Introduction to Immune System

The **immune system** is a host defense system comprising many biological structures and processes within an organism that protects against disease. To function properly, an immune system must detect a wide variety of agents, known as pathogens, from viruses to parasitic worms, and distinguish them from the organism's own healthy tissue. In many species, the immune system can be classified into subsystems, such as the innate immune system versus the adaptive immune system, or humoral immunity versus cell-mediated immunity. In humans, the blood–brain barrier, blood–cerebrospinal fluid barrier, and similar fluid–brain barriers separate the peripheral immune system from the neuroimmune system which protects the brain.

Pathogens can rapidly evolve and adapt, and thereby avoid detection and neutralization by the immune system; however, multiple defense mechanisms have also evolved to recognize and neutralize pathogens. Even simple unicellular organisms such as bacteria possess a rudimentary immune system, in the form of enzymes that protect against bacteriophage infections. Other basic immune mechanisms evolved in ancient eukaryotes and remain in their modern descendants, such as plants and invertebrates. These mechanisms include phagocytosis, antimicrobial peptides called defensins, and the complement system. Jawed vertebrates, including humans, have even more sophisticated defense mechanisms,[1] including the ability to adapt over time to recognize specific pathogens more efficiently. Adaptive (or acquired) immunity creates immunological memory after an initial response to a specific pathogen, leading to an enhanced response to subsequent encounters with that same pathogen. This process of acquired immunity is the basis of vaccination.

Disorders of the immune system can result in autoimmune diseases, inflammatory diseases and cancer. Immunodeficiency occurs when the immune system is less active than normal, resulting in recurring and life-threatening infections. In humans, immunodeficiency can either be the result of a genetic disease such as severe combined immunodeficiency, acquired conditions such as HIV/AIDS, or the use of immunosuppressive medication. In contrast, autoimmunity results from a hyperactive immune system attacking normal tissues as if they were foreign organisms. Common autoimmune diseases include Hashimoto's thyroiditis, rheumatoid arthritis, diabetes mellitus type 1, and systemic lupus erythematosus.

The immune system protects organisms from infection with layered defenses of increasing specificity. In simple terms, physical barriers prevent pathogens such as bacteria and viruses from entering the organism. If a pathogen breaches these barriers, the innate immune system provides an immediate, but non-specific response. Innate immune systems are found in all plants and animals.[10] If pathogens successfully evade the innate response, vertebrates possess a second layer of protection, the adaptive immune system, which is activated by the innate response. Here, the immune system adapts its response during an infection to improve its recognition of the pathogen. This improved response is then retained after the pathogen has been eliminated, in the form of an immunological memory, and allows the adaptive immune system to mount faster and stronger attacks each time this pathogen is encountere.

Innate immune system: Fast and broadly effective

The strength of the innate, general defense is to be able to take action very quickly. It makes sure, for example that bacteria that have entered the skin through a small wound are detected and partly destroyed on the spot within a few hours. As the innate immune response is not specialized for specific pathogens, it does not need a long start-up phase. Because of this broad effect, it is only capable to a certain degree of stopping germs from entering and spreading in the body.

The innate defense consists of several elements:

- The skin and all mucous membranes in the body openings, which form external barriers
- Different defense cells from the white blood cell group (leukocytes)
- Various substances in the blood and in body fluids

Protection from the outside: Skin and mucous membranes

All external and internal surfaces of the human body are a key element of the innate immune system. The closed surface of the skin and of all mucous membranes already forms a mechanical barrier for pathogens, which prevents them from entering. Additionally, chemical substances like acid, enzymes or mucus prevent the bacteria or viruses from gaining a foothold. Movements created, for example, by hair-like structures in the bronchi (cilia) or by bowel muscles stop

germs from settling in the body. Tear fluid, sweat, or urine rinsing the urinary organs all have a similar effect.

Protection from the inside: Defense cells and proteins

If, despite all obstacles, pathogens make it past the skin or mucous membranes and enter the body, the innate system's second line of defense comes into action. Inflammatory cells move to the site of infection, or defense cells that are already there are activated. Soluble protein substances of the complement system are activated, too, and help to defend the body. This leads to an inflammatory reaction where blood circulation is increased and the affected area becomes swollen and hot. Sometimes there is also a fever.

If bacteria or viruses manage to enter the body they can be eliminated directly on the spot by scavenger cells or phagocytes (from the Greek *phagein*, meaning: "to eat"). Two types of defense cells are the most effective ones: macrophages, which are found in the tissue, and neutrophil granulocytes, which are in the blood and tissue. These cells enclose the pathogens and digest them in their interior. Scavenger cells can work best if the pathogen has already been marked by antibodies or proteins of the complement system. This makes the pathogen more "palatable" for the scavenger cells.

So at this point, antibodies of the adaptive immune system support the innate defense. Vice versa, the scavenger cells can help the adaptive immune system by taking up and digesting the marked pathogens very quickly.

Complement system: Proteins in a chain reaction

Soluble substances support the defense cells of the innate immune system. A total of nine different enzymes activate one another in a process similar to a chain reaction: one enzyme of the first stage alerts several enzymes of the second stage, each of which again activates several enzymes of the third stage, and so on. This process quickly makes the defense reaction a lot stronger, because the production of these protein substances increases in such large jumps.

The tasks of these enzymes:

- They mark pathogens, making them more attractive for scavenger cells.
- They attract other immune cells from the blood.
- They dissolve the cell walls of bacteria, so that they lose fluid and minerals and die.
- They fight viruses directly by destroying the virus envelopes, or indirectly by destroying cells infected by viruses.

Natural killer cells: Searching for changed body cells

The natural killer cells are the third important part of the innate immune system. They specialize in identifying cells that are infected by a virus or that have become tumorous. They do this by looking for changes in cell surfaces. If natural killer cells find cells with a changed surface, they dissolve them using cell poisons, also called cytotoxins.

The adaptive immune system: Precision and a long memory

If the body's first line of defense – the innate immune system – is unsuccessful in destroying the pathogens, after about four to seven days the specific adaptive immune response sets in. This means that the adaptive defense takes longer, but it targets the pathogen more accurately. Another advantage: It can remember the aggressor and acts specifically against certain antigens. If there is new contact with an antigen that is already known, the defense response can then be quicker. This way the defense responses of the adaptive immune system are more efficient and faster than those of the innate defense, if the antigen is already known.

The adaptive immune system can remember the antigens because it produces memory cells. This is also the reason why there are some illnesses you can only get once in your life, because afterwards your body becomes "immune." While after first contact with the pathogen it takes several days for the immune system to respond, a second infection often has no consequences, or at least the symptoms are weaker.

The adaptive immune system has several parts that react in different ways, depending on the place in the body where the pathogen is. Antibodies are made available for germs outside the cells (in the blood and in body fluids). To eliminate pathogens that are inside the tissue, a cell-mediated immune response is necessary.

These parts of the adaptive defense include:

- T lymphocytes
- B lymphocytes
- antibodies as soluble proteins in the blood
- cytokines in the blood and tissue as hormone-like messenger substances

T lymphocytes

In the adaptive immune system, T lymphocytes (T cells) are responsible for the special defense in the tissue, which is carried out by cells. They recognize infected cells and are responsible for their destruction and elimination from the body.

T lymphocytes belong to the group of white blood cells and, in adults, are produced in the bone marrow. In the thymus gland, they mature into cells that are capable of recognizing self from non-self cells. T cells have characteristic structures on their surfaces that pathogens can bind to – similar to a lock that a specific key fits to.

