not immunogenic in cow but is in rabbit.
- In addition BSA shows more immunogenicity in chicken as compared to a goat.

Molecular size

- Most antigens are large, complex molecules with a molecular weight generally greater than about 100,000 daltons.
- In general large molecules are better immunogens as compared to smaller molecules.

Chemical composition

- Simple repetitive polysaccharides do not assume a stable configuration hence are poor immunogens.
- Copolymers instead of homopolymers are found to be good immunogens.
- all four levels of protein organization—primary, secondary, tertiary, and quaternary—contribute to the structural complexity of a protein and hence affect its immunogenicity.

Degardability

- The T helper cells (Th) cells are required to be activated and help in the development of both the humoral and cell mediated immune response.
- an antigen has to be processed and presented with MHC molecules for development of immune response,
- the macromolecules that cannot be degraded and presented are poor immunogens. e.g., Polymers containing D-amino acids cannot be degraded and processed by the degradative enzymes of the antigen presenting cells (APCs) and serve as poor immunogens.
- Large, insoluble macromolecules generally are more immunogenic than small, soluble ones because the larger molecules are more readily

Infected host and immunogenicity

- the genetic makeup of the host also contributes to the immunogenicity.

Genotype of the recipient individual

- The genes that encode MHC, B cell and T cell receptors and various proteins for immune regulation are known to influence the type and the intensity of the immune response

Dosage and route of administration

- ✚ An insufficient dose and an excessive high dose act as poor antigens.
- ✚ A lower dose can either fail to activate enough lymphocytes or they induce a nonresponsive state.
- ✚ Too high a dose also fails to induce an immune response because it induces a nonresponsive state in the lymphocytes.
- ✚ Thus a minimal optimal dose and also a repeated administration (booster doses) are required to stimulate a strong immune response by increasing the proliferation of specific T and B cells.

Contd...

- The route of administration influences the cells and organs involved in an immune response.
- Most experimental immunogens are administered parenterally (around the gut). Other common routes of administration are intravenous, intradermal, subcutaneous, intramuscular and intraperitoneal.
- It is the route of administration which decides the immune organs and cell populations which will take part in the immune response.
- Antigen administered intravenously is carried first to the spleen, whereas antigen administered subcutaneously moves first to local lymph nodes. Differences in the lymphoid cells that populate these organs may be reflected in the subsequent immune response.

Cell-mediated Immunity

T-cell subsets/ T-cell heterogeneity

- T cells are so called because they are predominantly produced in the thymus. They recognize foreign particles (antigen) by a surface expressed, highly variable, T cell receptor (TCR). T cells express distinctive membrane molecules. All T-cell subpopulations express the T-cell receptor, a complex of polypeptides that includes CD3; and most can be distinguished by the

Con't..

- In addition, most mature T cells express the following membrane molecules:
- CD28, a receptor for the co-stimulatory B7 family of molecules present on B cells and other antigen presenting cells CD45, a signal-transduction molecule
- There are two major types of T cells: the helper T cell and the cytotoxic T cell. As the names suggest helper T cells 'help' other cells of the immune system, whilst cytotoxic T cells kill virally infected cells and tumours

Con't..

- Unlike antibody, the TCR cannot bind antigen directly. Instead it needs to have broken-down peptides of the antigen 'presented' to it by an antigen presenting cell (APC).
- The molecules on the Antigen Presenting Cells that present the antigen are called major histocompatibility complexes (MHC).

Con't..

- T cells that express the membrane glycoprotein molecule CD4 are restricted to recognizing antigen bound to class II MHC molecules, whereas T cells expressing CD8, a dimeric membrane glycoprotein, are restricted to recognition of antigen bound to class I MHC molecules.
- Thus the expression of CD4 versus CD8 corresponds to the MHC restriction of the T

- In general, expression of CD4 and of CD8 also defines two major functional subpopulations of T lymphocytes. CD4+ T cells generally function as T helper (TH) cells and are class-II restricted; CD8+ T cells generally function as T cytotoxic (TC) cells and are class-I restricted. Thus the ratio of TH to TC cells in a sample can be approximated by assaying the number of

Con't..

- There are two types of MHC: MHC class I and MHC class II. MHC class I presents to cytotoxic T cells; MHC class II presents to helper T cells.
- The binding of the TCR to the MHC molecule containing the antigen peptide is a little unstable and so co-receptors are required. The CD4 co-receptor is expressed by helper T cells and the CD8

Con't..

- TH cells are activated by recognition of an antigen-class II MHC complex on an antigen-presenting cell. After activation, the TH cell begins to divide and gives rise to a clone of effector cells, each specific for the same antigen-class II MHC complex.
- These TH cells secrete various cytokines, which play a central role in the activation of B cells, T cells, and other cells that participate in the immune response.

Con't..

- TC cells are activated when they interact with an antigen-class I MHC complex on the surface of an altered self-cell (e.g., a virus-infected cell or a tumor cell) in the presence of appropriate cytokines. This activation, which results in proliferation, causes the TC cell to differentiate into an effector cell called a cytotoxic T lymphocyte (CTL).

Con't..

- In contrast to TH cells, most CTLs secrete few cytokines. Instead, CTLs acquire the ability to recognize and eliminate altered self-cells.
- Another subpopulation of T lymphocytes—called T suppressor (TS) cells—has been present. These T cells help to suppress the humoral and the cell-mediated branches of the immune system,

Delayed hypersensitivity reaction

- When certain antigens are injected into the skin of a sensitized animal, an inflammatory response, taking many hours to develop, may occur at the injection site. This is a T cell- mediated response called delayed hypersensitivity.
- Delayed hypersensitivity reactions are classified as type IV hypersensitivity reactions.

Con't..

- An important example of a delayed hypersensitivity reaction is the tuberculin response—the skin reaction that follows an intradermal injection of tuberculin.
- A different form of type IV hypersensitivity occurs in allergic contact dermatitis. This is a slowly developing inflammatory response that occurs when reactive chemicals bind to skin cells and trigger T

Cytokines

- The immune responses result from multiple interactions among many different cell populations.
- One way that these cells interact is by secreting specialized signaling molecules such as cytokines and hormones.
- These signaling molecules bind to specific receptors on target cells. When signaling molecules bind to their receptors,

- These transcription factors then activate the transcription of selected genes.
- As a result of changes in gene transcription, new proteins are produced and secreted, and affected cells alter their behavior, perhaps by dividing or even dying on command.

Con't..

- Cytokines differ from conventional hormones in several important respects. For example, unlike conventional hormones, which tend to affect a single target cell type, cytokines can affect many different cell types.
- Second, immune system cells rarely secrete a single cytokine at a time. For instance, macrophages secrete at least four interleukins (IL-1, IL-6, IL-12, IL-18) as well as tumor necrosis factor- α (TNF- α).

- Third, cytokines are “redundant” in their biological activities in that many different cytokines have similar effects. For example, IL-1, TNF- α , TNF- β , IL-6, high mobility group box protein-1 (HMGB1), and the chemokine CCL3 all act on the brain to cause a fever. Finally, cytokine-mediated signals are transient, and the messages delivered may vary over time as the cytokine environment changes.

- The nomenclature and classification of the cytokines is not based on any systematic relationship among these proteins. Many were originally named after their cell of origin or the bioassay used to identify them. The interleukins, for example, are cytokines that mediate signaling between lymphocytes and other leukocytes. They are numbered sequentially in the order of

Con't..

- The interferons are cytokines produced in response to virus infection or immune stimulation. Their name is derived from the fact that they interfere with viral RNA and protein synthesis and so have antiviral activity. There are three types of interferon. Type I interferons are a diverse family, the most important of which are interferon- α (IFN- α) and IFN- β .

- There is a single type II interferon, called IFN- γ . Three type III interferons (IFN- λ) have been identified. Type I interferons are primarily antiviral with a secondary immunoregulatory role.
- Tumor Necrosis Factors, TNFs are cytokines secreted by macrophages and T cells. As their name suggests, they can kill tumor cells, although this is not their primary function. Thus TNF- α is the key mediator of acute inflammation.

Organ graft rejections

- Organ grafts between two unrelated individuals of the same species are called allografts. Allografts are transplanted between genetically different members of the same species. Most grafts performed on animals or humans for therapeutic reasons are of this type because tissues are obtained from a donor who is usually

Con't..

- Allografts are rejected by the recipient as a result of strong immune responses directed against donor blood group antigens and histocompatibility antigens.
- The response to donor histocompatibility antigens causes acute rejection and is mainly mediated by cytotoxic T cells attacking graft vascular endothelium.
- This rejection process must be suppressed if

Con't..

- The allograft rejection process is directed against the dominant antigens on the cells of the graft. The MHC molecules tend to trigger a T cell-mediated rejection response, whereas the blood group antigens tend to trigger antibody formation.
- The rejection process may be divided into two stages. First, the host's lymphocytes encounter the antigens of the graft and

The Major Histocompatibility Complex (MHC)

- In order to trigger an adaptive immune response, antigen molecules must be broken up inside cells, and the fragments generated must then be bound to appropriate antigen-presenting receptors. These antigen-presenting receptors are glycoproteins encoded by genes located in a large gene cluster called the major histocompatibility complex (MHC). The receptors are therefore called MHC molecules.

Con't..

- Antigen fragments can trigger an immune response only after they have bound to MHC molecules, and these antigen-MHC complexes have bound to T cell antigen receptors.
- Since the MHC molecules serve as specific antigen receptors, MHC genes determine which antigens can trigger adaptive immunity. Thus MHC can be considered an organized cluster of genes that control antigen presentation and so determine an animal's susceptibility to infectious or autoimmune diseases.

MHC complex

- ♣ Group of genes on a single chromosome encoding the MHC antigens
 - **HLA** (human leukocyte antigens): MHC antigens of man (first detected on leukocytes)
 - H-2 antigens**: MHC antigens of mouse
 - B antigens**: MHC antigens of chickens
 - BoLA**: MHC antigens of cattle
 - DLA**: MHC antigens of dogs
 - SLA**: MHC antigens of swine, and so forth

General organization and Inheritance of the MHC

- All vertebrate possess **histocompatibility** molecules that are normally clustered within the MHC complex
- Whose products play a role in interacellular recognition and discrimination between self and non-self
- The MHC encodes three major classes of molecules
- The major histocompatibility complex is a collection of genes arranged within a long continuous stretch of DNA on chromosomes although the arrangement of genes is what different in both cases the MHC genes are

MHC genes are organized into 3 classes of gene loci encoding 3 classes of molecules

- A. **Class I MHC genes:** encode glycoprotein expressed on the surface of most nucleated cells
 - The major function of the MHC class I gene products is the presentation of peptide antigens to Tc cells (cytotoxic T cells)
- B) **Class II MHC genes:** encode glycoprotein (MHC molecules) expressed primarily on antigen-presenting cells (macrophage, dendritic cells and B-cells) where they present processed antigen peptides to T helper cells
- C) **Class III MHC genes:** encode complement component (C4, C2 and factor B), several inflammatory cytokines, including TNF, and other products that have no immune function

Therefore the two major biological functions of MHC are in

- Antigen processing and presentation and organ graft rejection

Antigen processing and presentation

Antigen Presenting Cells (APCs)

Cells that are specialized to initiate or promote the development of lymphocyte activation

Include Dendritic Cells, Macrophages and B cells

Dendritic Cells (DCs) are the most effective of the APCs

Reasons- Constitutively express a high level of class

II MHC molecules

- Have costimulatory membrane molecules such as B7

Macrophages must be activated by phagocytosis of particulate antigens before they express class II MHC molecules or costimulatory molecules (B7)

4.2.1. Antigen-Processing and Presentation

- Processing of antigen is required for recognition by T cells
- Most cells can present antigen with class I MHC; presentation with class II MHC is restricted to APCs
- As a general rule,
 -] **Endogenous antigen** is processed in the **cytosolic pathway** and presented on the membrane with **class I MHC molecules or**
 -] **Antigen that have been produced within the infected cell (e.g; normal cell proteins, tumor protein, viral or bacterial protein) and expressed bound with class I MHC**
 -] **Exogenous antigen** is processed in the **endocytic pathway** and presented on the membrane with **class II MHC molecules or**

- APC carries about 2×10^5 MHC class II molecules
 - one APC can present many different antigens simultaneously
- A cell can express about 10^6 MHC class I-peptide complexes

Immunodiagnostic techniques

By Betelihem Tegege (DVM, MVSc in vet. Microbiology)

Immunodiagnostic techniques

- Immune responses are used in two ways to diagnose disease.
- First, specific antibodies may be used to detect or identify an antigen of interest. These antigens can be associated with an infectious agent, or can simply be molecules that need to be located or measured.
- Second, by detecting specific antibodies in serum it is possible to determine whether

- This may establish a diagnosis or determine the degree of exposure of the population to that agent.
- The measurement of antigen-antibody interactions for diagnostic purposes is called serology.

- ⌘ Serological techniques can be classified into three broad categories.
 - Primary binding tests directly measure the binding of antigen to antibody.
 - Secondary binding tests measure the results of antigen-antibody interaction in vitro.
 - The third category, in vivo tests, measure the actual protective effect of antibodies

I. Primary binding tests

- Performed by allowing antigen (Ag) and antibody (Ab) to combine and then measuring the immune complexes formed
- In order to measure these reactions, one of the reactants must be chemically labeled
 - ↳ Radioisotopes (Tritium [H^3], carbon 14, iodine 125)

- Fluorescent dyes (Fluorescein isothiocyanate [FITC], Rhodamine)
- Enzymes (Horseradish peroxidase [HRP], alkaline phosphatase)

Examples of primary binding tests:

- Radioimmunoassays
- Immunofluorescence Assays
- Enzyme-Linked Immunosorbent Assays (ELISA)
- Western Blotting

Immunofluorescence Assays

1. Fluorescent antibody tests (FAT)

- Widely used in the serological diagnosis of bacterial, viral, fungal, and parasitic diseases

Advantage

- Sensitive and give reproducible results.
- **Sensitivity** is the ability of a test to correctly classify an animal as diseased (detect the positive results)

Disadvantages

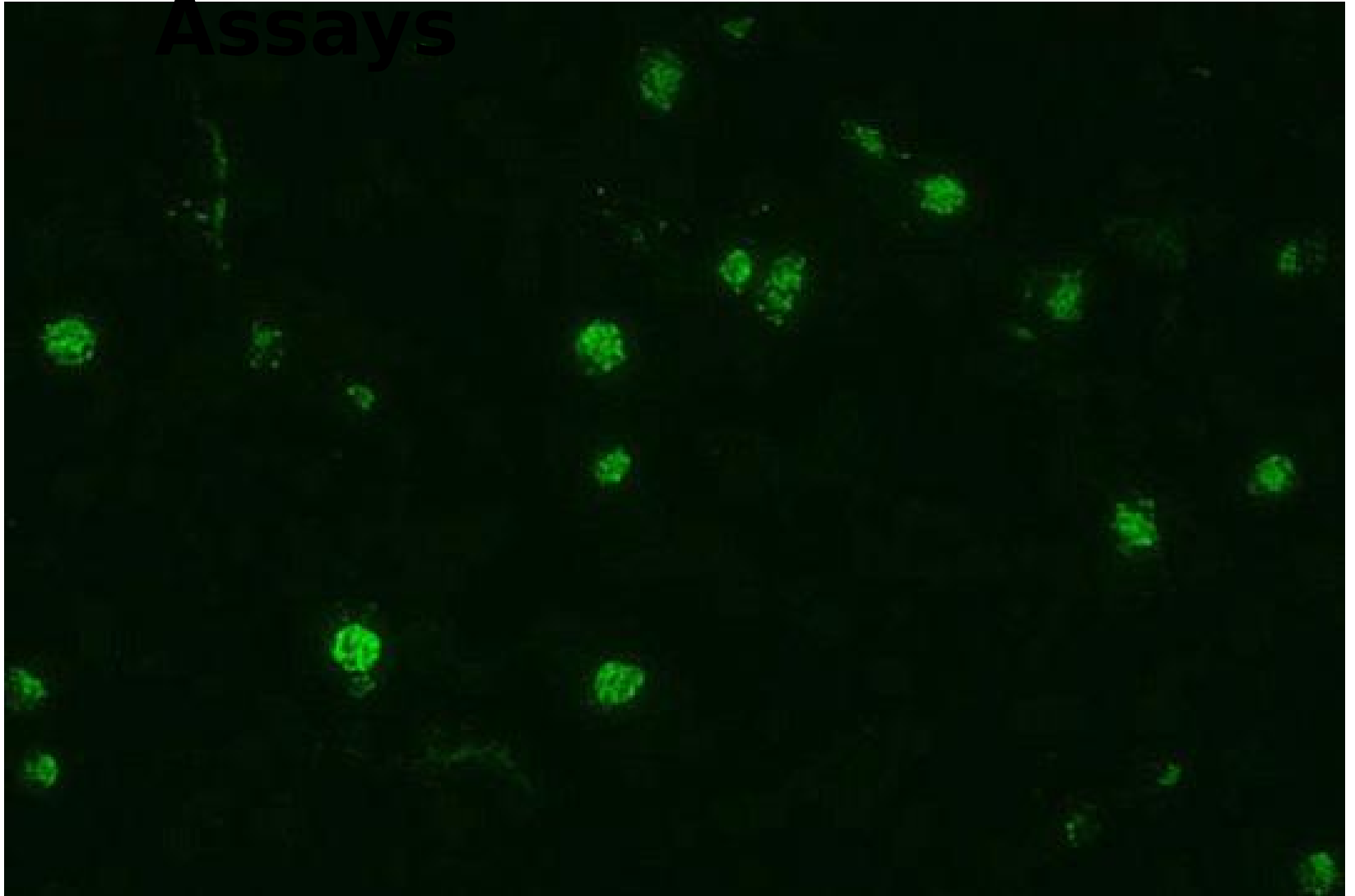
- Special training is needed to perform and read Immunofluorescence tests.
- Fluorescence microscope and high quality reagents are required.