A pathogen that exactly fits a T cell stimulates this T cell to multiply quickly and to develop into specialized T cells. At the same time, the great number of newly produced T cells triggers other defense reactions. This leads to the pathogens being destroyed and eliminated from the body.

During the course of a defense reaction, T lymphocytes develop into specialized cells. These include:

- T helper cells
- T killer cells or cytotoxic T cells
- memory T cells
- regulatory T cells

B lymphocytes

B lymphocytes are an important pillar of the adaptive defense: They produce antibodies, which are in the blood as soluble proteins and are specialized for exactly one pathogen.

The cells of the adaptive immune systems interact either directly by binding to the surface of different defense cells or they use soluble messenger substances like the cytokines. These messenger substances are mostly proteins and are produced by different cells in the organism.

Cells of the Immune System

Leukocytes (white blood cells) are immune system cells involved in defending the body against infectious disease and foreign materials. Five different types of leukocytes exist, all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. The innate leukocytes include the phagocytes, mast cells, eosinophils, basophils, and natural killer cells. These cells identify and eliminate pathogens and are important mediators in the activation of the adaptive immune system.

Neutrophils and macrophages are phagocytes that travel throughout the body in pursuit of invading pathogens. Neutrophils are normally found in the bloodstream and are the most abundant type of phagocyte. During the acute phase of inflammation neutrophils migrate toward the site of inflammation and are usually the first cells to arrive at the scene of infection . Macrophages reside within tissues and produce a wide array of chemicals. They also act as scavengers, ridding the body of worn-out cells and other debris, and as antigen-presenting cells that activate the adaptive immune system. Dendritic cells are phagocytes in tissues that are in contact with the external environment, and are located mainly in the skin, nose, lungs, stomach, and intestines. These cells serve as a link between the bodily tissues and the innate and adaptive immune systems, as they present antigen to T-cells, one of the key cell types of the adaptive immune system.

Natural killer cells are leukocytes that attack and destroy tumor cells, or cells that have been infected by viruses.

The cells of the adaptive immune system are special types of leukocytes, called lymphocytes . B cells and T cells are the major types of lymphocytes and are derived from hematopoietic stem cells in the bone marrow.

Blood Cells

Red blood cells, several white blood cells including lymphocytes, a monocyte, a neutrophil, and many small disc-shaped platelets.

T cells recognize a "non-self" target, such as a pathogen, only after antigens have been processed and presented in combination with a "self" receptor, called a major histocompatibility complex (MHC) molecule. There are two major subtypes of T cells: the killer T cell, which kills cells that are infected with viruses (and other pathogens) or are otherwise damaged or dysfunctional, and the helper T cell, which regulates both innate and adaptive immune responses and helps determine which immune responses the body makes to a particular pathogen. These cells have no cytotoxic activity and do not kill infected cells or clear pathogens directly. A third, minor subtype are the γ T cells that recognize intact antigens not bound to MHC receptors.

In contrast, the B cell antigen-specific receptor is an antibody molecule on the B cell surface, which recognizes whole pathogens without any need for antigen processing. Each lineage of B cell expresses a different antibody, so the complete set of B cell antigen receptors represent all the antibodies that the body can manufacture.

Organs of the Immune System

The organs of the immune system are positioned throughout the body. They are called lymphoid organs because they are home to lymphocytes, small white bloodcells that are the key players in the immune system.

There are two groups of immune system organs.

- Primary (central)--organs where *immature* lymphocytes develop
    - Thymus
    - Bone marrow
- Secondary (peripheral)--tissues where antigen is localized so that it can be effectively exposed to *mature* lymphocytes
    - Lymph nodes
    - Appendix

- Peyer's Patches (of GI tract)
- Tonsils
- Adenoids
- Spleen
- MALT (**M**ucosal-**A**ssociated **L**ymphoid **T**issue)
    - GALT (**Gut**-**A**ssociated **L**ymphoid **T**issue)
    - BALT (**B**ronchial/Tracheal-**A**ssociated **L**ymphoid **T**issue)
    - NALT (**N**ose-**A**ssociated **L**ymphoid **T**issue)
    - VALT (**V**ulvovaginal-**A**ssociated **L**ymphoid **T**issue)

Thymus

The thymus (from θυμος, thumos, Greek for warty outgrowth) is the site of T cell maturation. T cells become immunocompetent here; that is, they develop their ability to mount an effective immune response against foreign invaders without attacking the host's own tissues. The thymus lies just above the heart in the mediastinum. It is largest in childhood, and it begins to shrink significantly as a person ages. The organ itself contains two lobes, and each lobe contains numerous lobules, separated from each other by connective tissue septa known as trabeculae. Each lobule is separated into an inner medulla (with few immature thymocytes) and an outer cortex (with large numbers of immature thymocytes). In the thymus, the many different T cells (produced via somatic genetic mutation, discussed below) are exposed to MHC/Ag and MHC/self-Ag. If they do *not* react to MHC/Ag, they are destroyed due to their ineffectiveness (**positive selection**). On the other hand, if they *do* react to MHC/self-AG, they are destroyed in order to stop them from becoming traitorous autoimmune responses against the body's own tissue(**negative selection**). Only 1 out of 20 immature thymocytes will pass successfully through this vetting process and become functional T cells. Dendritic cells, macrophages, and epithelial cells are interspersed throughout both the medulla and cortex; special epithelial nurse cells surround clusters of thymocytes in the cortex.

Myasthenia gravis is an autoimmune disease where the body creates antibodies against acetylcholine receptors at post-synaptic neuromuscular junctions. In up to 25% of MG cases, there is a tumor of the thymus present (thymoma); the exact reasons for this tumor development

is unknown. Additionally, removal of the thymoma will halt the disease's progress; this deserves further study as antibodies are released from plasma B cells rather than T cells, yet T helper cells may mediate the autoimmunity.

DiGeorge's Syndrome is a congenital lack of a thymus, and causes in increase in infections and a depressed immune system (especially the cell-mediated response is retarded by the lack of a thymus).

Bone Marrow

Bone marrow (medulla ossea) is the site of B cell maturation in mice and humans. B cells undergo both positive and negative selection, similar to T cell maturation in the thymus. Bone marrow is also the site of hematopoiesis, the development of the myriad blood cells from progenitor cells. The site of B cell maturation in birds is the bursa of Fabricius, after which B cells are named. The tissue of bone marrow where leukocytes, red blood cells, and platelets develop (i.e., the site of hematopoiesis) is known as myeloid tissue.

Lymph Nodes

Extracellular fluid flows from capillary beds into tissue; from this tissue it enters lymphatic capillaries that are "pumped" along with the movement of skeletal muscle towards lymph nodes. The paracortical areas of the nodes contain T cells, and the central areas contain **germinal centers**, where B cells are contained. APCs and antigen are sent from the tissue into the lymphatics, eventually reaching the lymph nodes where they can be exposed to the T and B cell populations. This allows a faster response, as the many combinations of T and B cell specificities are able to reside in several locations throughout the body (the lymph nodes) rather than relying on random meetings of antigen and lymphocytes throughout the tissues themselves.

Spleen

The spleen acts as a site of hematopoiesis during the second and third trimesters of development, before the long bones have fully developed. In the adult, the spleen acts as a site for breakdown of dying red blood cells (lifespan 120 days). For this reason, enlargement of the spleen

(splenomegaly) can occur in sickle cell anemia or in certain infections. White pulp, near the arteriolar entry points into the spleen, is where lymphocytes reside and are degraded. The central red pulp is the site of RBCs breakdown. The white pulp region has a central part, with the T cells residing in the **PALS, or PeriArteriolar Lymphoid Sheath** and a B cell ring (or corona) surrounding the PALS.

Primary and Secondary Immune Responses

Immune responses to antigens may be categorised as primary or secondary responses. The primary immune response of the body to antigen occurs on the first occasion it is encountered. Depending on the nature of the antigen and the site of entry this response can take up to 14 days to resolve and leads to the generation of memory cells with a high specificity for the inducing antigen. The humoral response, mediated by B cells with the help of T cells, produces high-affinity and antigen-specific antibodies. This is in contrast with the CD8 T-cell response which leads to the generation of large numbers of antigen-specific cells that are capable of directly killing infected cells. Antigen-specific CD4 T cells, which provide help to B cells in the form of cytokines and other stimulatory factors, can also be expanded upon antigenic stimulation.

The secondary response of both B- and T cells is observed following subsequent encounter with the same antigen and is more rapid leading to the activation of previously generated memory cells. This has some quantitative and qualitative differences from the primary response.

- The innate immune system is the first line of defence against infectious agents. When this is breached, the adaptive immune system provides a more efficient response to clearing pathogens.
- The adaptive immune system has the capacity to ‘remember’ previous antigens, a process termed immunological memory.
- Antigen-specific T cells are selected during a primary immune response and expand to produce clones of T cells with high specificity for the activating antigen.
- In a B cell primary response to a thymus-dependent antigen, the immune system selects B cells with a high affinity and specificity for the antigen and these become memory cells.

- The selection of B cells with a high affinity for a given antigen occurs in the germinal centres of secondary lymphoid follicles and requires the enzyme activation-induced cytidine deaminase (AID) and interactions with other immune cells.
- The ability to change the isotype of antibody produced (class switching) by a B cell also occurs in germinal centres and requires AID.
- In a secondary response to the same antigen, memory cells are rapidly activated. This process is quicker and more effective than the primary response.

Stages of Primary Immune Response

When somebody is exposed to an antigen they have never encountered before, a relatively brief, weak immune response, the primary immune response, develops. This can be broken down into four stages: the lag, exponential, steady state, and declining phases.

- **Lag (latent) phase**

This is the time from initial antigen exposure to when antibodies are detected in the blood, and takes about a week. In this time, specialized B and T cells are activated by contact with the antigen.

- **Exponential phase**

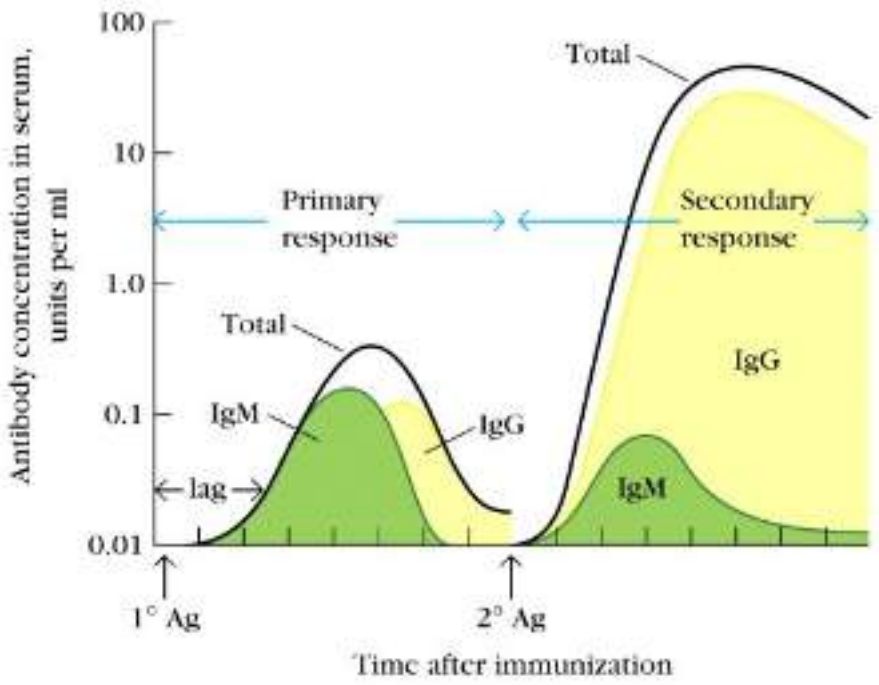
Here, there is a sharp rise in the levels of antibodies, which are secreted by large numbers of plasma cells (differentiated B cells).

- **Steady state (plateau) phase**

The antibody levels stay relatively constant due to the continuous secretion of antibodies to replenish any that have degraded.

- **Declining phase**

Antibody levels slowly decrease, due to existing plasma cells dying off, with no new plasma cells generated to replace them. The immunogen has probably been eliminated from the body, so no further antibody production is needed.



Secondary Immune Response

For second and subsequent encounters with similar antigens, secondary (anamnestic) immune responses occur. Here, the lag phase is shorter, and high and steady levels of antibodies are generated within a few days. This is due to antigen-specific memory T and B cells, originally produced during the primary response.

Due to the rapidness of the secondary immune response, the antigen can be eliminated from the body fairly soon after it has entered, and before it causes disease. The antibodies produced remain in circulation longer to ensure the infection has disappeared.

Antigen- Properties, Types and Determinants of Antigenicity

Antigen is a substances usually protein in nature and sometimes polysaccharide, that generates a specific immune response and induces the formation of a specific antibody or specially sensitized T cells or both

Although all antigens are recognized by specific lymphocytes or by antibodies, only some antigens are capable of activating lymphocytes. Molecules that stimulate immune responses are called I**mmunogens.**

Epitope is immunologically active regions of an immunogen (or antigen) that binds to antigen-specific membrane receptors on lymphocytes or to secreted antibodies. It is also called **antigenic determinants.**

Autoantigens, for example, are a person's own self antigens. Examples: Thyroglobulin, DNA, Corneal tissue, etc.

Alloantigens are antigens found in different members of the same species (the red blood cell antigens A and B are examples).

Heterophile antigens are identical antigens found in the cells of different species. Examples: Forrssman antigen, Cross-reacting microbial antigens, etc.

Adjuvants are substances that are non-immunogenic alone but enhance the immunogenicity of any added immunogen.

Chemical Nature of Antigens (Immunogens)

A. **Proteins**

   The vast majority of immunogens are proteins. These may be pure proteins or they may be glycoproteins or lipoproteins. In general, proteins are usually very good immunogens.

B. **Polysaccharides**

   Pure polysaccharides and lipopolysaccharides are good immunogens.

C. **Nucleic Acids**

   Nucleic acids are usually poorly immunogenic. However, they may become immunogenic when single stranded or when complexed with proteins

D. **Lipids**

   In general lipids are non-immunogenic, although they may be haptens

Types of antigen

On the basis of order of their class (Origin)

1. **Exogenous antigens**
   - These antigens enters the body or system and start circulating in the body fluids and trapped by the APCs (Antigen processing cells such as macrophages, dendritic cells, etc.)
   - The uptakes of these exogenous antigens by APCs are mainly mediated by the phagocytosis
   - Examples: bacteria, viruses, fungi etc
   - Some antigens start out as exogenontigens, and later become endogenous (for example, intracellular viruses)

2. Endogenous antigens

- These are body's own cells or sub fragments or compounds or the antigenic products that are produced.
- The endogenous antigens are processed by the macrophages which are later accepted by the cytotoxic T – cells.
- Endogenous antigens include xenogenic (heterologous), autologous and idiotypic or allogenic (homologous) antigens.
- Examples: Blood group antigens, HLA (Histocompatibility Leukocyte antigens), etc.