Principle

- Fluorescent dyes (fluorochromes) illuminated by UV lights are used to show the specific combination of an **antigen** with its **antibody**.
- The **antigen-antibody complexes** are seen fluorescing against a dark background.

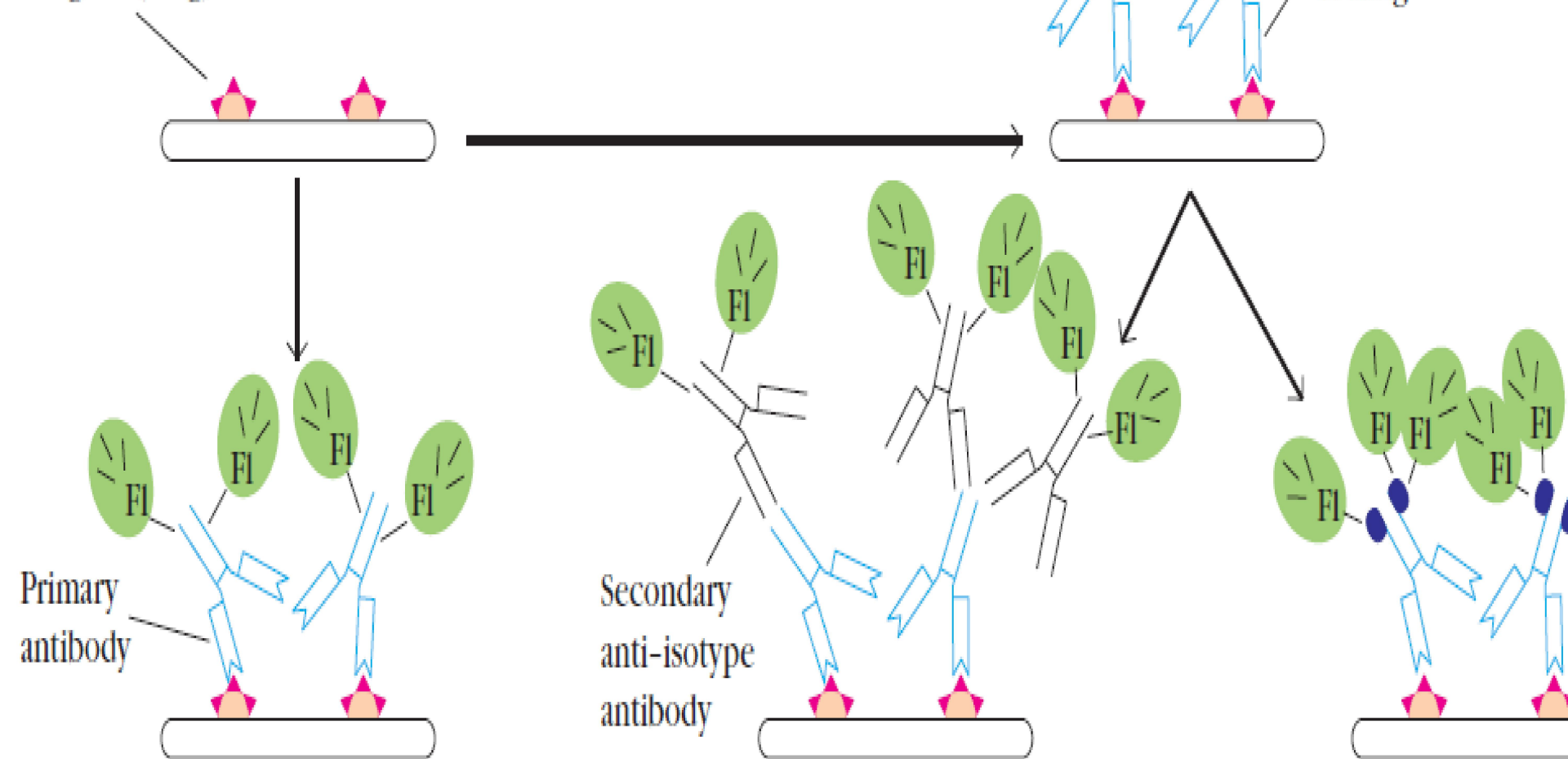
Fluorescein isothiocyanate [FITC]
emits visible green light

Immunofluorescence Assays



- **Direct FAT** is used to detect and identify an **unknown antigen** in specimens
- The **indirect FAT** is used in two main ways:
 - └ To detect and identify **unknown antigen** in specimens
 - └ To detect **antibodies** in a patient serum using a **known antigen** (microorganism).

Cells with membrane antigens (mAg)



(a) Direct method with fluorochrome-labeled antibody to mAg

(b) Indirect method with fluorochrome-labeled anti-isotype antibody

(c) Indirect method with labeled protein A

Enzyme-Linked Immunosorbent Assays (ELISA)

- ELISA techniques are becoming increasingly used in the diagnosis of microbial infections.
- They are **specific, sensitive**, and require only a small amount of specimen.
- **Sensitivity** is the ability of a test to correctly classify an animal as diseased
- **Specificity** is the ability of a test to correctly classify an animal as healthy.
- Large numbers of specimens can be tested

Principles

- As its name suggests, the **enzyme linked-immunosorbent assay** uses an **enzyme system** to show the specific combination of an **antigen** with its **antibody**.
- An **enzyme conjugated** with an **antibody** reacts with a **colorless substrate** to generate a **colored** reaction product.
- A number of variations of ELISA have been developed

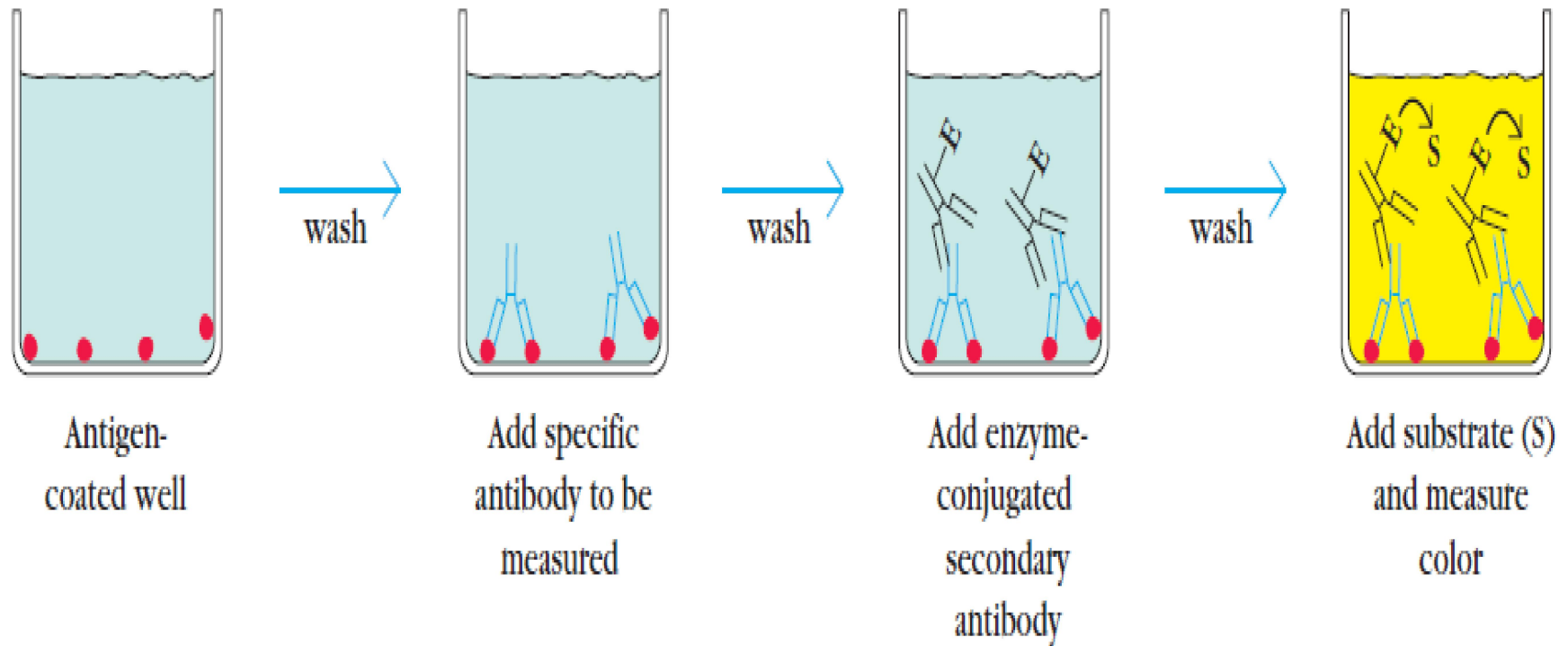
Indirect ELISA

- The most common form of ELISA is used to detect and measure specific antibodies.
- microwells in polystyrene plates are first filled with an antigen solution
- The serum under test is added to the wells. Any antibodies in the serum will

- After incubation and washing to remove unbound antibody, the presence of any bound antibodies can be detected by adding a solution containing an antiglobulin chemically linked to an enzyme.
- This labeled antiglobulin binds to the antibody and, following incubation and washing, can be detected and measured by adding a solution containing the enzyme substrate.

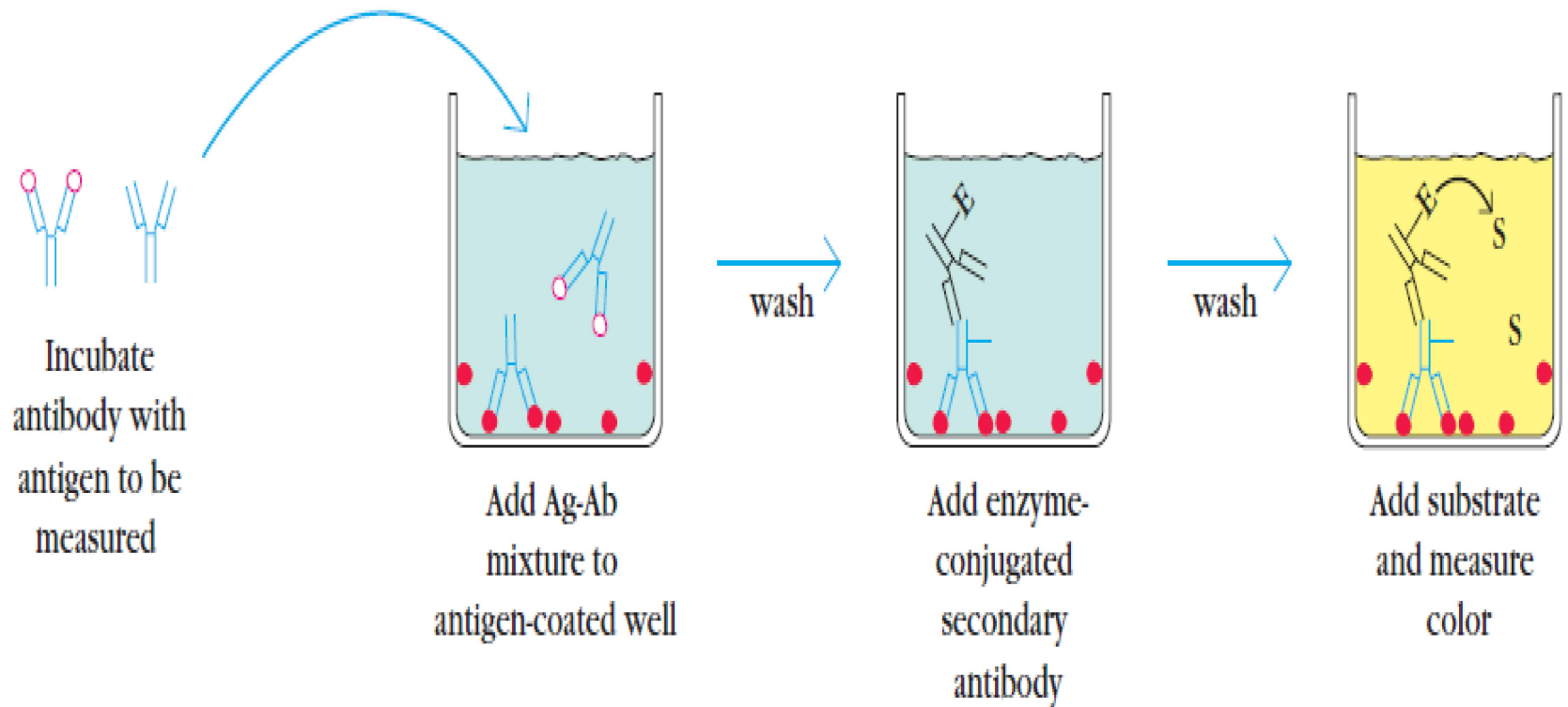
- The enzyme and substrate have been selected to ensure that a colored product develops in the tube.
- The intensity of the color that develops is therefore proportional to the amount of enzyme-linked antiglobulin that is bound, which in turn is proportional to the amount of antibody present in the serum under test.
- The color intensity may be estimated visually or, preferably, by

Indirect ELISA

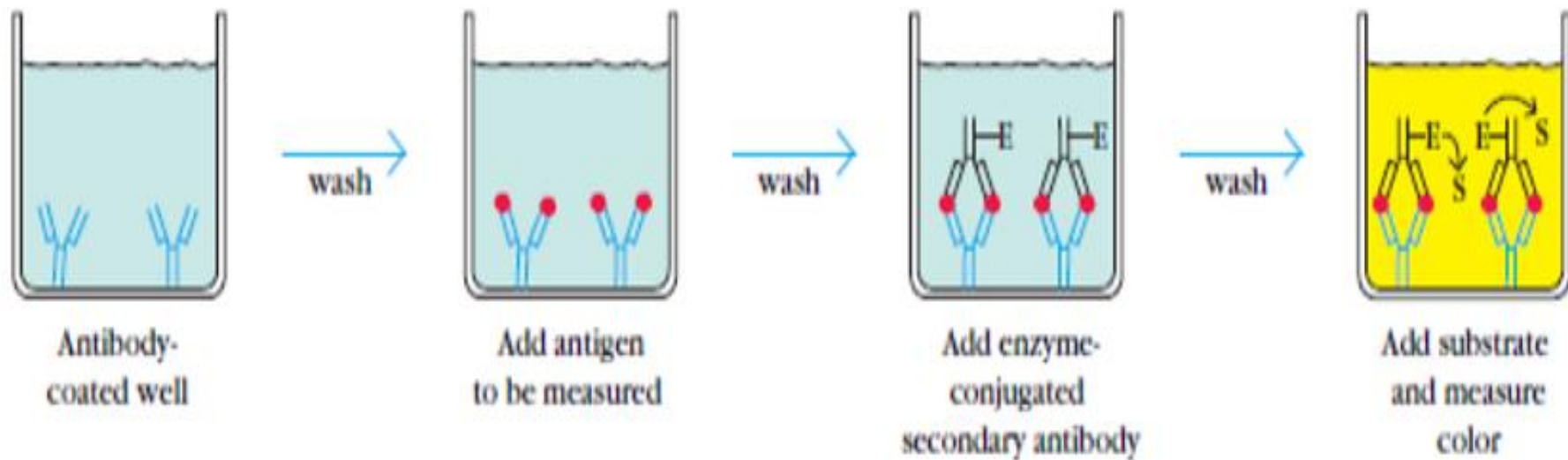


The indirect ELISA is used in the diagnosis of several parasitic infections and is being increasingly used in the diagnosis of bacterial, fungal and viral infections

Competitive ELISA



- In the competitive assay, the higher the concentration of antigen in the original sample, the lower the absorbance. It can be used to measure viral antigens



- Because this test involves the formation of antibody-antigen-antibody layers, it is called **sandwich ELISA**.
- It is used to detect circulating virus in blood from cats with feline leukemia.

Radioimmunoassays

- The principle of RIA involves competitive binding of radiolabeled antigen and unlabeled antigen to a high-affinity antibody.