3. Autoantigens

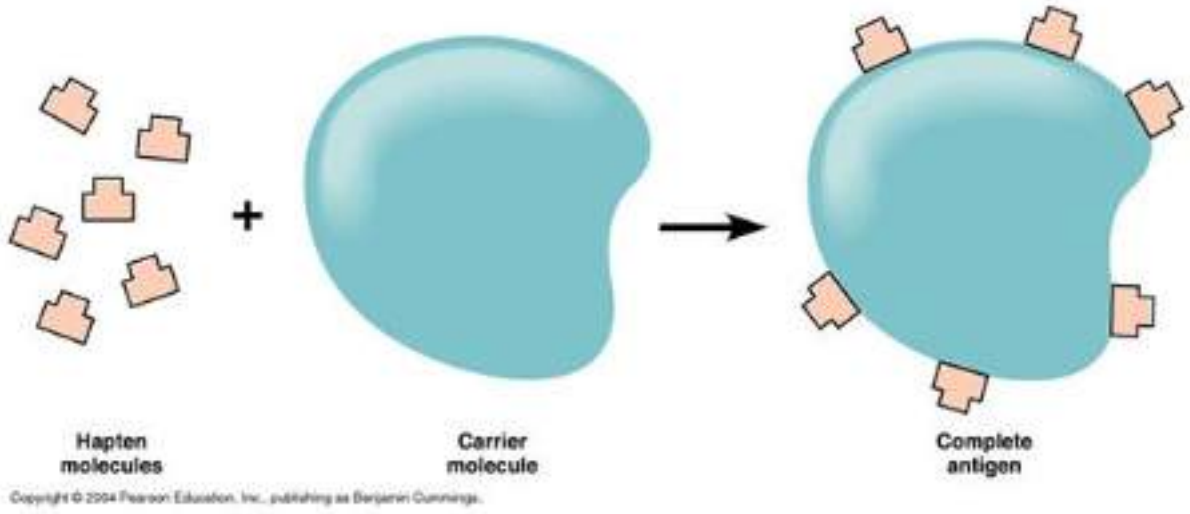
- An autoantigen is usually a normal protein or complex of proteins (and sometimes DNA or RNA) that is recognized by the immune system of patients suffering from a specific autoimmune disease
- These antigens should not be, under normal conditions, the target of the immune system, but, due mainly to genetic and environmental factors, the normal immunological tolerance for such an antigen has been lost in these patients.
- Examples: Nucleoproteins, Nucleic acids, etc.

On the basis of immune response

1. Complete Antigen or Immunogen

- Posses antigenic properties denovo, i.e. ther are able to generate an immune response by themselves.
- High molecular weight (more than 10,000)
- May be proteins or polysaccharides

2. Incomplete Antigen or Hapten



- These are the foreign substance, usually non-protein substances
- Unable to induce an immune response by itself, they require carrier molecule to act as a complete antigen.
- The carrier molecule is a non-antigenic component and helps in provoking the immune response. Example: Serum Protein such as Albumin or Globulin.
- Low Molecular Weight (Less than 10,000)
- Haptens can react specifically with its corresponding antibody.
- Examples: Capsular polysaccharide of pneumococcus, polysaccharide "C" of beta haemolytic streptococci, cardiolipin antigens, etc.

Property of antigens/ Factors Influencing Immunogenicity

Immunogenicity is determined by:

1. Foreignness

- An antigen must be a foreign substances to the animal to elicit an immune response.

2. Molecular Size

- The most active immunogens tend to have a molecular mass of 14,000 to 6,00,000 Da.
- Examples: tetanus toxoid, egg albumin, thyroglobulin are highly antigenic.
- Insulin (5700) are either non-antigenic or weakly antigenic.

3. Chemical Nature and Composition

- In general, the more complex the substance is chemically the more immunogenic it will be.
- Antigens are mainly proteins and some are polysaccharides.
- It is presumed that presence of an aromatic radical is essential for rigidity and antigenicity of a substance.

4. Physical Form

- In general particulate antigens are more immunogenic than soluble ones.
- Denatured antigens are more immunogenic than the native form.

5. Antigen Specificity

- Antigen Specificity depends on the specific actives sites on the antigenic molecules (Antigenic determinants).
- Antigenic determinants or epitopes are the regions of antigen which specifically binds with the antibody molecule.

6. Species Specificity

- Tissues of all individuals in a particular species possess, species specific antigen.
- Human Blood proteins can be differentiated from animal protein by specific antigen-antibody reaction.

7. Organ Specificity

- Organ specific antigens are confined to particular organ or tissue.

- Certain proteins of brain, kidney, thyroglobulin and lens protein of one species share specificity with that of another species.

8. Auto-specificity

- The autologous or self antigens are ordinarily not immunogenic, but under certain circumstances lens protein, thyroglobulin and others may act as ***autoantigens.***

9. Genetic Factors

- Some substances are immunogenic in one species but not in another .Similarly, some substances are immunogenic in one individual but not in others (i.e. responders and non-responders).
- The species or individuals may lack or have altered genes that code for the receptors for antigen on B cells and T cells.
- They may not have the appropriate genes needed for the APC to present antigen to the helper T cells.

10. Age

- Age can also influence immunogenicity.
- Usually the very young and the very old have a diminished ability to elicit and immune response in response to an immunogen.

11. Degradability

- Antigens that are easily phagocytosed are generally more immunogenic.
- This is because for most antigens (T-dependant antigens) the development of an immune response requires that the antigen be phagocytosed, processed and presented to helper T cells by an antigen presenting cell (APC).

12. Dose of the antigen

- The dose of administration of an immunogen can influence its immunogenicity.
- There is a dose of antigen above or below which the immune response will not be optimal.

13. Route of Administration

- Generally the subcutaneous route is better than the intravenous or intragastric routes.
- The route of antigen administration can also alter the nature of the response.
- Antigen administered intravenously is carried first to the spleen, whereas antigen administered subcutaneously moves first to local lymph nodes.

14. Adjuvants

- Substances that can enhance the immune response to an immunogen are called adjuvants.
- The use of adjuvants, however, is often hampered by undesirable side effects such as fever and inflammation.
- Example: aluminum hydroxide.

Antibody

An antibody is a Y-shaped protein that is produced by B cells to identify and neutralize antigens in the body.

- An antibody, also known as an immunoglobulin, is a large Y-shaped protein produced by B-cells that is used by the immune system to identify and neutralize foreign objects such as bacteria and viruses.
- Each tip of the "Y" of an antibody contains a paratope (a structure analogous to a lock) that is specific for one particular epitope (similarly analogous to a key) on an antigen, allowing these two structures to bind together with precision.
- Though the general structure of all antibodies is very similar, a small region at the tip of the protein is extremely variable, allowing millions of antibodies with different antigen binding sites, to exist. This region is known as the hypervariable region.
- There are 5 isotypes of antibodies that are found in different locations and perform different specific functions.
- The base of the Y plays a role in modulating immune cell activity. This region is called the Fc region, and phagocytes may bind to it to initiate phagocytosis.

- Antibodies that bind to surface antigens on, for example, a bacterium attract the first component of the complement cascade with their Fc region and initiate activation of the "classical" complement system.

An antibody (formally called immunoglobulin) is a large Y-shaped glycoprotein produced by B-cells and used by the immune system to identify and neutralize pathogens. Antibodies are produced by B cells, and are either secreted into circulation, or remain expressed on the surface of the B cell.

Structure

The antibody recognizes a unique part of an antigen (foreign object). Each tip of the "Y" of an antibody contains a paratope (a structure analogous to a lock) that is specific for one particular epitope (similarly analogous to a key) on an antigen, allowing these two structures to bind together with precision. Using this binding mechanism, an antibody can neutralize its target directly, or tag it for attack by other parts of the immune system.

Isotypes

There are five different isotypes of antibodies. They all perform different functions and found are generally found in different parts of the body.

- **IgA:** A dimer that is secreted into mucosal surfaces, such as the gut, respiratory tract, and urogenital tract, and prevents mucosal invasion into the body by pathogens. It is resistant to the proteolytic enzymes found in the gastrointestinal mucosae.
- **IgD:** Functions mainly as an antigen receptor on B cells that have not been exposed to antigens. It has been shown to activate basophils and mast cells to produce antimicrobial factors.
- **IgE**: Found in circulation, and binds to allergens and triggers histamine release from mast cells and basophils, and is involved in allergy. Also protects against parasitic helminthes worms.
- **IgG:** Has four different forms, and provides the majority of antibody-based immunity against invading pathogens, because it is the best opsonin of any type of antibody. This is because it expresses a tail for Fc receptors on phagocytes to bind to, which activates

phagocytosis. It is the only antibody capable of crossing the placenta to give passive immunity to fetus, and can activate the classical complement system.

- **IgM:** Expressed on the surface of B cells (monomer) and in a secreted pentamer with very high avidity. Eliminates pathogens in the early stages of B cell mediated (humoral) immunity before there is sufficient IgG. Like IgG, it can also activate the classical complement system.

Function of Antibodies

Circulating antibodies are produced by clonal B cells that specifically respond to only one antigen. Antibodies contribute to immunity in three ways: preventing pathogens from entering or damaging cells by binding to them (neutralization); stimulating removal of pathogens by macrophages and other cells by coating the pathogen (opsonization); and triggering destruction of pathogens by stimulating other immune responses such as the complement pathway. The complement system starts a long cascade of protein productions that either opsonize a pathogen for phagocytosis, or lyse it directly by forming a membrane attack complex. During opsonization, the antibody will express the tail for an Fc receptor on a macrophage, neutrophil, or natural killer cell. The immune cell will then bind to the antibody's Fc tail instead of the pathogen itself, which speed up the process of finding pathogens to phagocytize. Additionally, because antibodies have two or more paratopes, they can sometimes link pathogens together, which makes phagocytosis more efficient.

Antibodies: Characteristics and Functions of Immunoglobulin's (Igs) or Antibodies!

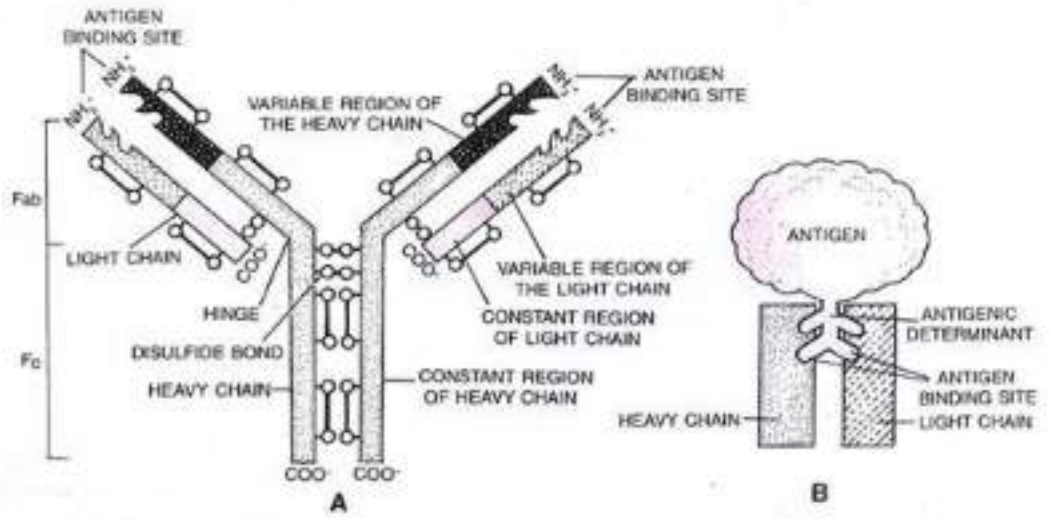
Antibodies are immunoglobulin's (Igs) which are produced in the body in response to the antigen or foreign bodies.

Thus all antibodies are immunoglobulin's but all immunoglobulin's are not antibodies.

Location and Formation:

The antibodies may be bound to a cell membrane or they may remain free. Antibodies are produced by B lymphocytes and plasma cells. In fact B-lymphocytes get transformed into plasma

cells. The mature plasma cell produces antibodies at an extremely rapid rate— about 2000 molecules per second. Antibodies direct the antibody- mediated immunity (= humoral immunity).



A, structure of an antibody molecule. B, Antigen binding site.

Types of Antibodies:

There are five types of antibodies viz:

1. IgA (Ig alpha);

2. IgD (Ig delta);

3. IgE (Ig epsilon);

4. IgG (Ig gamma) and

5. IgM (Ig mu).

Among the antibodies, IgG forms 80% of the antibodies in the body.

Antibody Structure:

IgG has been studied extensively and serves as a model of basic structural unit of all Igs.

An antibody molecule consists of the following parts.

(i) Heavy and Light Chains:

An antibody molecule is made up of 4 peptide chains, two small called light chains and two longer called heavy chains. Hence an antibody is represented as H_2L_2. The heavy chain has larger number of amino acids while light chain has smaller number of amino acids. Heavy and light chains may be either lambda or Kappa type.

(ii) Constant and Variable Regions:

There are two different regions the constant region and variable region in each chain of the antibody.

(iii) Disulfide Bonds and Hinge Region:

A disulfide bond joins a light chain with a heavy chain. Two disulfide bonds also link the two heavy chains. This part of the antibody displays considerable flexibility and is called the hinge region. Because the antibody "arms" can move somewhat as the hinge region bends, an antibody can assume a Y shaped molecule.

(iv) Fragment Antigen Binding (Fab) and Fragment Crystallisable (Fc):

Two identical fragments of Y-shaped molecule possess the antigen-binding sites and are thus named fragment-antigen binding (Fab). The antigen-binding sites bind to the specific antigens in a lock and key pattern, forming an antigen-antibody complex. The third fragment which lacks the ability to bind to antigen and can be crystallized, is, therefore, known as fragment crystallizable (Fc).

The stem of the Y-shaped antibody monomer is called the F_c region, so named because when antibody structure was first being identified, it was a fragment (F) that crystallized (c) in cold storage.

Antibodies show the following characteristics and perform different functions.

(i) IgA:

It is the second most abundant class, constituting about 10 to 15 per cent of antibodies of serum. It is mainly found in sweat, tears, saliva, mucus, colostrum (first milk secreted by a mother) and gastrointestinal secretions.

Smaller quantities are present in blood and lymph. IgA has an extra polypeptide called a J-(joining) chain and extra protein known as secretory component. Levels decrease during stress, lowering resistance to infection. Provides localized protection in external secretions (tears, intestinal secretions, etc.) against bacteria and viruses. When IgA is excreted through faeces, it is called coproantibody.

(ii) IgD:

It is mainly found on the surfaces of B cells as antigen receptors, where it activates B cells for antigen recognition. It is about 0.2% of all antibodies in the blood.

(iii) IgE:

It is less than 0.1% of all antibodies in the blood; located on mast cells and basophils releasing histamine from mast cells and basophils. It is involved in allergic and hypersensitivity reactions; provides protection against parasitic worms. This immunoglobulin was discovered in 1966 by Ishizaka.