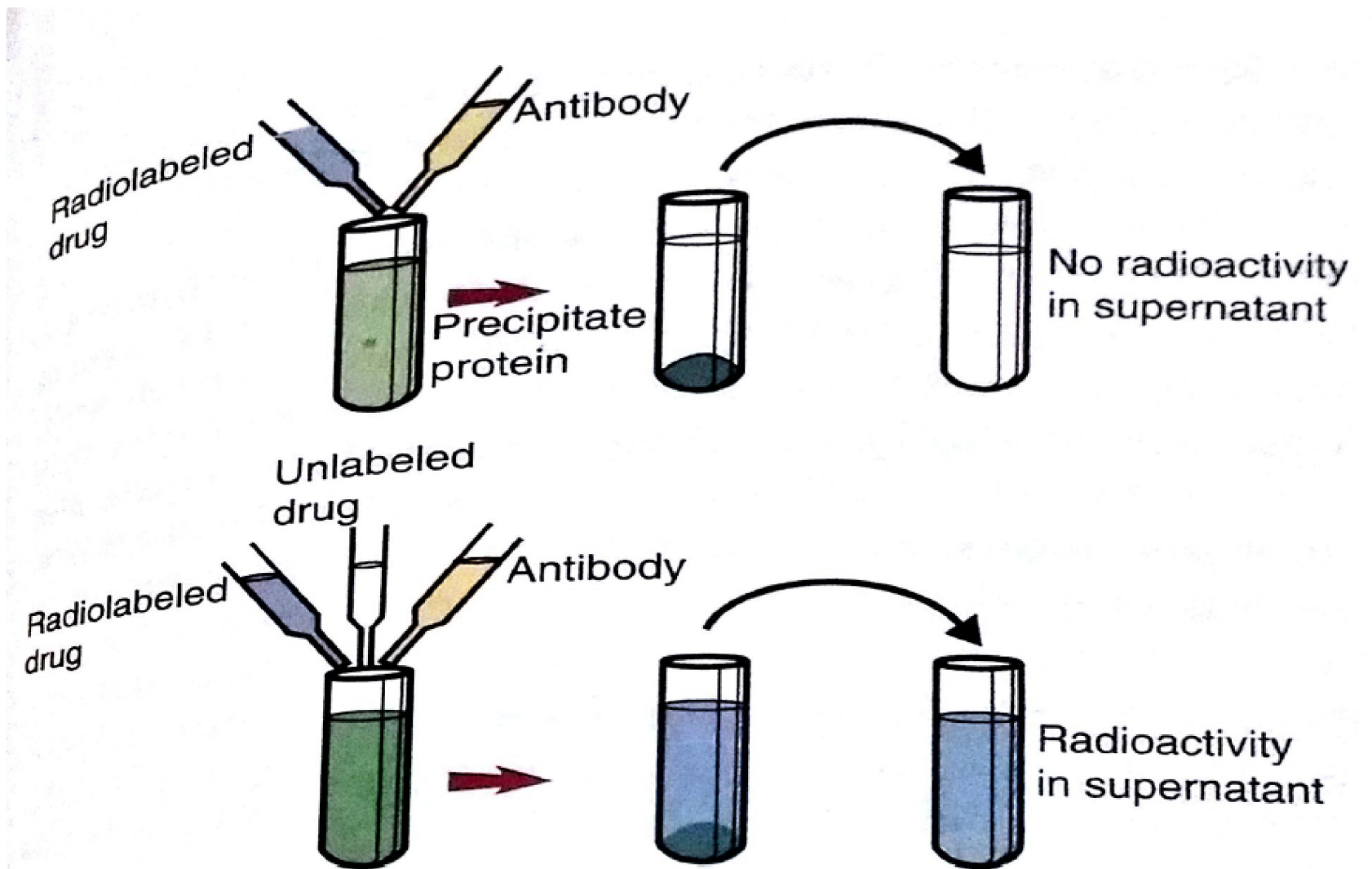
Advantage

- Very sensitive ($0.00005\mu\text{g/mL}$)

Disadvantage

- Expensive and Hazards of radioactivity and the need to safely disposal of radioactive material

- When radio labeled antigen is mixed with its specific antibody, the two combine to form immune complexes that can be precipitated out of solution. Any **radioactivity** remaining in the supernatant fluid is due to the presence of **unbound antigen**.
- If **unlabeled antigen** is added to the mixture, before adding the antibody, it will **compete** with the **radioactive antigen** for antibody-binding sites. As a result, some labeled



Pictorial presentation of Radioimmunoassay

II. Secondary binding tests

- The reactions between **Ags** and **Abs** are commonly followed by a **secondary reaction**.

If **Abs** combine with soluble **Ags** in solutions, the resulting complexes may

Precipitate

- **Abs** that aggregate soluble **Ags** are called **Precipitins**

Abs binding to particulate Ags (e.g. bacteria or RBCs), may make them **clump** or **agglutinate**

- o **Abs** that produce **agglutination** reactions are called **agglutinins**

Neutralization of bacteria, viruses, or toxins; and

Activation of the complement system.

Agglutination Tests

Principle of agglutination tests

- ♣ Agglutination is the visible clumping together of bacteria, cells, or particles, by an antigen combining with its specific antibody. The resulting clumps are referred to as agglutinates.
- ♣ Agglutination tests can be performed:
 - a) On slides
 - b) In tubes
 - b) In microtitration plates

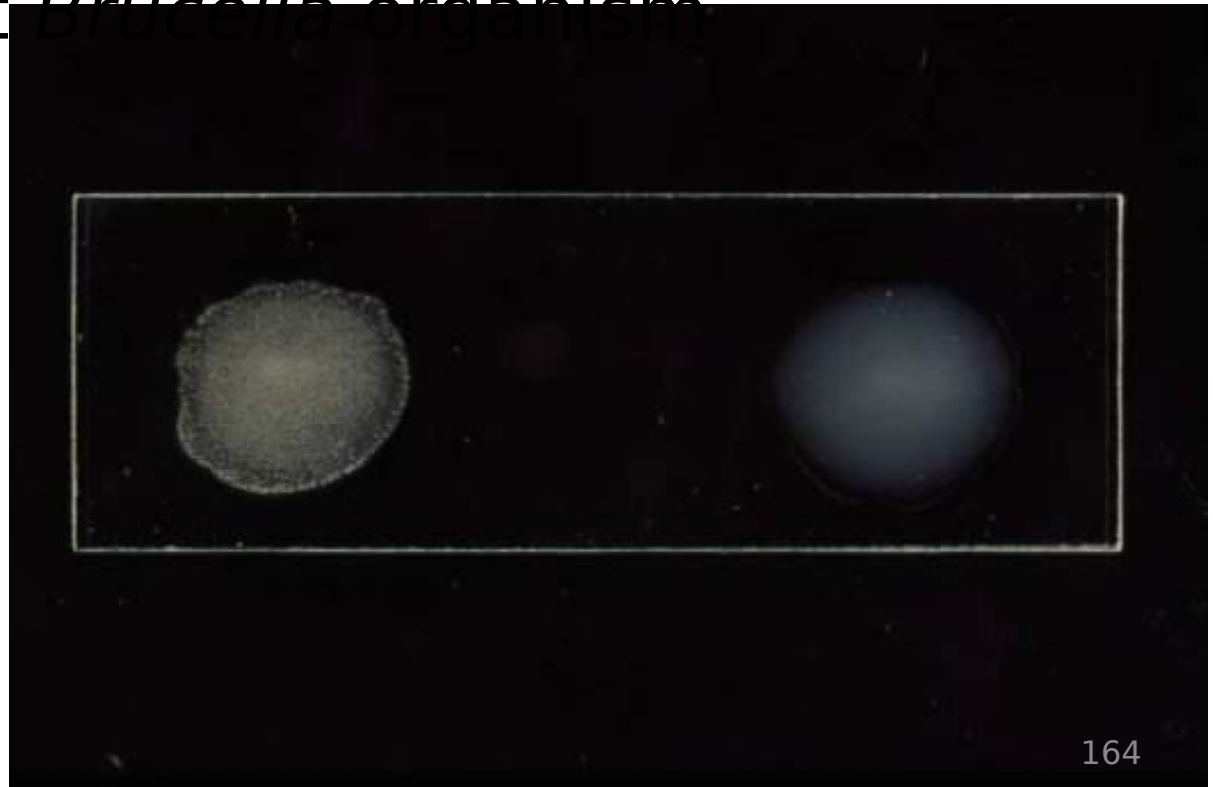
A) **Slide agglutination tests**

- These are rapid, easily performed techniques that give a reaction in minutes or even seconds. Example: Rose Bengal Plate Test (RBPT)
- **Principle:** RBPT is a rapid plate agglutination test for the detection of agglutinating antibodies by using inactivated *Brucella* cells, stained with Rose Bengal and suspended in an acid buffer

- The acid pH of the suspension prevents the non-specific agglutination of the bacteria, increasing the specificity of the test. Rose Bengal is used to mark the antigen and make its clumping visible agglutinating IgM, IgG and IgA antibodies contribute to the reactions.

Test Interpretation

- Any degree of agglutination = presence of antibodies directed against *Brucella* organism
- No agglutination = absence of antibodies directed against *Brucella* organism



B) Tube agglutination tests

- In tube tests, agglutination occurs in a larger volume of fluid
- Tube tests are usually more sensitive than slide tests. In this tube agglutination test, serum is diluted serially and then antibody level is measured by adding standard antigenic suspension.

C) Microtitration agglutination tests

- These techniques are performed in microtitration plates. They are more sensitive, more economical, easier to perform, and usually give quicker results

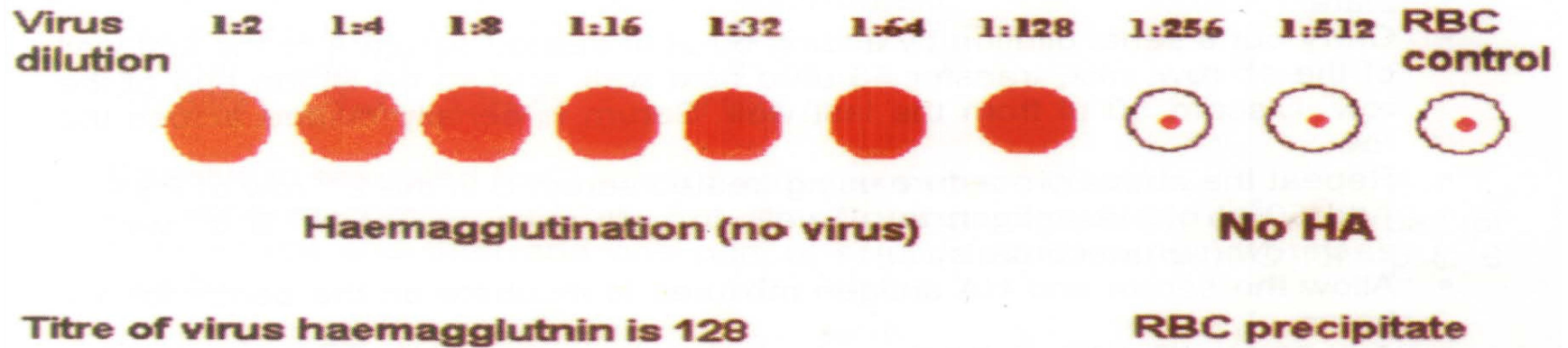
Example: Haemagglutination inhibition test (HI)

- This technique is used to detect antibodies against Arboviruses and Influenza viruses.

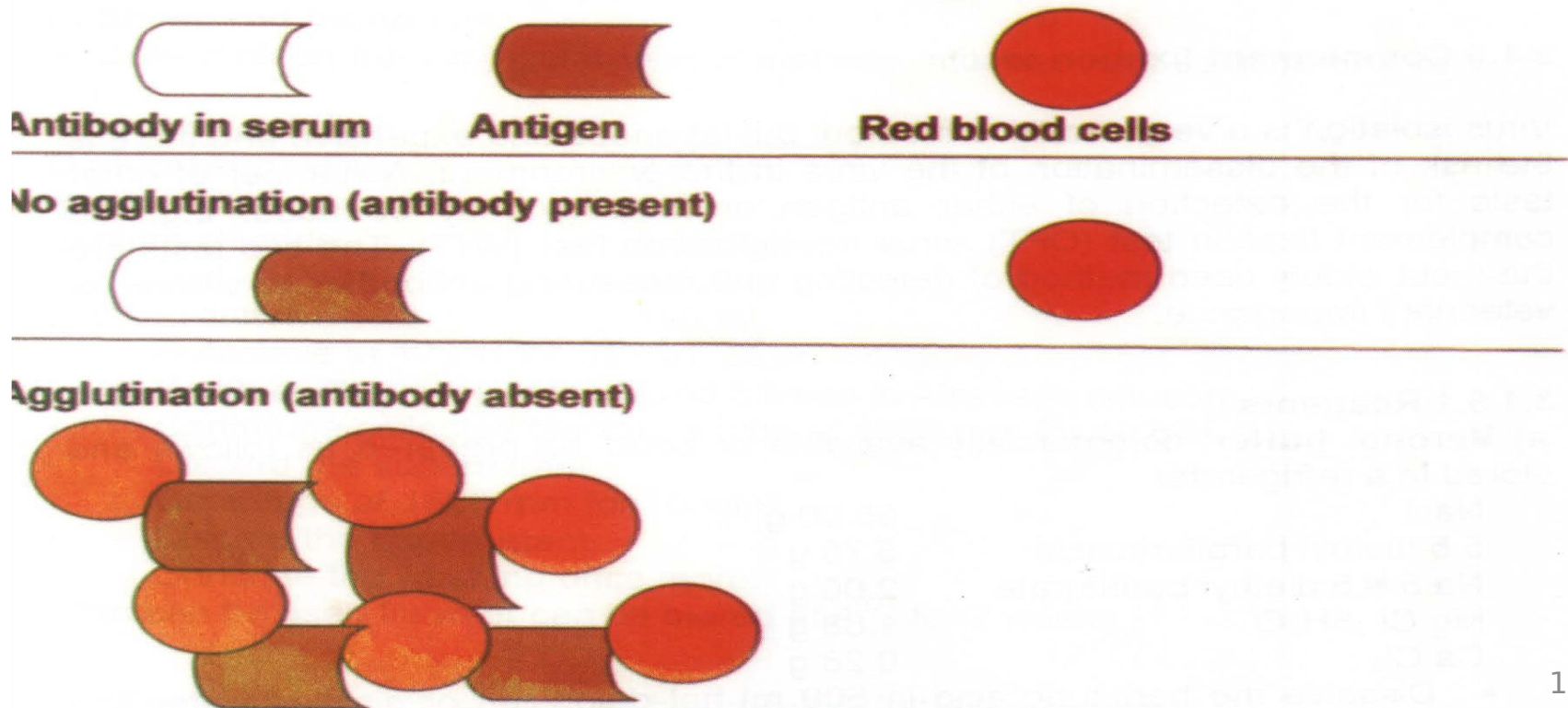
These viruses are able to agglutinate red

- In the haemagglutination inhibition (HI) test, the animal's serum is reacted with a suspension of known viral antigen. If the corresponding antibody is present, it will coat the haemagglutinins (antigens) on the viral particles and so prevent haemagglutination when red cells are added. The viral antigen should be titrated.

A)



B)



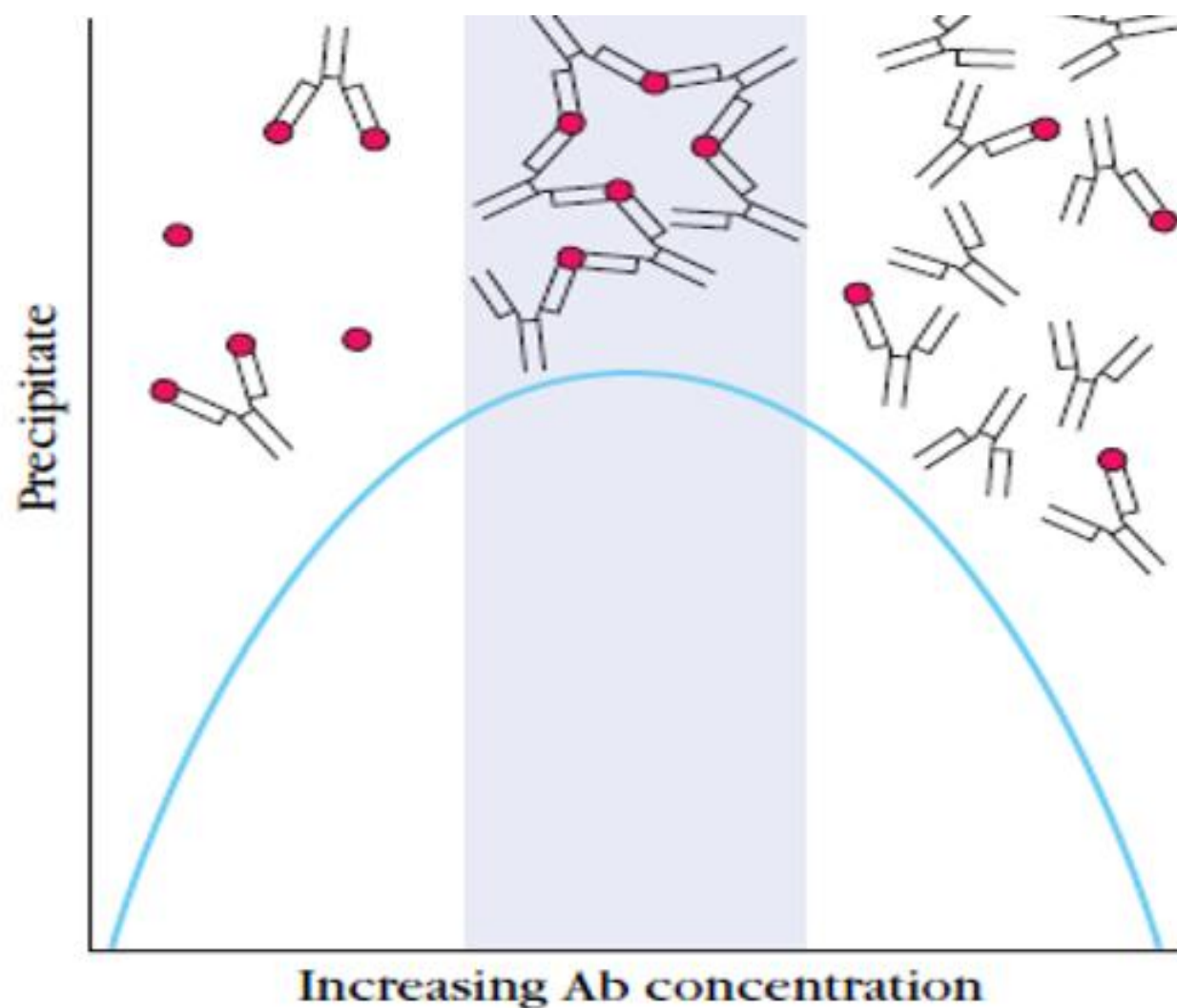
Precipitation Tests

- Precipitin techniques are used to detect and identify antigens in specimens, extracts, and cultures, and to detect and quantify antibodies in serum. Compared with agglutination tests, precipitin techniques require more experience in their performance and interpretation.

Principle

- In precipitation tests, the antigen and antibody are in a soluble form and combine to form a visible precipitate. Various systems are available in which precipitation tests are performed, either in semisolid media such as agarose, or cellulose acetate. Agarose is a transparent, colorless, neutral gel.

- Immunoprecipitation techniques rely on the ability of antibodies to bind to more than one antigenic determinant on a single antigen, thus forming a large complex that will fall out of solution.
- The test can be done in solution, or on solid agar/gel matrices. In solution, it forms precipitates.

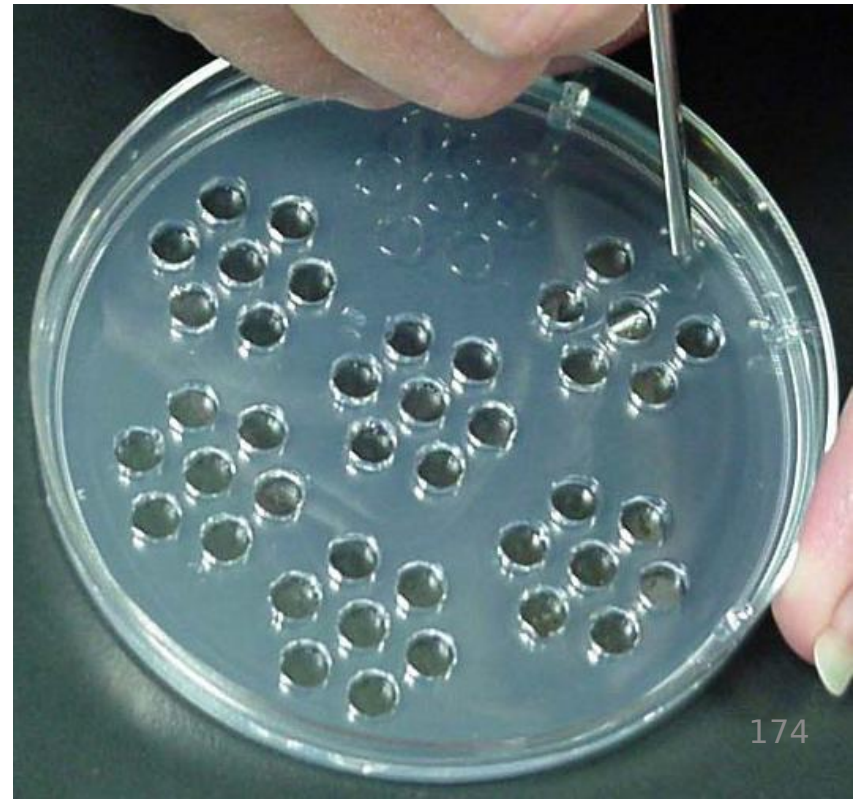


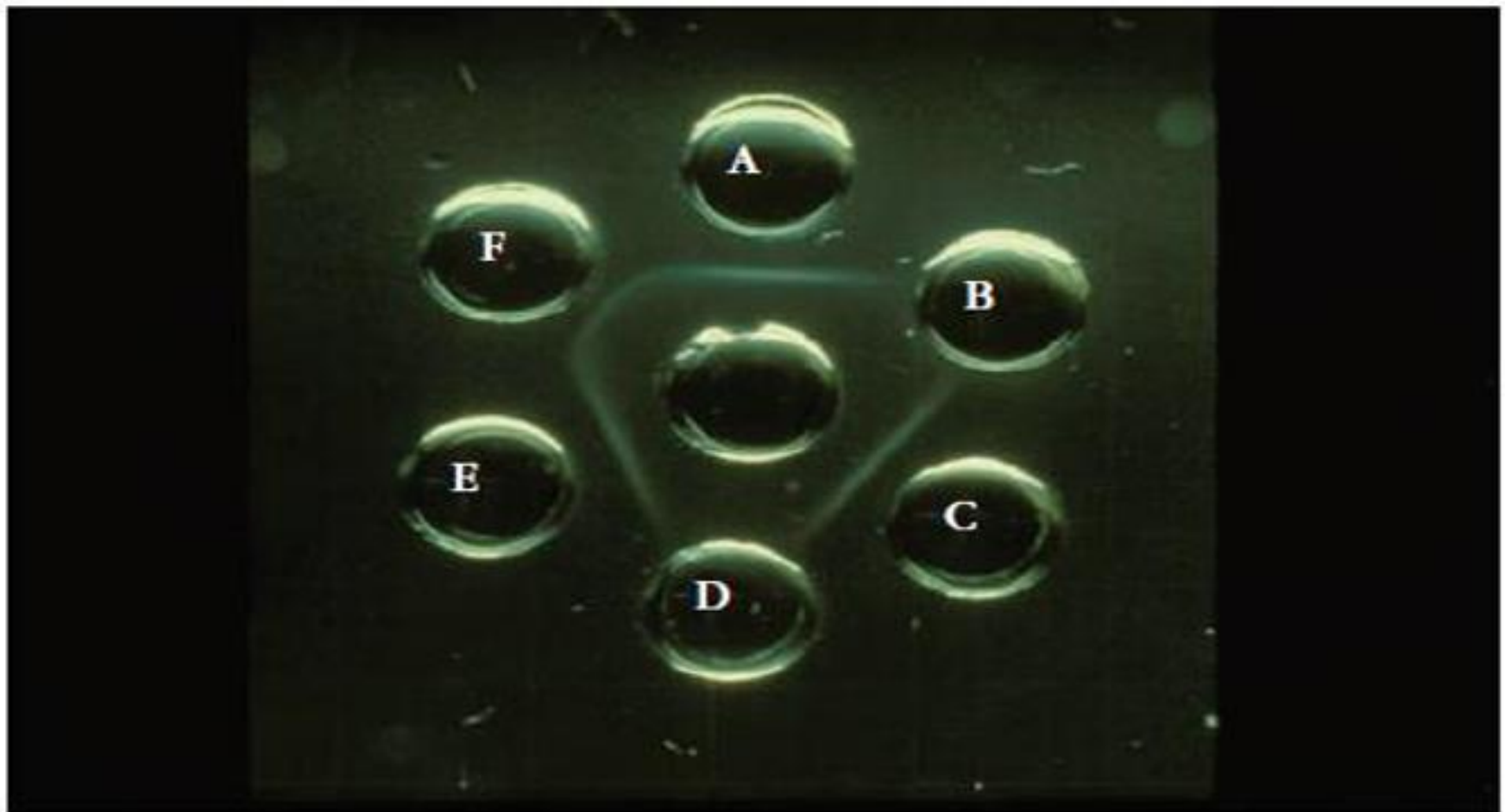
Immunoprecipitation in solution. When bi- or multivalent antibodies are mixed in solution with antigen, the antibodies can form cross-linkages with two or more antigen molecules, leading to the formation of a cross-linked precipitate (middle

- Immune precipitates can form not only in solution but also in an agar matrix. When antigen and antibody diffuse toward one another in a gel matrix, a visible line of precipitation (lattice) will form.
- As in a precipitation reaction in solution, visible precipitation occurs when the concentrations of antibody and antigen are equivalent to one another.
- Both antigen and antibody diffuse radially

E.g. Agar Gel Immunodiffusion (AGID) Test

- The diffusion of soluble antigens and/or antibodies toward each other leading to their precipitation in a gel matrix.

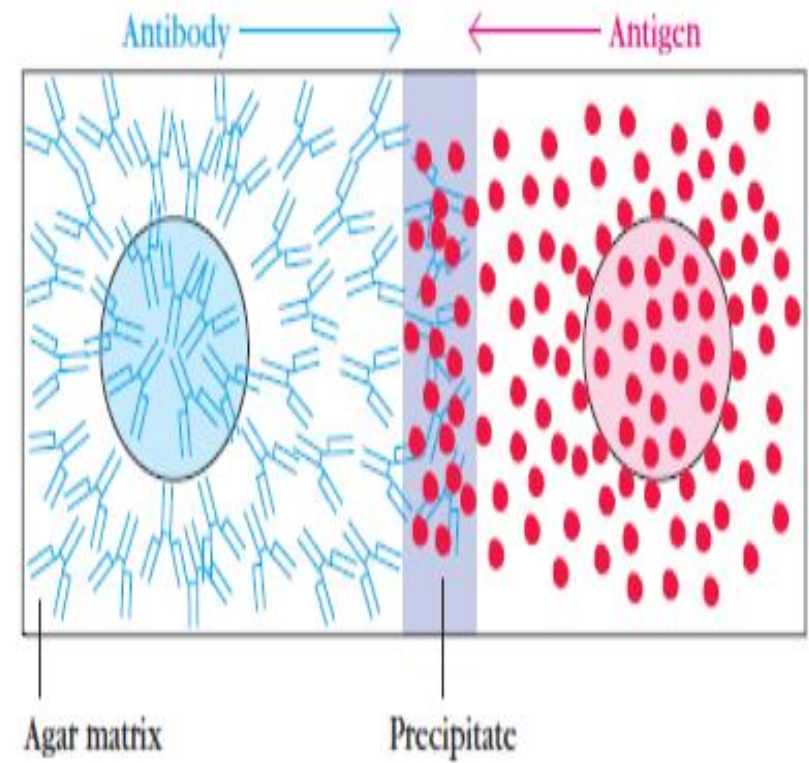




Immunodiffusion in agar gels can be used to assay for the presence of antibodies and determine cross-reactivity patterns between complex antigens and antibody samples. In this example, viral antigen was placed in the center well, and serum samples from different individuals were introduced into each of the surrounding wells. Note that the serum samples of individuals B and D are negative for antiviral antibodies, in that



DOUBLE IMMUNODIFFUSION



Complement Fixation Tests (CFT)

- CFT is best performed in reference laboratories where facilities exist for the careful standardization and control of reagents, which these tests require. Usually used as confirmatory test for bacteria like Brucella

Principle of CFT

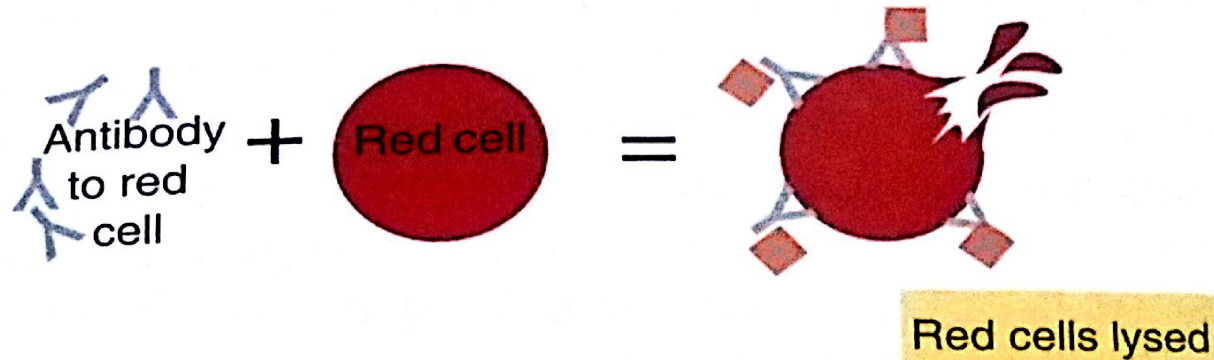
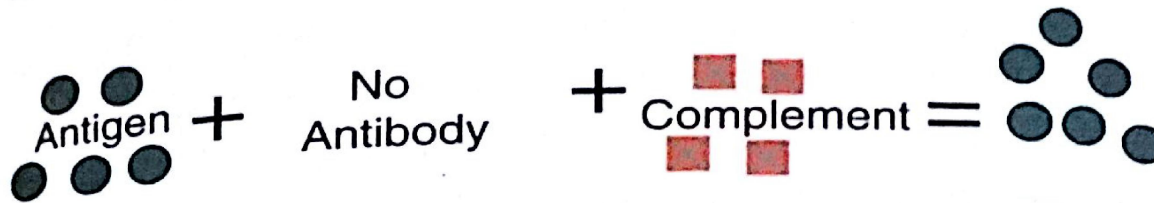
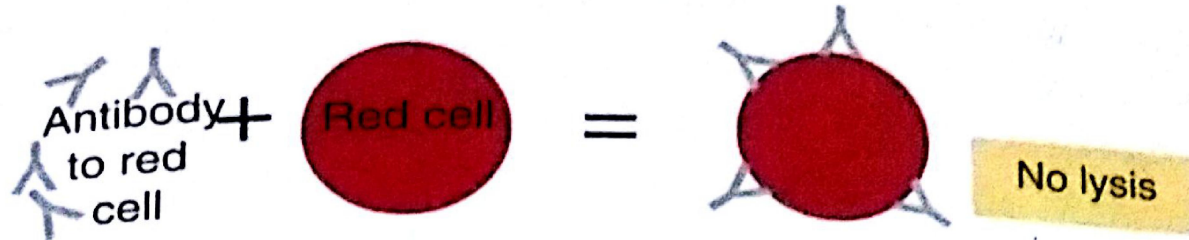
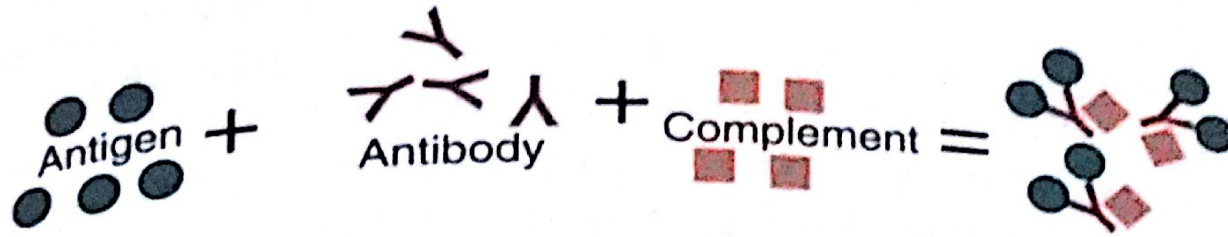
- It is used to detect and quantify antibody that does not agglutinate or precipitate when reacted with its antigen, but can be

The complement fixation test system consists of two components:

- The first component is an indicator system consisting of a combination of sheep red blood cells, anti sheep red cells antibody and exogenous source of complement, usually guinea pig serum. When these three components are combined in an optimum concentration, the anti sheep red blood cell antibody complexes and causes cell lysis.

- The second component consists of a known antigen and inactivated animal's serum (the serum under test deprived of its complement by heating at 56°C).
- Antigen-antibody reactions lead to immune complex formation that produces complement fixation via the classical pathway. When these complexes are on bacteria, red cells or other cells, the

Complement fixation test



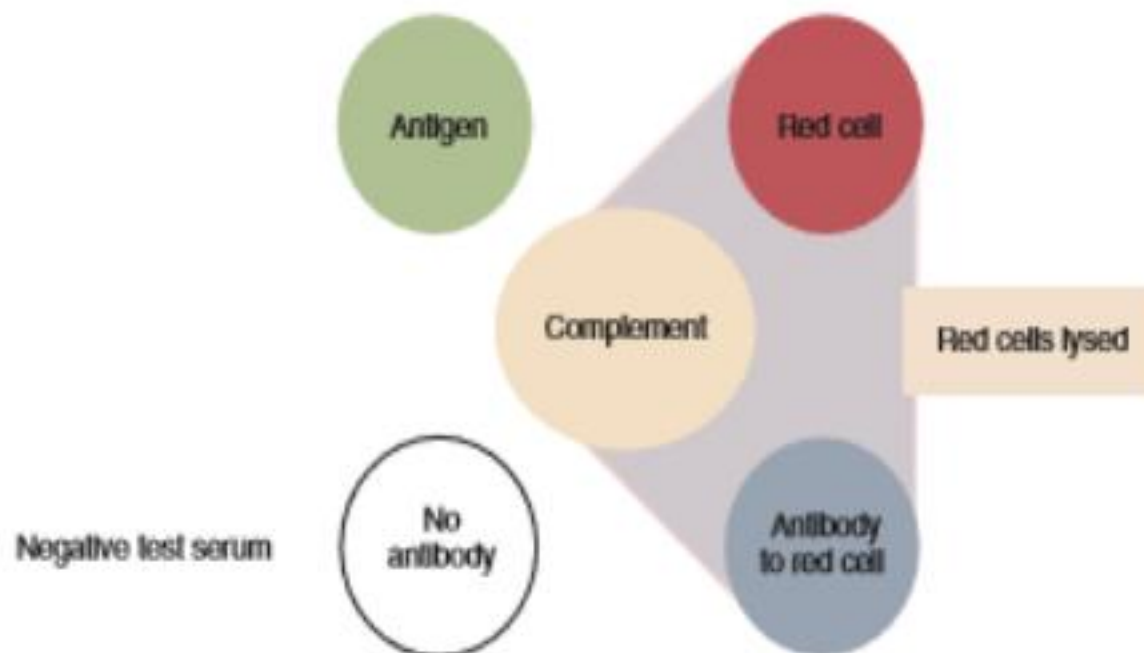
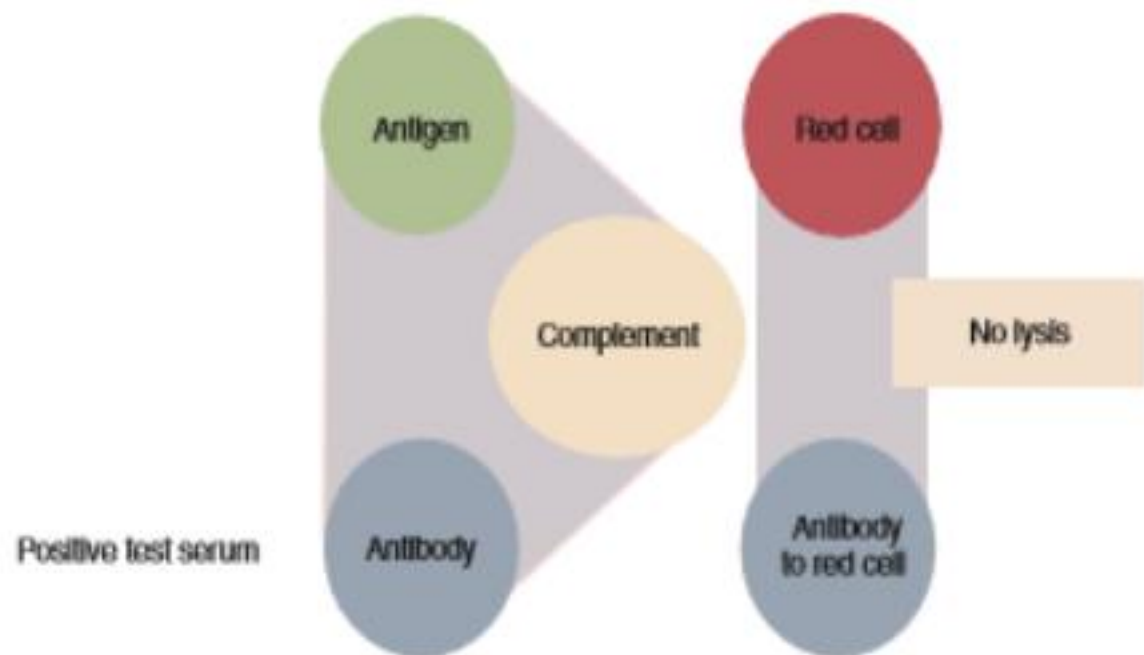


FIGURE 41-30 The principle of the complement fixation test. Complement, if fixed by antigen and antibody, is unavailable to lyse the target cells in the indicator system. In the absence of antibody the complement remains unfixed and is available to lyse the indicator system.