It exhibits unique properties such as heat lability (inactivated at 56°C in one hour). IgE mediates type I hypersensitivity (anaphylaxis). Prausnitz and Kustner in 1921 demonstrated transmission of IgE-mediated type I hypersensitivity. It is called Prausnitz-Kustner (PK) reaction. Thus IgE acts as mediator in allergic response.

(iv) IgG:

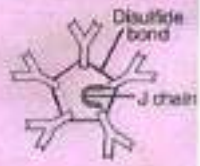
This is the most abundant class of Ig in the body constituting approximately 80% of the total Igs. It is found in the blood, lymph and intestine. It protects against bacteria and viruses by enhancing phagocytosis, neutralizing toxins and complement activation. It is the only class of antibody to cross the placenta from mother to foetus thereby conferring considerable immune protection in new-borns.

(v) IgM:

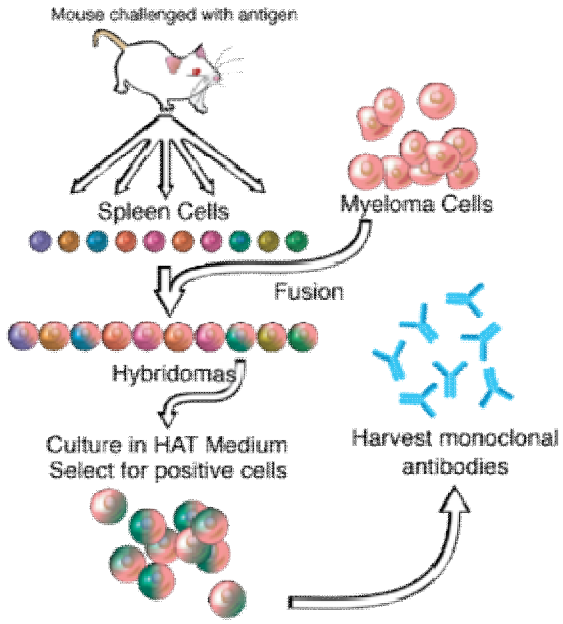
IgM is about 5 to 10% of all antibodies in the blood. It is also found in lymph. It is the largest Ig which is secreted first by the plasma cells. It is so named because it is a macroglobulin at least five times larger than IgG. IgM is the oldest immunoglobulin class. It activates the B cells. It is also the earliest immunoglobin to be synthesised by the foetus, IgM has a J chain and its each dimer contains polypeptide called a secretory component.

It cannot cross the placental barrier. IgM is 500-1000 times more effective than IgG in opsonisation (to be described ahead), in bacterial action and in bacterial agglutination. But in neutralization of toxins and viruses, it is less active than IgG. It helps in complement activation.

Summary of Human Immunoglobulins (Antibodies)

Characteristics	IgA	IgD	IgE	IgG	IgM
					
Structure	Dimer (with secretory component) and J-chain	Monomer	Monomer	Monomer	Pentamer with J chain
Percentage of total serum antibody	10–15%	0.2%	Less than 0.1%	80%	5–10%
Location	Secretions (tears, saliva, mucus, intestine, colostrum), blood, lymph	B cell surface, blood, lymph	Bound to mast and basophil cells throughout body, blood	Blood, lymph, intestine	Blood, lymph, B cell surface (as monomer)
Function	Localized protection in external secretions (tears, intestinal secretions, etc.	Antigen recognition by B cells	it is involved in allergic reactions	Complement activation	Complement activation
Placental transfer	No	No	No	Yes	Yes

Monoclonal antibody



A general representation of the method used to produce monoclonal antibodies.

Monoclonal antibodies (**mAb** or **moAb**) are antibodies that are made by identical immune cells that are all clones of a unique parent cell, in contrast to polyclonal antibodies, which are made from several different immune cells. Monoclonal antibodies can have monovalent affinity, in that they bind to the same epitope (the part of an antigen that is recognized by the antibody). Engineered bispecific monoclonal antibodies also exist, where each "arm" of the antibody is specific for a different epitope.

Given almost any substance, it is possible to produce monoclonal antibodies that specifically bind to that substance; they can then serve to detect or purify that substance. This has become an important tool in biochemistry, molecular biology and medicine. When used as medications, non-proprietary drug names end in **-mab**.

Production

Monoclonal antibodies are typically made by cell culture that involves fusing myeloma cells with mouse spleen cells immunized with the desired antigen. Rabbit B-cells can be used to form a rabbit hybridoma. Polyethylene glycol is used to fuse adjacent plasma membranes,[4] but the

success rate is low, so a selective medium in which only fused cells can grow is used. This is possible because myeloma cells have lost the ability to synthesize hypoxanthine-guanine-phosphoribosyl transferase (HGPRT), an enzyme necessary for the salvage synthesis of nucleic acids. The absence of HGPRT is not a problem for these cells unless the de novo purine synthesis pathway is also disrupted. Exposing cells to aminopterin (a folic acid analogue, which inhibits dihydrofolate reductase, DHFR), makes them unable to use the de novo pathway and become fully auxotrophic for nucleic acids, thus requiring supplementation to survive.

The selective culture medium is called HAT medium because it contains hypoxanthine, aminopterin and thymidine. This medium is selective for fused (hybridoma) cells. Unfused myeloma cells cannot grow because they lack HGPRT and thus cannot replicate their DNA. Unfused spleen cells cannot grow indefinitely because of their limited life span. Only fused hybrid cells, referred to as hybridomas, are able to grow indefinitely in the media because the spleen cell partner supplies HGPRT and the myeloma partner has traits that make it immortal (similar to a cancer cell).

This mixture of cells is then diluted and clones are grown from single parent cells on microtitre wells. The antibodies secreted by the different clones are then assayed for their ability to bind to the antigen (with a test such as ELISA or Antigen Microarray Assay) or immuno-dot blot. The most productive and stable clone is then selected for future use.

The hybridomas can be grown indefinitely in a suitable cell culture medium. They can also be injected into mice (in the peritoneal cavity, surrounding the gut). There, they produce tumors secreting an antibody-rich fluid called ascites fluid.

The medium must be enriched during *in vitro* selection to further favour hybridoma growth. This can be achieved by the use of a layer of feeder fibrocyte cells or supplement medium such as briclone. Culture-media conditioned by macrophages can be used. Production in cell culture is usually preferred as the ascites technique is painful to the animal. Where alternate techniques exist, ascites is considered unethical.

Applications

Diagnostic tests

Once monoclonal antibodies for a given substance have been produced, they can be used to detect the presence of this substance. The Western blot test and immuno dot blot tests detect the protein on a membrane. They are also very useful in immunohistochemistry, which detect antigen in fixed tissue sections and immunofluorescence test, which detect the substance in a frozen tissue section or in live cells.

Analytic and chemical uses

Antibodies can also be used to purify their target compounds from mixtures, using the method of immunoprecipitation.

Therapeutic treatment

Therapeutic monoclonal antibodies act through multiple mechanisms, such as blocking of targeted molecule functions, inducing apoptosis in cells which express the target, or by modulating signalling pathways.

T-cell receptor

The **T-cell receptor**, or **TCR**, is a molecule found on the surface of T cells, or T lymphocytes,[1] that is responsible for recognizing fragments of antigen as peptides bound to major histocompatibility complex (MHC) molecules. The binding between TCR and antigen peptides is of relatively low affinity and is degenerate: that is, many TCRs recognize the same antigen peptide and many antigen peptides are recognized by the same TCR.