(Modified from Roitt I: *Essential Immunology*. Blackwell Science, 1971, Oxford.)

Interpretation:

Lysis of the indicator sheep cells indicates both a lack of antibody and negative complement fixation test.

If the sick animal's serum does contain a complement-fixing antibody, a positive result will be demonstrated by the lack of hemolysis

VACCINES

- **Immunization:** The process of producing a state of immunity in a subject

Types of immunization procedures

- There are two basic methods by which any animal may be made immune to an infectious disease:

a) Passive immunization

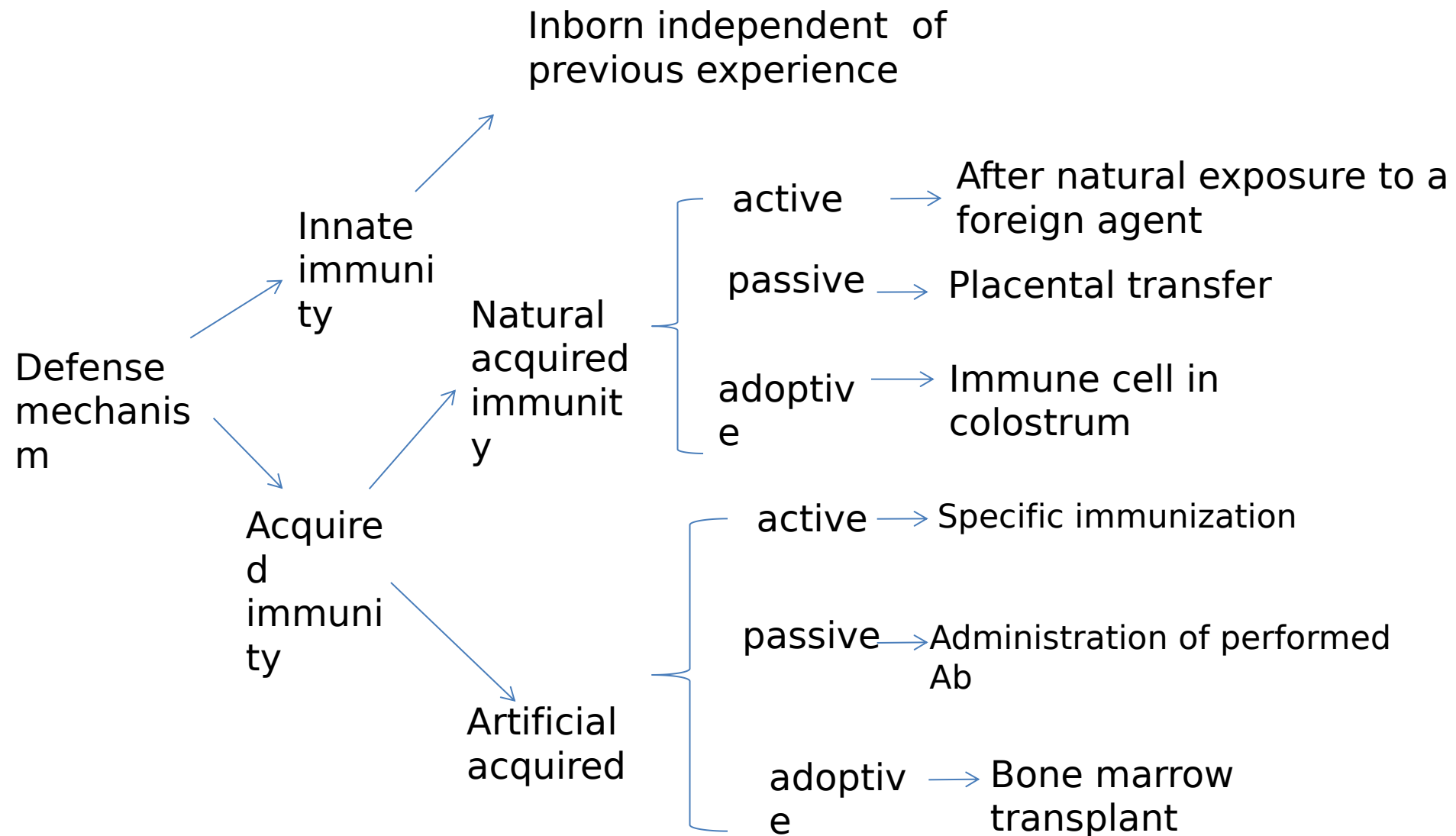
b) Active immunization

A) Passive immunization

Achieved by:

Transfer of preformed antibodies from dam to the developing fetus across placenta

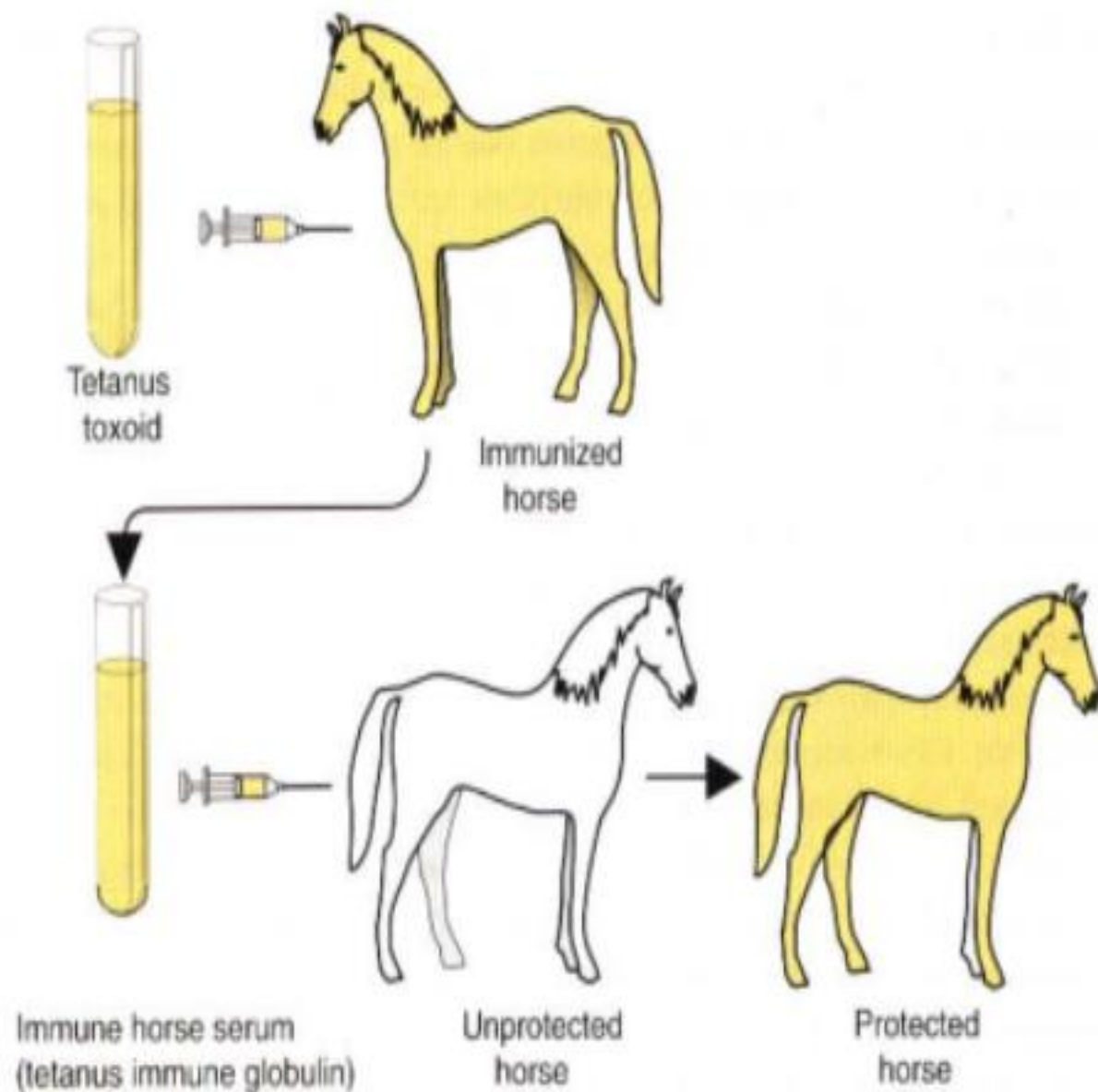
Transfer of maternal antibodies to newborns through colostrum



Injecting a recipient animal with preformed antibodies (immunoglobulins) in donor animals by active immunization

e.g. anthrax (cattle), distemper (dog), panleukopenia (cat), Tetanus (horses)

Figure 1-6. Transfer of immunity to tetanus by means of serum derived from an immunized horse. This clearly demonstrates that antibodies in serum are sufficient to confer immunity to tetanus.



Advantage of passive immunity

- Confer immediate protection

Disadvantage

- No memory response and the protection provided is short-lived
- Elicit an immune response when given to other species of animal (immunoglobulins, mAbs)
- Transmission of **infections** from donor to

B) Active immunization

- Achieved by **natural infection** with a **microorganism**, or
- **Acquired artificially** by administration of a **vaccine**

Advantage

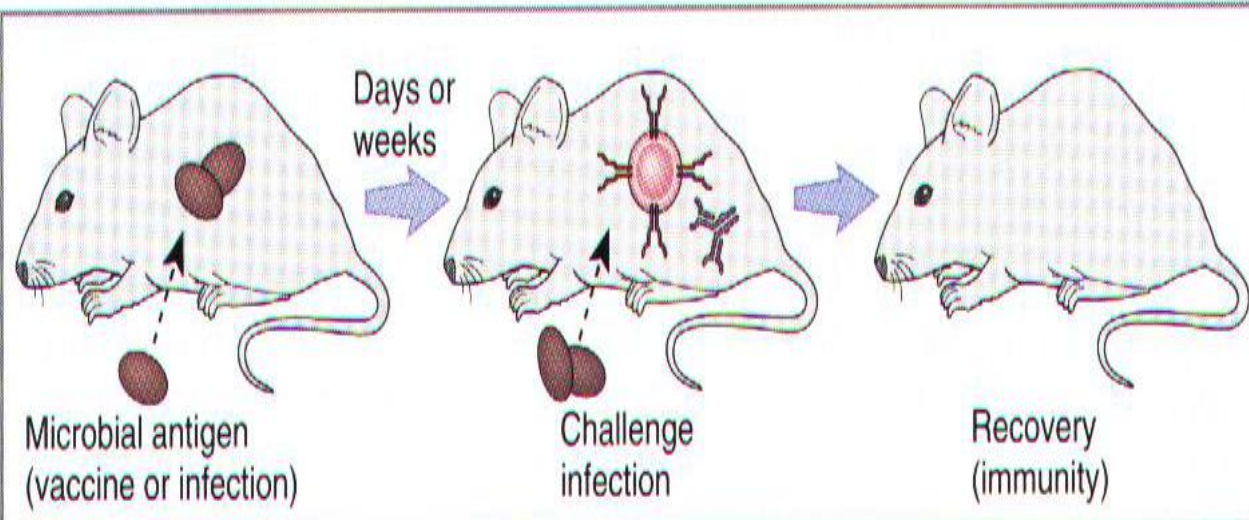
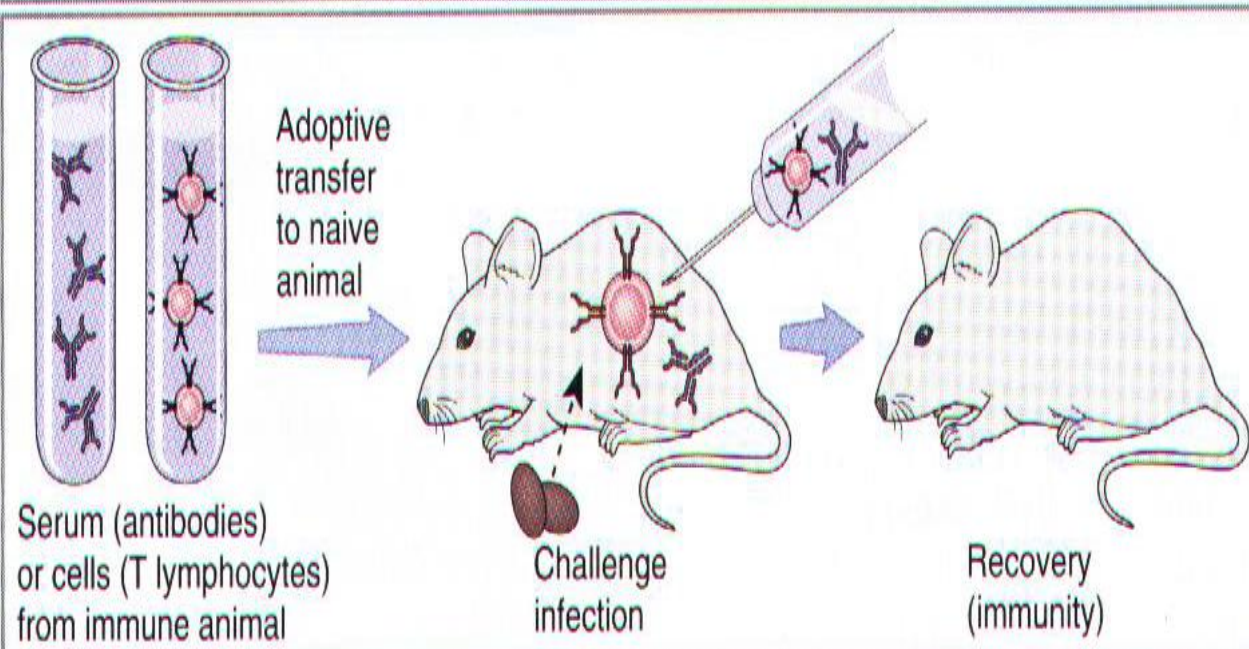
Provide long-lasting immunity

Capable of re-stimulation by repeated injections of antigen or exposure to infection

Disadvantage

Passive Vs Active Immunization

Feature	Passive	Active
Protection	Immediate	Requires 7-10 days
Duration	Days to months	Years
Host immune system	Not involved	Required
Elements of response	Anti-pathogen Ab only	Anti-pathogen Ab, CTLs, Th cells
Memory	No	Yes
Uses	Immunocompromised individual unvaccinated individual where no vaccine exist	Immunocompetent individual individuals not naturally exposed to pathogen

			Specificity	Memory	
Active immunity	 Microbial antigen (vaccine or infection) Days or weeks Challenge infection Recovery (immunity)			Yes	Yes
Passive immunity	 Serum (antibodies) or cells (T lymphocytes) from immune animal Adoptive transfer to naive animal Challenge infection Recovery (immunity)			Yes	No

191

So what is vaccine?

Vaccine: A preparation of immunogenic material used to induce immunity against pathogenic organisms

Vaccination: Intentional administration of a harmless or less harmful form of a pathogen to induce a specific immune response that protects the individual against later exposure to the pathogen

Principle of vaccination

1. Primary immune response

- ♣ Response that occurs after the **first exposure** of an individual to a **foreign antigen**
- ♣ Characterized by relatively **slow kinetics** and **low magnitude** compared with the response after a **subsequent exposure** to the same antigen

2. Secondary immune response

- Response that occurs on **second exposure** to **previously** encountered antigen
- Characterized by **more rapid kinetics** and **greater in magnitude** than the **primary immune response**

- The principle of vaccination follows the above types of immune response:

That is, **initial administration** of the vaccine against specific pathogen will initiate **primary immune response**.

Then, **natural infection** by the **same pathogen** will induce **secondary immune response**, with ultimate elimination of the pathogen.

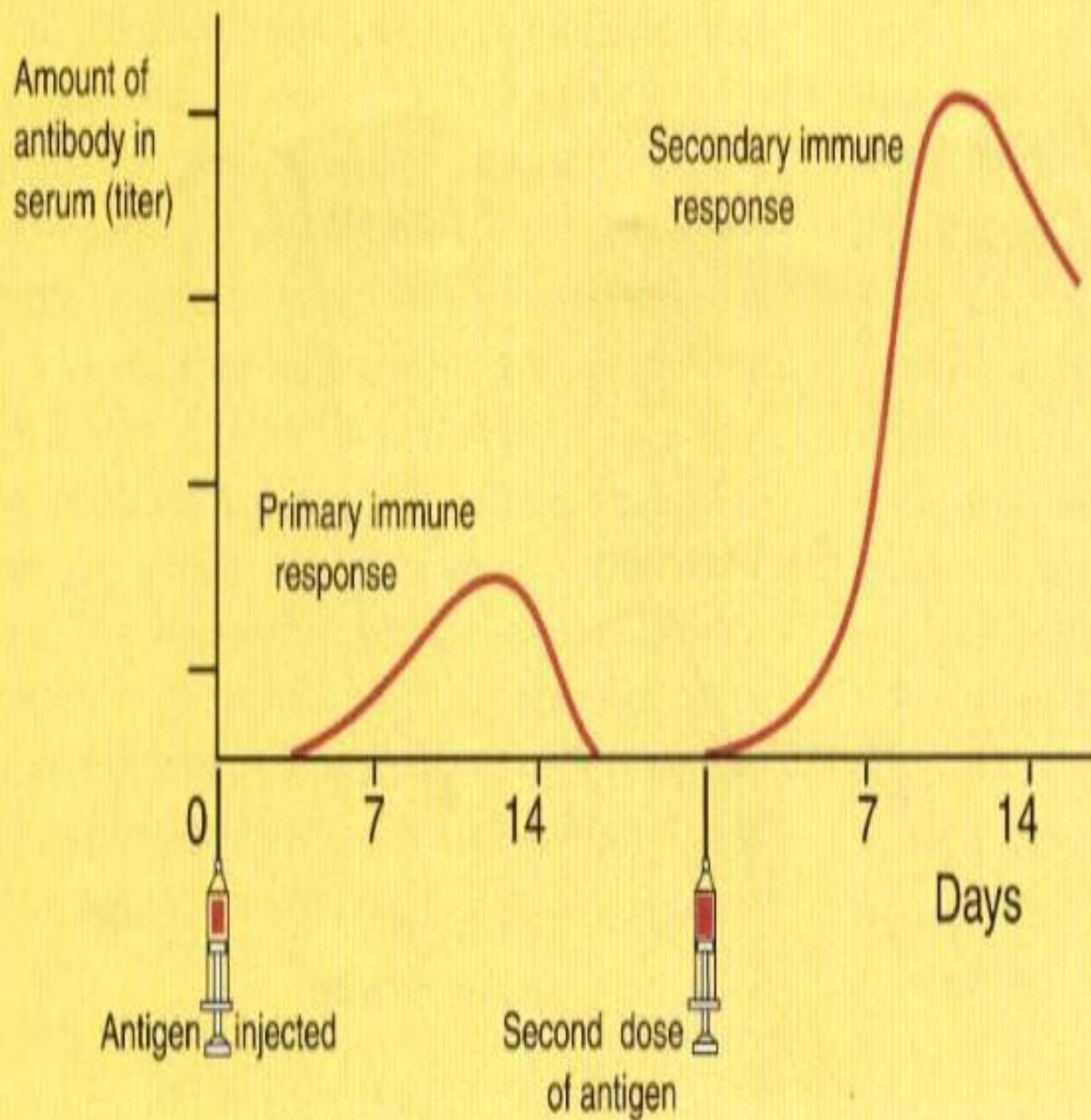
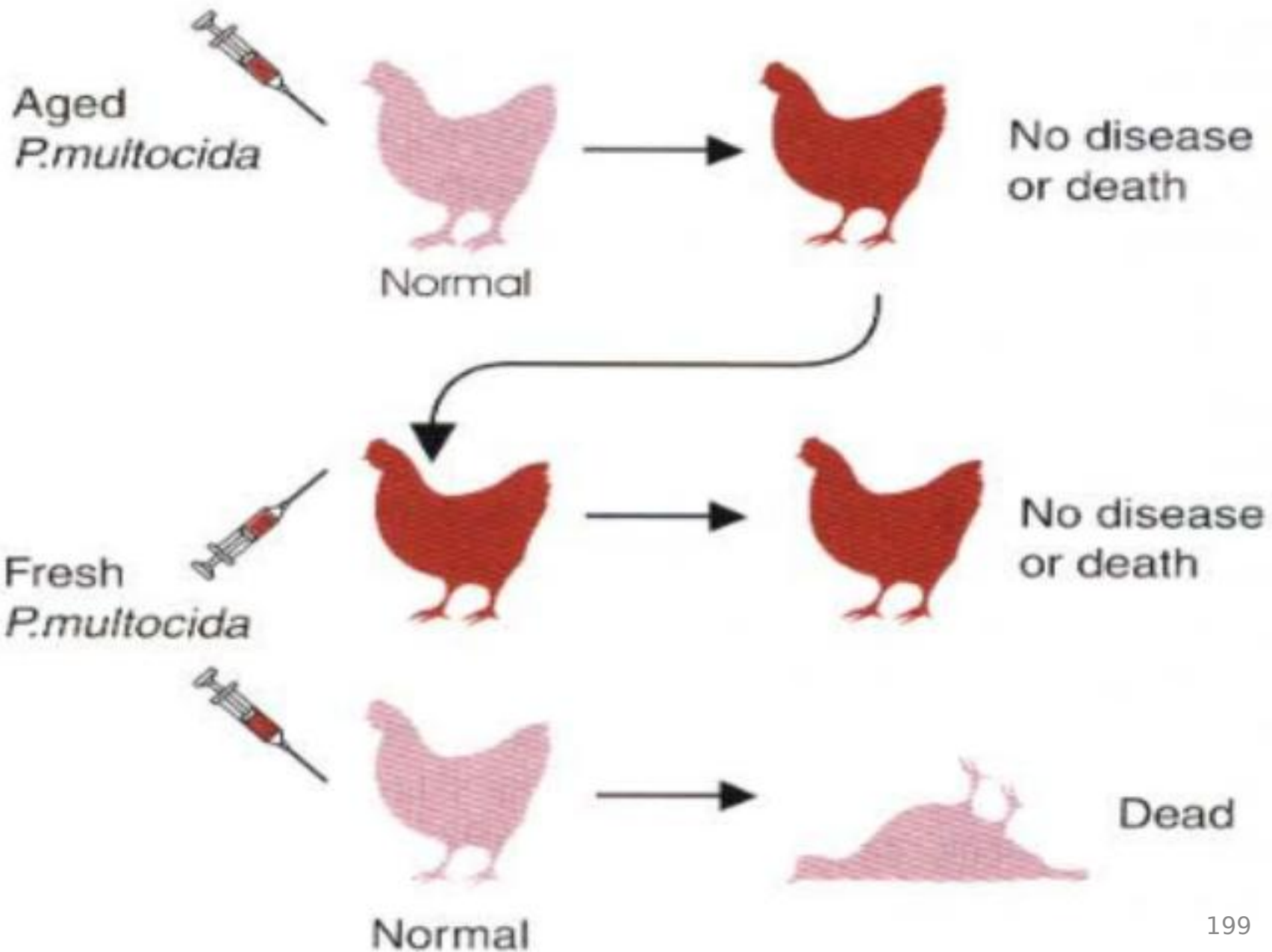


Figure 1-7. The characteristic time course of the immune response to an antigen as measured by serum antibody levels. Note the key differences between a primary and a secondary immune response.

Characteristics of ideal /perfect/ vaccine:

- It should give prolonged **strong immunity**.
- The immunity should be conferred on both the **animal immunized** and any **fetus** carried by it
- It should induce both **humoral** and **cellular** immunity

- It should be free of **adverse side effects**
- It should be cheap, stable, and adaptable to mass vaccination
- It should stimulate an **immune response** distinguishable from that resulting from **natural infection**



Routes of vaccine administration

♣ Most vaccines are administered by injection

♣ **Precaution while injection**

- ‖ All needles must be clean and sharp
- ‖ Skin at the injection site must be clean and dry
- ‖ Vaccines are provided in a standard dose, and this dose should not be divided to account for body weight or age

A. Subcutaneous or intramuscular routes

- The simplest and most common method of vaccine administration
- Excellent for small numbers of animals and for disease in which systemic immunity is important
e.g. Rabies vaccine, Rinderpest, Anthrax

B) Intranasal route

- Induce both systemic and mucosal immune responses
- Obviate/ avoid/the need for sterile syringes and needles

e.g. *Streptococcus equi* infection in horses,

- Infectious bronchitis and Newcastle disease in poultry

C) Aerosolization

- Enables the vaccines to be inhaled by all the animals in a group

D) Oral route

→ Putting vaccines in feed and drinking water

- Induce both **systemic** and **mucosal immune** responses
- Avoid the need for sterile syringes and needles

e.g. Newcastle disease and infectious laryngotracheitis vaccines in poultry

E) Intradermal and epidermal routes

- Avoid needle dangers and drawbacks
- Dose-sparing ability (smaller quantities of vaccine antigen could be delivered)
e.g. BCG (Bacille Calmette-Guerin), smallpox

F) Immersion

- Achieved by immersing fish in a dilute antigen solution
- Immersion results in the antigen being deposited on mucosal surfaces such as gills or oral cavity and some may be swallowed

First generation vaccines

- Consist of many of the common vaccines currently in use
- Its formulation involves
 - Cultivation and harvesting of organism,
 - purification and conversion either as suspension form or to freeze dried products

Con't..

- Originate from viruses or bacteria and can be divided into
 - A. Killed vaccines
 - B. Live attenuated vaccines

A) Killed vaccines

- ♣ Composed of killed or inactivated normal (wild type) infectious, pathogenic microorganisms

- ♣ **Methods of inactivation:**

- └ Heat treatment

- └ Gamma irradiation

Chemical treatment

\ Formaldehyde

\ Acetone

\ Alcohol

Alkylating agents

- Ethylene Oxide
- Ethyleneimine
- Acetyleneimine and β -propiolactone)

Advantages

- Unlikely to cause disease through residual virulence
- No risk of reversion to wild type pathogen
- Lower development cost
- Safe to use and can be given to immunodeficient and pregnant individuals
- Stable on storage
- Unlikely to contain live contaminating

- **Disadvantages:**

- o Require use of adjuvant
- o Since the microorganisms cannot multiply, a larger quantities are required to stimulate immunity

- o Higher risk of producing hypersensitivity reaction
- o Require repeated boosters to maintain the immune status of the host
- o Induce a predominantly humoral antibody response
- o Produce relatively short-lived immunity

Examples:

- ‖ Rabies vaccine
- ‖ FMD vaccine
- ‖ Equine herpesvirus-4 vaccine

Vaccines containing **killed bacteria** are called **bacterins**

- B. abortus* strain 45/20 vaccine
- E. coli* bacterins
- Mannhemia bacterins, etc

B) Live attenuated vaccines

- Composed of live, attenuated microorganisms that cause a limited infection in their hosts sufficient to induce an immune response, but insufficient to cause disease
- The process of reducing virulence is called attenuation
- Under-attenuation will result in residual virulence and disease

Methods of attenuation

I. Adapting organisms to growth in unusual condition

E.g.1. **BCG strain** of *M. bovis* was rendered avirulent by being grown for **13 years** on **bile saturated medium**

2. ***B. abortus*** strain 19 was grown under conditions in which there was a **shortage of nutrient**
3. **Sterne Strain *B. anthracis*** was rendered **avirulent** by growing in **50% serum agar** under an atmosphere rich in **CO₂** so that it lost its ability to form a **capsule**

II. Growth in species to which they are not naturally adapted

E.g. 1. Rinderpest virus was attenuated by growing in **rabbit**

2. African horse sickness virus to **mice**

3. Canine distemper virus to **ferrets**

III. Growth in eggs

E.g. Flury strain of **rabies** was attenuated by prolonged passage in eggs

IV. Prolonged tissue culture

E.g. **Canine distemper** virus in **canine kidney** cells

Advantages

- o Stimulate both humeral and cell-mediated responses
- o Do not require use of adjuvant
- o Confer long-lasting protection
- o Some live vaccines can be given orally and induce mucosal immunity, which gives more protection at the site of entry
- o Since the organisms can multiply in the host, fewer quantities are required to induce protection

- o Fewer doses required
- o Less chance of hypersensitivity
- o Relatively cheap

Disadvantages

- o Risk of **reversion** to **virulent form**
- o Not safe in **immunosuppressed** individuals
- o Proper storage is critical. Cold chain is required
- o Risk of contamination with live organism
- o Risk of spread to other animals

♣ Examples

- └ Strains 19 and RB51 of *B. abortus* vaccine
- └ Rinder pest vaccine
- └ Anthrax vaccine
- └ African horse sickness vaccine, etc

Second generation vaccines (Subunit vaccines)

- Composed of semi-pure or purified antigens instead of whole organisms
- Consists of only those antigens that elicit protective immunity

Forms of subunit vaccines:

I) Inactivated exotoxins or toxoids

- Formaldehyde treated exotoxin is called toxoid
- Vaccination with toxoid induces antitoxoid antibodies, which are capable of binding to the toxin and neutralizing its effect
- Example: Tetanus and diphtheria vaccines

II) Capsular polysaccharide

- Capsular polysaccharides are plentiful on the bacterial surface and thus easy to purify
- Vaccination with vaccine consisting of purified capsular polysaccharide induces opsonizing antibodies, which coat the capsule and render the pathogen susceptible to phagocytosis by macrophage

Example: *Streptococcus pneumoniae* vaccine

III) Recombinant protein antigen

- Large quantities of purified immunogenic proteins (antigens) can be produced by recombinant DNA technology

- DNA coding for an Ag of interest is first isolated
- Then the DNA is inserted into a bacterium, yeast or mammalian cell
- The recombinant Ag then expressed
- The expressed Ag is used for vaccine development

Examples:

1. FMD vaccine (VP1 [protective antigen])
2. Feline leukemia vaccine (gp70 [protective antigen], commercially available)
3. Lyme vaccine: *Borrelia burgdorferi* (OspA [immuno-dominant outer surface lipoprotein])

Advantages

- Induce strong humoral and cell-mediated immune response
- They can safely be given to immunosuppressed individual
- Have an excellent safety profile
- Can be used in combination with other subunit vaccines

Disadvantages

- May require the use of adjuvants to enhance immune responses
- Antigens may not retain their native conformation, so that antibodies produced against the subunit may not recognize the same protein on the pathogen surface.