The TCR is composed of two different protein chains (that is, it is a heterodimer). In humans, in 95% of T cells the TCR consists of an alpha (α) and beta (β) chain, whereas in 5% of T cells the TCR consists of gamma and delta (γ/δ) chains. This ratio changes during ontogeny and in diseased states as well as in different species.

When the TCR engages with antigenic peptide and MHC (peptide/MHC), the T lymphocyte is activated through signal transduction, that is, a series of biochemical events mediated by

associated enzymes, co-receptors, specialized adaptor molecules, and activated or released transcription factors.

Structural characteristics of the TCR[edit]

The TCR is a disulfide-linked membrane-anchored heterodimeric protein normally consisting of the highly variable alpha (α) and beta (β) chains expressed as part of a complex with the invariant CD3 chain molecules. T cells expressing this receptor are referred to as α:β (or αβ) T cells, though a minority of T cells express an alternate receptor, formed by variable gamma (γ) and delta (δ) chains, referred as γδ T cells.[2]

Each chain is composed of two extracellular domains: Variable (V) region and a Constant (C) region, both of Immunoglobulin superfamily (IgSF) domain forming antiparallel β-sheets. The Constant region is proximal to the cell membrane, followed by a transmembrane region and a short cytoplasmic tail, while the Variable region binds to the peptide/MHC complex.

The variable domain of both the TCR α-chain and β-chain each have three hypervariable or complementarity determining regions (CDRs), whereas the variable region of the β-chain has an additional area of hypervariability (HV4) that does not normally contact antigen and, therefore, is not considered a CDR.

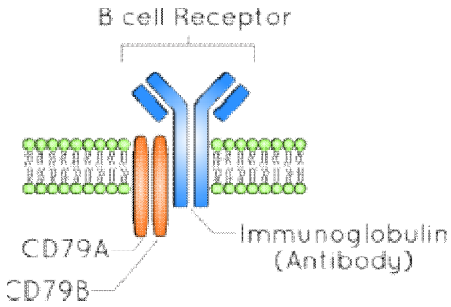
The residues are located in two regions of the TCR, at the interface of the α- and β-chains and in the β-chain framework region that is thought to be in proximity to the CD3 signal-transduction complex.[3] CDR3 is the main CDR responsible for recognizing processed antigen, although CDR1 of the alpha chain has also been shown to interact with the N-terminal part of the antigenic peptide, whereas CDR1 of the β-chain interacts with the C-terminal part of the peptide.

CDR2 is thought to recognize the MHC. CDR4 of the β-chain is not thought to participate in antigen recognition, but has been shown to interact with superantigens.

The constant domain of the TCR domain consists of short connecting sequences in which a cysteine residue forms disulfide bonds, which forms a link between the two chains.

The TCR being a member of the IgSF protein means it may be compared to antibodies and BCR. In terms of similarity, TCR is like half an antibody with a heavy and a light chain, except the heavy chain is without its crystallisable fraction (Fc) (Note: ontogenically TCR alpha undergo VJ recombination, so it is like a light chain; TCR beta undergoes VDJ recombination, so it is like a heavy chain). So the TCR is ontologically like one of the antibody-binding fragments of the antibody. The two subunits of TCR are twisted together. Where as the antibody uses its Fc region to bind to Fc Receptors on innate leukocytes, TCR is already docked onto the cell membrane. However, it is not able to mediate signal transduction itself due to its short cytoplasmic tail, so TCR still requires CD3 and zeta to carry out the signal transduction in its place, just as antibodies requires binding to FcRs to initiate signal transduction. In this way the MHC-TCR-CD3 interaction for T cells is functionally similar to the Ag-Ig-FcR interaction for myeloid leukocytes, and Ag-Ig-CD79 interaction for B cells.

B-cell receptor



The B-cell receptor includes both CD79 and the immunoglobulin. The plasma membrane of a B cell is indicated by the green phospholipids. The B cell receptor extends both outside the cell (above the plasma membrane) and inside the cell (below the membrane).

The **B-cell receptor** or **BCR** is a transmembrane receptor protein located on the outer surface of B cells. The receptor's binding moiety is composed of a membrane-bound antibody that, like all antibodies, has a unique and randomly determined antigen-binding. When a B cell is activated by its first encounter with an antigen that binds to its receptor (its "cognate antigen"), the cell proliferates and differentiates to generate a population of antibody-secreting plasma B cells and memory B cells. The B cell receptor (BCR) has two crucial functions upon interaction with the antigen. One function is signal transduction, involving changes in receptor oligomerization. The

second function is to mediate internalization for subsequent processing of the antigen and presentation of peptides to helper T cells.[1] BCR functions are required for normal antibody production, and defects in BCR signal transduction may lead to immunodeficency, and B-cell malignancy.[1]

Components of the B-cell receptor

The B-cell receptor is composed of two parts:[1] i) A membrane-bound immunoglobulin molecule of one isotype (IgD, IgM, IgA, IgG, or IgE). With the exception of the presence of an integral membrane domain, these are identical to their secreted forms. ii) Signal transduction moiety: A heterodimer called Ig-α/Ig-β (CD79), bound together by disulfide bridges. Each member of the dimer spans the plasma membrane and has a cytoplasmic tail bearing an *immunoreceptor tyrosine-based activation motif (ITAM).*

Signaling pathways of the B-cell receptor

There are several signaling pathways that the B-cell receptor can follow through. The physiology of B cells is intimately connected with the function of their B-cell receptor.[1] In B cells, the balance of initiation, amplitude and duration of BCR activation can be influenced by a specific immunoglobulin structure, the expression adaptor molecules (like GAB1, BLNK, GRB2, CARD11), the activity of kinases (like LYN, SYK, PI3K) or phosphatases (like SHIP-1, SHP-1 and PTEN) and levels of microRNAs. [2] [3] The miR-150 and miR-155 were shown to be regulators of proteins that affect the propensity of BCR signalling pathway.[4][5]

1. *IKK/NF-κB Transcription Factor Pathway:* CD79 and other proteins, microsignalosomes, go to activate PLC-γ after antigen recognition by the BCR and before it goes to associate into the c-SMAC. It then cleaves PIP2 into IP3 and DAG (diacylglycerol). IP3 acts as a second messenger to dramatically increase ionic calcium inside the cytosol (via release from the endoplasmic reticulum or influx from the extracellular environment via ion channels). This leads to eventual activation of PKCβ from the calcium and DAG. PKCβ phosphorylates (either directly or indirectly) the NF-κB signaling complex protein CARMA1 (the complex itself comprising CARMA1, BCL10, and MALT1). These result in recruitment and summoning of the IKK (IkB

kinase), TAK1, by several ubiquitylation enzymes also associated with the CARMA1/BCL10/MALT1 complex. MALT1 itself is a caspase-like protein that cleaves A20, an inhibitory protein of NF-κB signaling (which acts by deubiquitylating NF-κB's ubiquitylation substrates, having an inhibitory effect). TAK1 phosphorylates the IKK trimer after it too has been recruited to the signaling complex by its associated ubiquitylation enzymes. IKK then phosphorylates IkB (an inhibitor of and bound to NF-κB), which induces its destruction by marking it for proteolytic degradation, freeing cytosolic NF-κB. NF-κB then migrates to the nucleus to bind to DNA at specific response elements, causing recruitment of transcription molecules and beginning the transcription process.