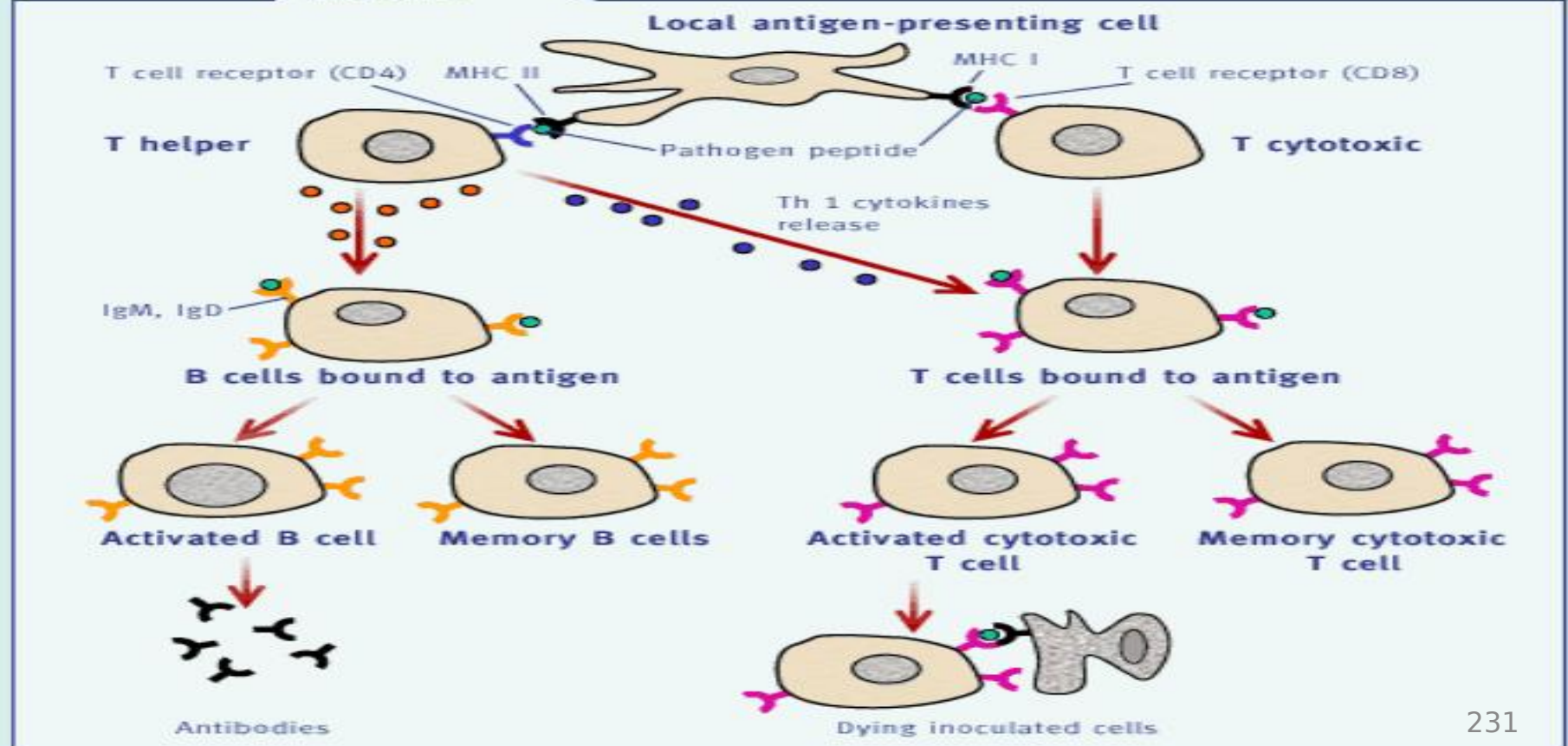
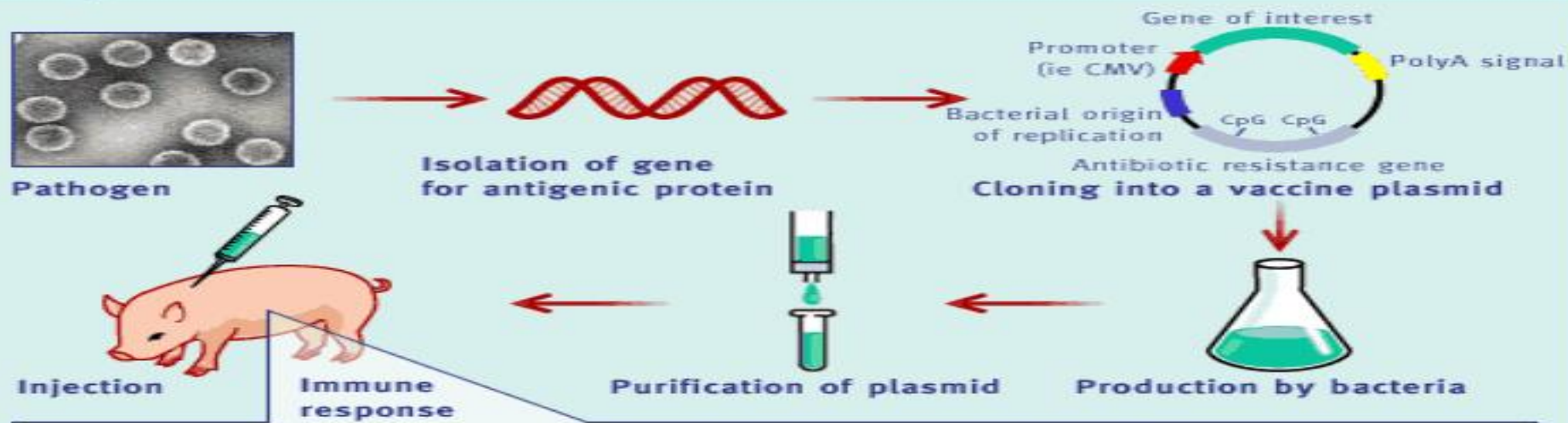
Third generation vaccines (DNA vaccine)

- ♣ **DNA vaccination**;- is the direct introduction of a bacterial plasmid DNA into host cells that expresses an antigenic protein under the control of a eukaryotic promoter./vector/
- ♣ As a consequence, the foreign antigen is expressed within the host cell and can simulate the induction of both humeral and cell-mediated immune responses

Delivery of DNA vaccine

- Intramuscular, intradermal or intranasal inoculation
- Particle-mediated intradermal delivery using a gene gun, in which the DNA is precipitated onto gold microspheres
- Using attenuated intracellular bacteria, such as *Shigella flexneri* or *Salmonella typhimurium*

Principle of DNA vaccination



Advantages

- Induce both humeral and cell-mediated immune responses
- The risks associated with live vaccines are absent
- Inexpensive and relatively easy to prepare
- There is no protein component and so there will be no immune response against the vector itself.

- Combination DNA vaccines containing the genes for multiple antigens of one pathogen, or single antigen from more than one pathogen, are also relatively easy to prepare
- DNA vaccines are very stable and therefore have a long shelf life and can be transported without a cold chain.

Disadvantages

- The plasmid persists for a long time, there is a potential for vaccine DNA to integrate into the host genome and activate oncogenesis or inhibit tumour suppressor genes
- The presence of an antibiotic-resistant gene in these plasmids also runs the risk of transferring this resistance to bacteria
- Large quantities of DNA are required to induce immune response

Examples may include:

- FMD, Anthrax, TB, Influenza, Newcastle disease, Rabies, Canine parvovirus etc.
- DNA vaccines are in experiment

Table 4. DNA vaccines approved for veterinary use.

Type	Species	Target	Product/Company	License date/ country	Route of administration	Benefits
Prophylactic vaccine	Horses	West Nile Virus	West Nile-Innovator [®] / Fort Dodge Animal Health	2005 USA	IM ¹	Production of protective antibodies
Prophylactic vaccine	Salmon	Infectious haematopoietic necrosis virus (IHNV)	Apex-IHN [®] /Novartis Animal Health	2005 Canada	IM	Stimulation of innate and adaptive immune responses improving the welfare and product yield
Immunotherapy of cancer	Dogs	Melanoma	Oncept [™] /Merial	2010 USA	ID ² needle-free.	Production of antibodies capable of preventing the progress of the disease and prolonging the animal's life
Gene therapy	Swine	Growth hormone releasing hormone (GHRH)	LifeTide [®] SW5/VGX [™] Animal Health	2008 Australia	IM followed by electroporation	Decrease in perinatal mortality and morbidity, increase of the number of pigs weaned per sow and productivity ³⁶

Immune Response to Infectious Diseases

By Betelihem T. (DVM, MVSc)

Lesson objectives

After the end of this class, the students will be able to

- ‖ Discuss the immunity for viruses, bacteria, fungi and parasites
- ‖ Distinguish the types of immunity against those pathogens
- ‖ Describe type of immunity for each pathogen

1) Immune response to viral infections

- Viruses are small segments of **nucleic acid** with a **protein** or **lipoprotein** coat
- They require host resources for their **replication**
- Since viruses are **obligate intracellular** organisms, their very existence is threatened /endanger/ if they are destroyed by the **immune system** or by

- Because of these opposing factors, both viruses and their hosts have been subjected to rigorous/hard/ selection and adaptation
- Viruses are selected for their ability to evade the host's immune responses, while at the same time animals are selected for **resistance to virus-induced disease**
- Viruses that are **eliminated** before they **replicate** cannot spread

- Hosts **eliminated** by **viruses** can no longer serve as hosts
- Among the survival strategies available to viruses are:
 - 1) A **long latency** period before severe illness or death occurs. E.g. HIV
 - 2) Facile /superficial/ transmission, in which infection is efficiently transferred during even a short **acute illness**. E.g. **Influenza** and **smallpox viruses**

- A number of specific **immune effector mechanisms**, together with **nonspecific defense mechanisms**, are called into play to prevent or eliminate **viral infection**

I. Innate immunity

- Rapid, powerful **innate immune** responses limit many viral infections by **inhibiting viral replication**

↳ **Type I interferon's** (IFN- α and IFN- β) are especially important to **viral resistance**.

They are produced by **virus infected cells**

- NK cells kill **virus infected cells**.
- NK cell cytotoxicity is stimulated by **Type I interferons** and **IL-12**.
- **NK cells** produce **IFN- γ** (Type II interferon) and this too has a direct **antiviral effect**

- **Lysozyme** can destroy several viruses, as can many intestinal enzymes and bile
- **Collectins** bind to **viral glycoproteins** and block **virus interaction** with **host cells**
- **Defensins** from **leukocytes** and **mucosal epithelial cells** play a dual role in **antiviral defenses** since they can act both on the **virus** and on the **host cells**
- Cells invaded by viruses may undergo **premature apoptosis**, thus preventing successful **viral invasion** and replication

ii) Acquired immunity

A. Antibody -mediated immunity

- Viral capsids are antigenic and it is against these and the envelope proteins that antiviral immune responses are largely mounted

Antibodies can prevent cell invasion by:

- ‖ Blocking the adsorption of **virions** to **target cells**
- ‖ Stimulating **phagocytosis** of viruses
- ‖ Triggering **complement-mediated** virolysis
- ‖ Causing viral clumping and so reducing the number of infectious units available for cell invasion

- Antibodies are also directed against **viral antigens** expressed by infected cells. As a result, these infected cells may also be destroyed.
- E.g. NCD, rabies, bovine viral diarrhoea, infectious bronchitis of birds, etc
- Antibodies may kill infected cells by complement mediated cytolysis or by antibody-dependent cell-mediated cytotoxicity (ADCC)

- These **cytotoxic cells** include **lymphocytes, macrophage, and neutrophils** with **Fc receptors** through which they can bind to **antibody-coated target cells**
- The **immunoglobulins** that **neutralize viruses** include **IgG** and **IgM** in **serum**, and **IgA** in **secretions**.
- IgE may also play a **protective role**

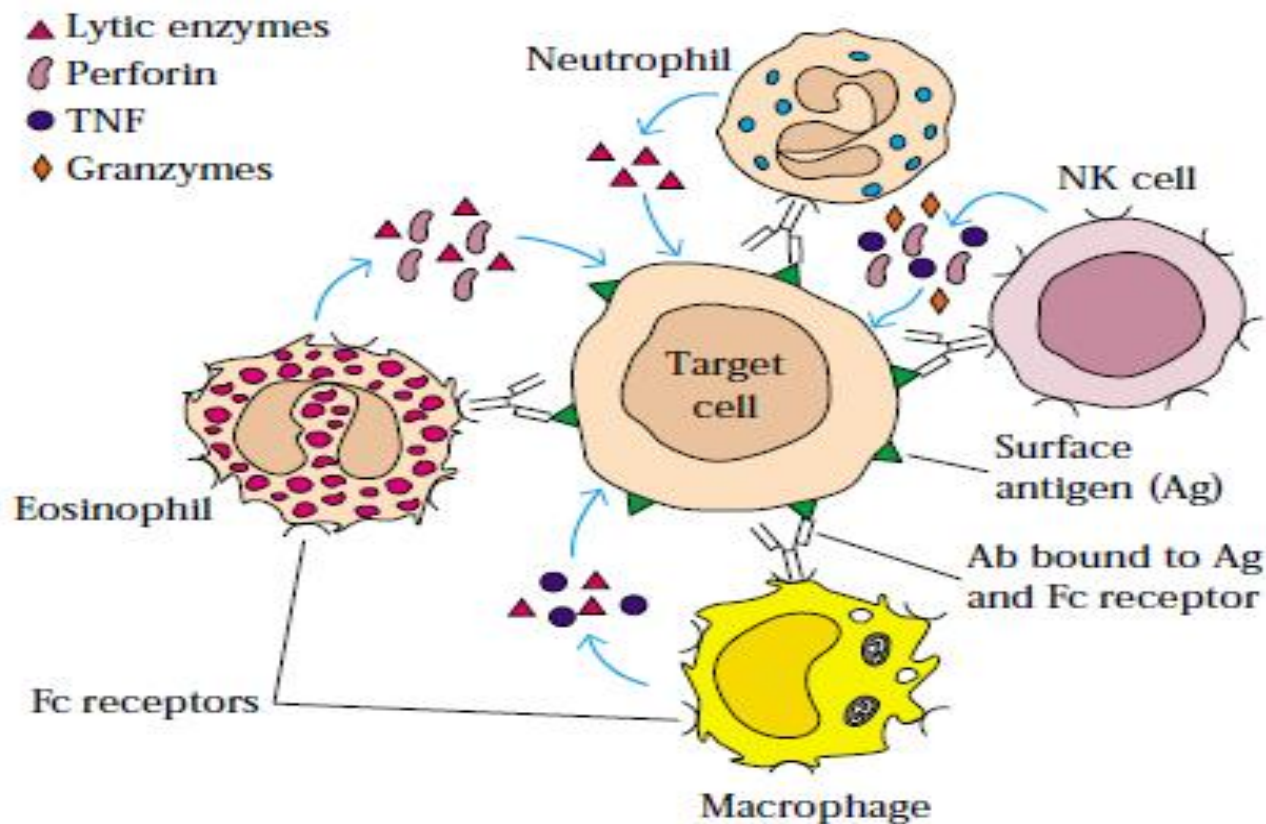


FIGURE 14-15 Antibody-dependent cell-mediated cytotoxicity (ADCC). Nonspecific cytotoxic cells are directed to specific target cells by binding to the Fc region of antibody bound to surface antigens on the target cells. Various substances (e.g., lytic enzymes, TNF, perforin, granzymes) secreted by the nonspecific cytotoxic cells then mediate target-cell destruction.

B) Cell-Mediated Immunity

- **Antibodies** are not usually able to eliminate the virus once infection has occurred
- Once an infection is established, **cell-mediated immune mechanisms** are most important in host defense.
- In general, CD_8^+ T_C cells and CD_4^+ T_H^1 cells are the main components of **cell-mediated antiviral defense**
- Activated T_H^1 cells produce a number of

- **IFN- γ** acts directly by inducing an **antiviral state** in cells.
- **IL-2** acts indirectly by assisting in the recruitment of **CTL precursors** into an effector population.
- Both IL-2 and IFN- γ activate **NK cells**, which play an important role in host defense during the first days of many viral infections until a specific CTL response develops

- In most viral infections, specific CTL activity arises within 3–4 days after infection, peaks by 7–10 days, and then declines.
- Within 7–10 days of primary infection, most virions have been eliminated, paralleling the development of CTLs.

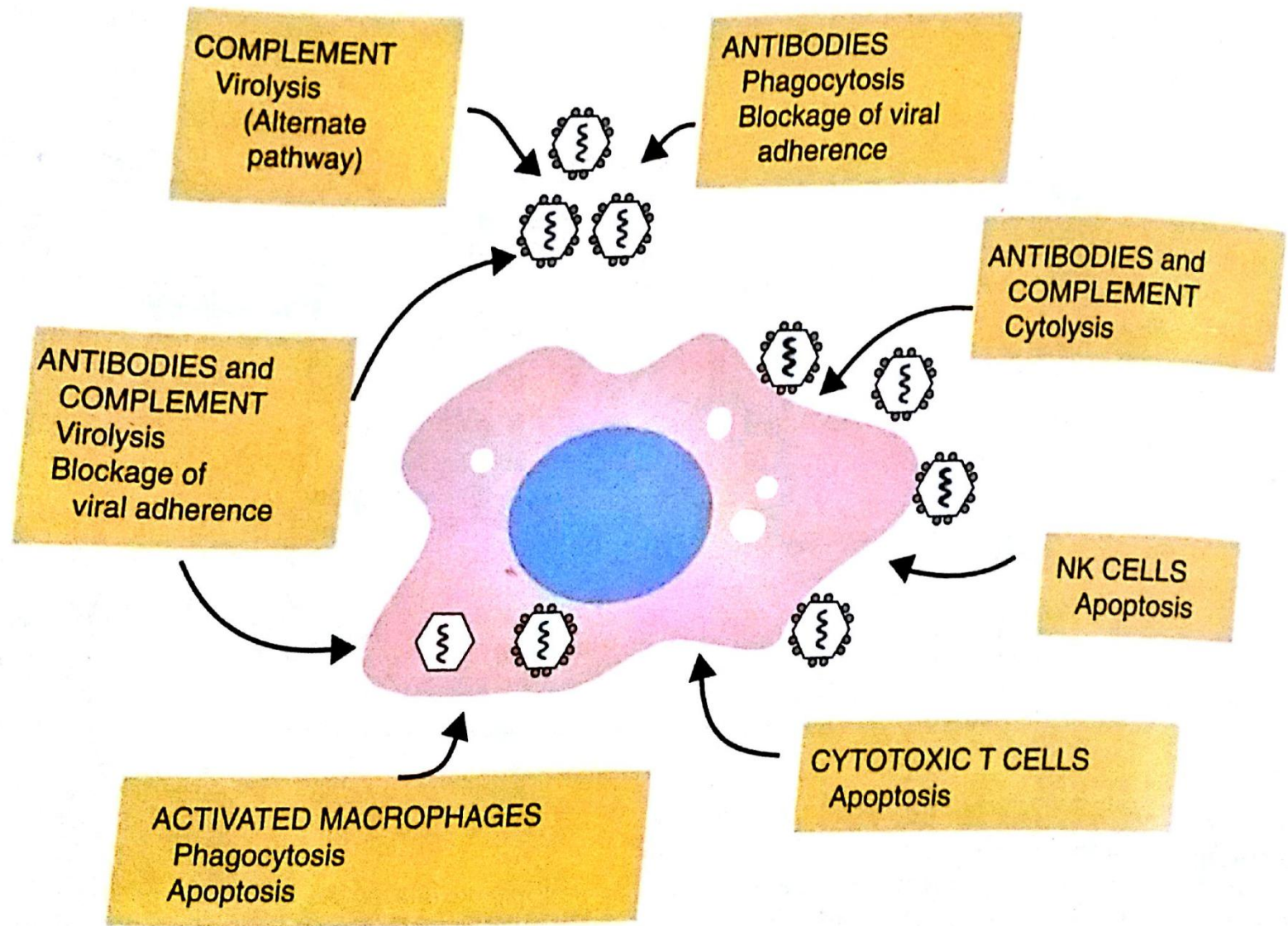


FIGURE 23-6 ■ The ways in which the immune system can protect the body against viruses.

2) Immune response to bacterial infection

- Antimicrobial immunity consists of an **early innate response** followed by a more sustained **adaptive response**
- Recognition of invading bacteria through **toll-like receptors** (TLRs) and other receptors induces, **inflammation, cytokine release** and **complement activation**
- If this is **insufficient** to eliminate the

- Dendritic cells and macrophages ingest invading bacteria and initiate acquired immunity by secreting cytokines and triggering both T and B cells responses.

- TLRs play a role in recognizing invading microbes and in triggering inflammation
- These receptors are expressed on (sentinel cells) macrophages and mast cells, as well as on dendritic cells, eosinophils and epithelial cells in the respiratory tract and intestine

- Pathogen Associated Molecular Patterns include the Lipopolysaccharides of gram negative bacteria, the lipoteichoic acids of gram positive bacteria.
- The mannans of yeasts and the glycolipids of mycobacteria as well as bacterial DNA.
- All of these molecules are restricted to microorganism and are not found in animal tissue

- Bacterial DNA-is a significant stimulator of innate immunity. It differ from eukaryotic DNA in that it contains a large proportion of the dinucleotide cytosine -guanosine (CpG).
- In addition while the cytosine in eukaryotic DNA is normally methylated this is not the case in bacterial DNA
- Thus unmethylated CpG dinucleotides can trigger innate immune response

- There are five basic mechanism by which the acquired immune responses combat bacterial infections
 - i) Neutralization of toxins or enzymes by antibody
 - ii) Killing of bacteria by antibodies and complement
 - iii) Opsonization of bacteria by antibodies and complement, resulting in their phagocytosis and destruction

- iv) Destruction of intracellular bacteria by activated macrophages
- v) Direct killing of bacteria by cytotoxic T cells and natural killer cells

Immune response to extracellular bacteria

① Toxin neutralization

② Complement-mediated lysis

③ Opsonization and phagocytosis

④ Anaphylatoxins mediate mast cell degranulation

⑤ Chemotaxis

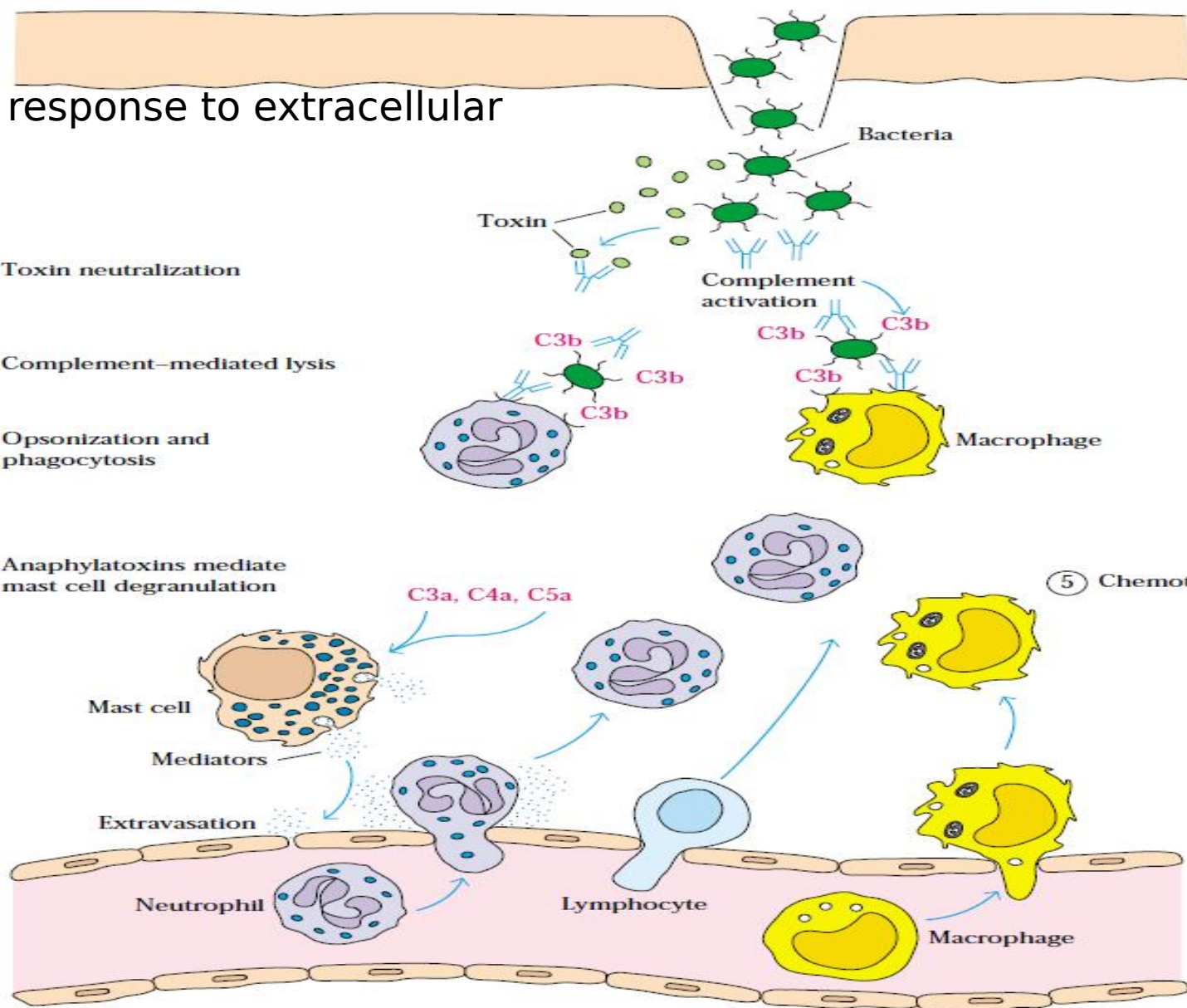


FIGURE 17-8 Antibody-mediated mechanisms for combating infection by extracellular bacteria. (1) Antibody neutralizes bacterial toxins. (2) Complement activation on bacterial surfaces leads to complement-mediated lysis of bacteria. (3) Antibody and the complement split product C_3b bind to bacteria, serving as opsonins to

increase phagocytosis. (4) C_3a , C_4a , and C_5a , generated by antibody-initiated complement activation, induce local mast cell degranulation, releasing substances that mediate vasodilation and extravasation of lymphocytes and neutrophils. (5) Other complement split products are chemotactic for neutrophils and macrophages.

⌘ **Immune response to intracellular bacteria**

- While innate immunity is not very effective against intracellular bacterial pathogens, intracellular bacteria can activate NK cells, which, in turn, provide an early defense against these bacteria.
- Intracellular bacterial infections tend to induce a cell-mediated immune response, specifically, delayed type hypersensitivity.

- In this response, cytokines secreted by CD_4^+ T cells are important—notably IFN- γ , which activates macrophages to kill ingested pathogens more effectively

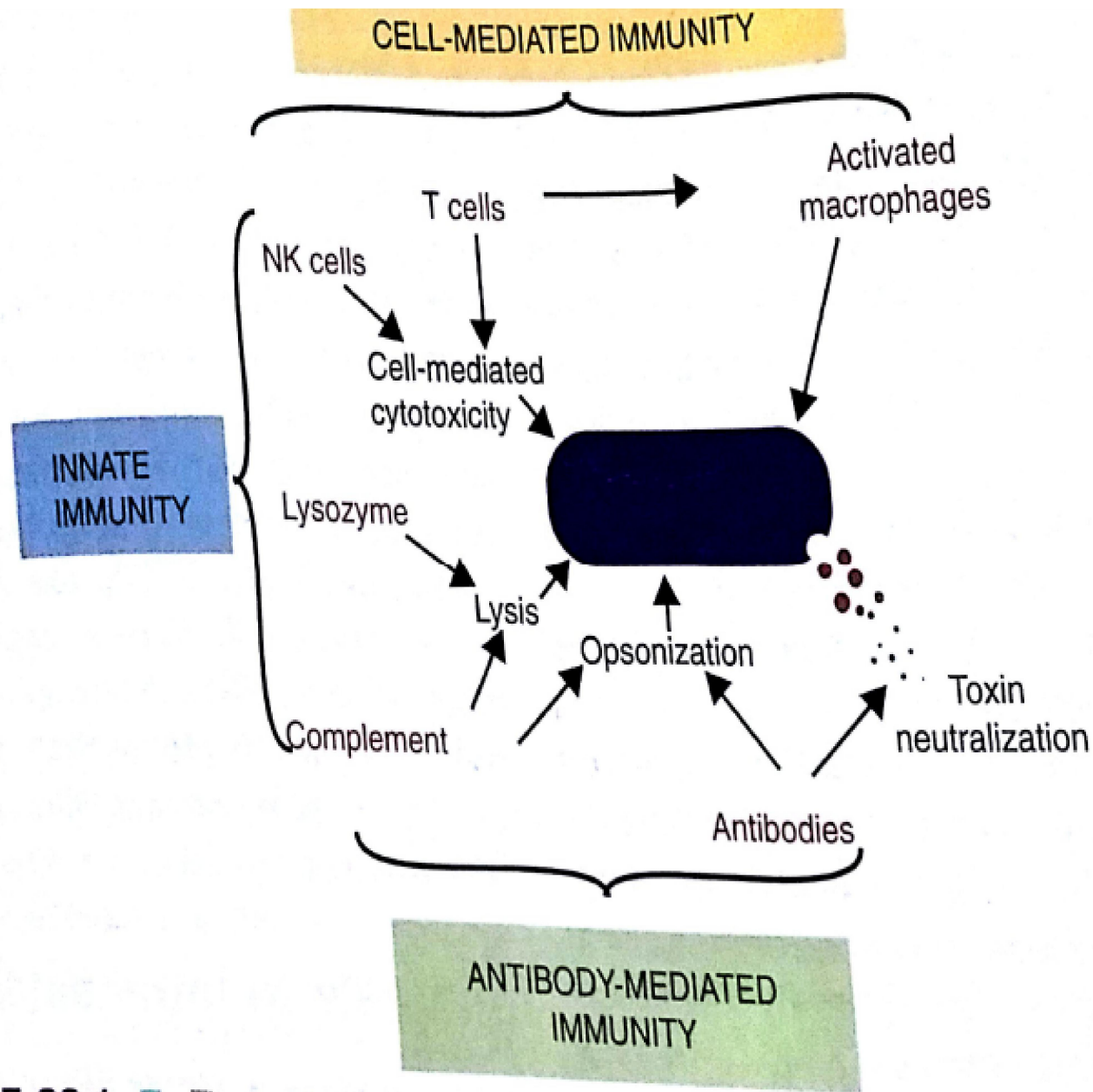


FIGURE 22-1 ■ The mechanisms by which the immune responses can protect the body against bacterial invasion.

3) Immune response to fungal infection

- The barrier of innate immunity control most fungi
- Innate immune mechanism against invasive fungi such as *Candida* spp or *Aspergillus* spp include activation of the alternative pathway of the complement system resulting in attraction of neutrophils and attempts by these neutrophils to ingest the invading hyphae or pseudohyphae

- Neutrophils are also activated by the IL-23/IL-17
- IL-23 activates T_h17 cells.
- The IL-17 produced by T_h17 cells then activates both neutrophils and endothelial cells and promote acute inflammation
- Once established, fungal infections can be destroyed only by Th1-mediated mechanisms

- T_h1 functions primarily in fungal infections by activating macrophages and by promoting epidermal growth and keratinization
- Some T and NK cells can exert a direct cytotoxic effect on yeast such as *Cryptococcus neoformans* and *Candida albicans*

4) Immune response to parasitic infection

a) Immunity to Protozoa

- The mechanism of innate resistance to protozoa appear to be similar to those of that prevent bacterial and viral invasion.
- Like other invaders, protozoa stimulate both antibody and cell-mediated immune response

- In general, antibodies control the numbers of parasites in blood and tissue fluids, whereas cell-mediated responses are directed largely against intracellular parasites
 - └ Serum antibodies directed against protozoan surface antigens may opsonize, agglutinate, or immobilize them
 - └ Antibodies together with complement and cytotoxic cells may kill them

- Some antibodies may inhibit their division
- In babesiosis, the infective stages of the organisms (sporozoites) invade red blood cells.
- Infected red cells incorporate *Babesia* antigens into their membranes.

These in turn induce antibodies that opsonize the red cells and cause their removal by phagocytosis

- Infected red cells may also be destroyed by ADCC. (antibody-dependent cell-mediated cytotoxicity)
- Macrophages and cytotoxic lymphocytes can recognize the opsonizing-antibody complex on surface of infected erythrocytes
- Cytotoxic T cells may play a role early in infection, when the number of infected

- Protective immunity against *Cryptosporidia*, *Eimeria*, *Neospora*, *Plasmodia* and *Toxoplasma* is generally mediated by T_h1 response.
- T_h1 cells secrete $INF-\gamma$, $INF-\gamma$ activates macrophages enabling them to kill the intracellular organism by permitting lysosome-phagosome fusion.

b) Immunity to Helminths

- Although the complement and other factors of natural immune response might contribute to protecting against the helminth infections, specific immune response with the production of antibodies and cytokines is important.

- The Th² cells are producers of cytokines like IL-4, IL-5 and IL-13 which, among their other functions, induce IgE production by B cells and activate the fundamental components in protecting against helminths, namely eosinophils, mastocytes and basophiles, respectively.

- IgE antibodies bind to circulating basophiles or tissue mast cells.
- This induces the release of histamine and other reaction mediators of immediate hypersensitivity leading to the destruction of helminths

- Macrophage and eosinophils with CD23(Fc receptor), These cells can therefore bind to IgE-coated parasites, become activated, and kill them
- Th¹ cells responses may be little protective benefits
- Cytotoxic T cells may attack helminths that are deeply embedded in the intestinal mucosa or undergoing tissue migration

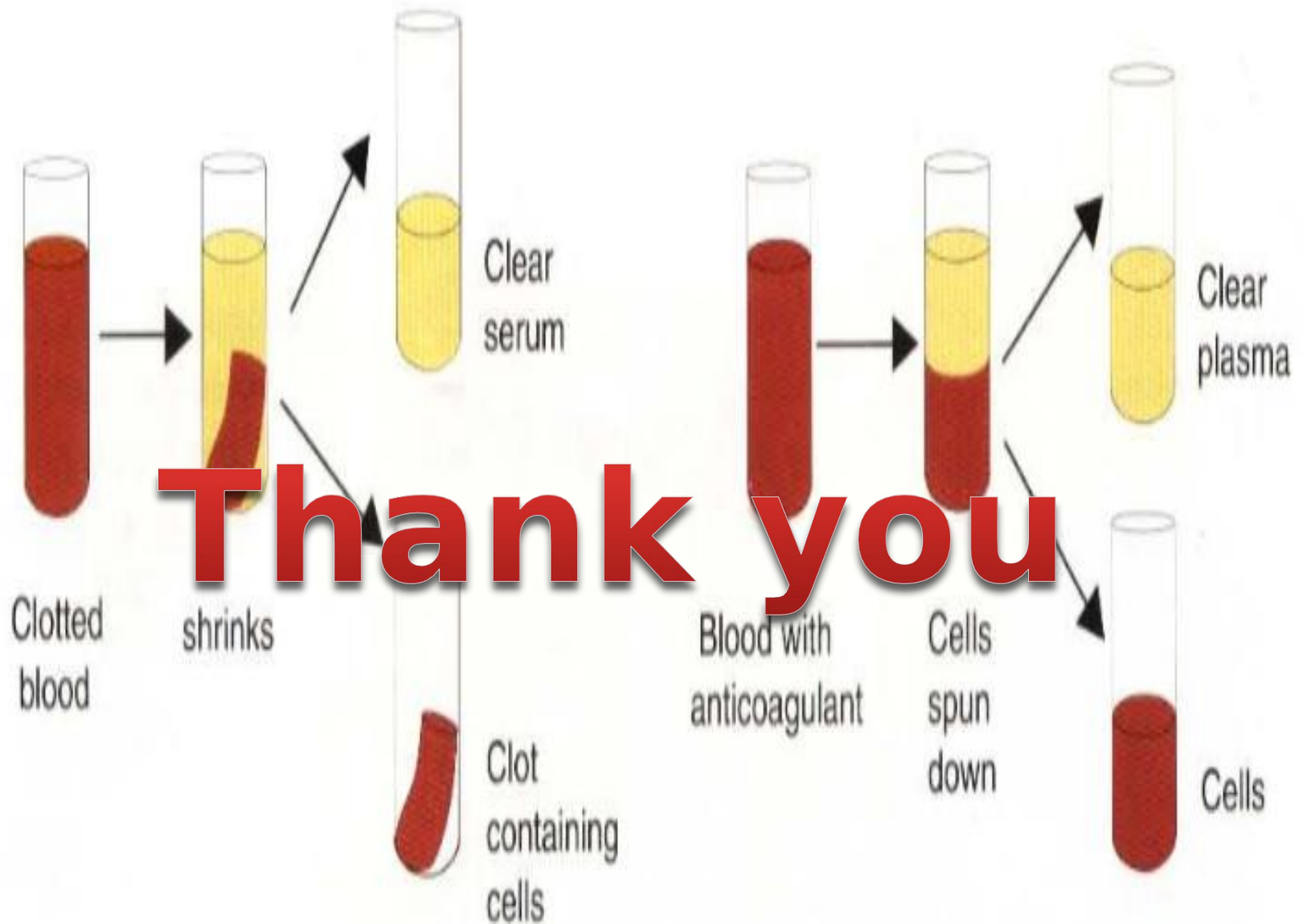


Figure 1-5. The difference between serum and plasma. Plasma contains blood-clotting components that are absent from serum.