2. Ligand binding to the BCR also leads to the phosphorylation of the protein BCAP. This leads to the binding and activation of several proteins with phosphotyrosine-binding SH2 domains. One of these proteins is PI3K. Activation of PI3K leads to PIP2 phosphorylation, forming PIP3. Proteins with PH (Pleckstrin homology) domains can bind to the newly created PIP3 and become activated. These include proteins of the FoxO family, which stimulate cell cycle progression, and protein kinase D, which enhances glucose metabolism. Another important protein with a PH domain is Bam32. This recruits and activates small GTPases such as Rac1 and Cdc42. These, in turn, are responsible for the cytoskeletal changes associated with BCR activation by modifying actin polymerisation.

Antigen-antibody interaction

Antigen-antibody interaction, or **antigen-antibody reaction**, is a specific chemical interaction between antibodies produced by B cells of the white blood cells and antigens during immune reaction. It is the fundamental reaction in the body by which the body is protected from complex foreign molecules, such as pathogens and their chemical toxins. In the blood, the antigens are specifically and with high affinity bound by antibodies to form an antigen-antibody complex. The immune complex is then transported to cellular systems where it can be destroyed or deactivated.

There are several types of antibodies and antigens, and each antibody is capable of binding only to a specific antigen. The specificity of the binding is due to specific chemical constitution of each antibody. The antigenic determinant or epitope is recognized by the paratope of the antibody, situated at the variable region of the polypeptide chain. The variable region in turn has hyper-variable regions which are unique amino acid sequences in each antibody. Antigens are bound to antibodies through weak and noncovalent interactions such as electrostatic interactions, hydrogen bonds, Van der Waals forces, and hydrophobic interactions.[4]

The principles of specificity and cross-reactivity of the antigen-antibody interaction are useful in clinical laboratory for diagnositic purposes. One basic application is determination of ABO blood group. It is also used as a molecular technique for infection with different pathogens, such as HIV, microbes, and helminth parasites.

Chemical basis of antigen-antibody interaction

Antibodies bind antigens through weak chemical interactions, and bonding is essentially non-covalent. Electrostatic interactions, hydrogen bonds, van der Waals forces, and hydrophobic interactions are all known to be involved depending on the interaction sites.[6][7]

CHAPTER 2

Passive immunity

Passive immunity is the transfer of active immunity, in the form of readymade antibodies, from one individual to another. Passive immunity can occur naturally, when maternal antibodies are transferred to the foetus through the placenta, and can also be induced artificially, when high levels of human (or horse) antibodies specific for a pathogen or toxin are transferred to non-immune individuals. Passive immunization is used when there is a high risk of infection and insufficient time for the body to develop its own immune response, or to reduce the symptoms of ongoing or immunosuppressive diseases.[7] Passive immunity provides immediate protection, but the body does not develop memory, therefore the patient is at risk of being infected by the same pathogen later.[8]

Naturally acquired passive immunity

Maternal passive immunity is a type of naturally acquired passive immunity, and refers to antibody-mediated immunity conveyed to a fetus by its mother during pregnancy. Maternal antibodies (MatAb) are passed through the placenta to the fetus by an FcRn receptor on placental cells. This occurs around the third month of gestation. IgG is the only antibody isotype that can pass through the placenta. Passive immunity is also provided through the transfer of IgA antibodies found in breast milk that are transferred to the gut of the infant, protecting against bacterial infections, until the newborn can synthesize its own antibodies.[8]

Artificially acquired passive immunity

Artificially acquired passive immunity is a short-term immunization induced by the transfer of antibodies, which can be administered in several forms; as human or animal blood plasma, as pooled human immunoglobulin for intravenous (IVIG) or intramuscular (IG) use, and in the form of monoclonal antibodies (MAb). Passive transfer is used prophylactically in the case of immunodeficiency diseases, such as hypogammaglobulinemia. It is also used in the treatment of several types of acute infection, and to treat poisoning. Immunity derived from passive

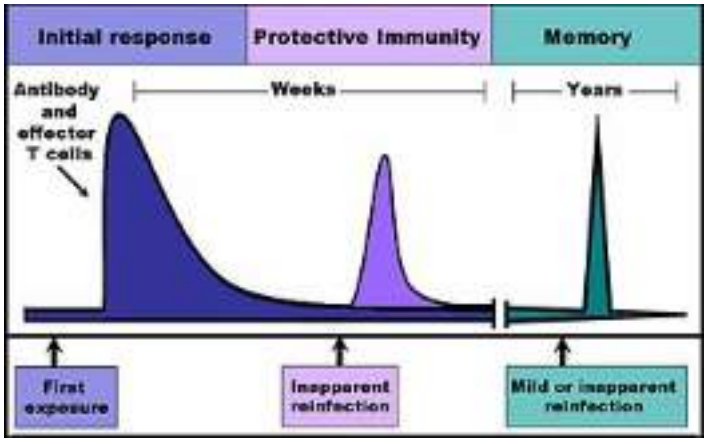
immunization lasts for only a short period of time, and there is also a potential risk for hypersensitivity reactions, and serum sickness, especially from gamma globulin of non-human origin.

The artificial induction of passive immunity has been used for over a century to treat infectious disease, and prior to the advent of antibiotics, was often the only specific treatment for certain infections. Immunoglobulin therapy continued to be a first line therapy in the treatment of severe respiratory diseases until the 1930s, even after sulfonamide lot antibiotics were introduced.

Passive transfer of cell-mediated immunity

Passive or "adoptive transfer" of cell-mediated immunity, is conferred by the transfer of "sensitized" or activated T-cells from one individual into another. It is rarely used in humans because it requires histocompatible (matched) donors, which are often difficult to find. In unmatched donors this type of transfer carries severe risks of graft versus host disease. It has, however, been used to treat certain diseases including some types of cancer and immunodeficiency. This type of transfer differs from a bone marrow transplant, in which (undifferentiated) hematopoietic stem cells are transferred.

Active immunity



The time course of an immune response. Due to the formation of immunological memory, reinfection at later time points leads to a rapid increase in antibody production and effector T cell activity. These later infections can be mild or even unapparent.

When B cells and T cells are activated by a pathogen, memory B-cells and T- cells develop, and the *primary* immune response results. Throughout the lifetime of an animal these memory cells will "remember" each specific pathogen encountered, and are able to mount a strong *secondary* response, if the pathogen is detected again. The primary and secondary responses were first described in 1921 by English immunologist Alexander Glenny although the mechanism involved was not discovered until later.This type of immunity is both *active* and *adaptive* because the body's immune system prepares itself for future challenges. Active immunity often involves both the cell-mediated and humoral aspects of immunity as well as input from the innate immune system.

Naturally acquired active immunity

Naturally acquired active immunity occurs when a person is exposed to a live pathogen, and develops a primary immune response, which leads to immunological memory.[7] This type of immunity is "natural" because it is not induced by deliberate exposure. Many disorders of immune system function can affect the formation of active immunity such as immunodeficiency (both acquired and congenital forms) and immunosuppression.

Artificially acquired active immunity

Artificially acquired active immunity can be induced by a vaccine, a substance that contains antigen. A vaccine stimulates a primary response against the antigen without causing symptoms of the disease.[7] The term *vaccination* was coined by Richard Dunning, a colleague of Edward Jenner, and adapted by Louis Pasteur for his pioneering work in vaccination. The method Pasteur used entailed treating the infectious agents for those diseases so they lost the ability to cause serious disease. Pasteur adopted the name vaccine as a generic term in honor of Jenner's discovery, which Pasteur's work built upon.

Immunological Tolerance: Mechanisms

Immunological tolerance refers to a reduction or complete inhibition of the ability of an individual to mount a specific immune response upon immunisation. Several mechanisms are involved in induction and maintenance of tolerance, including clonal deletion, clonal anergy, receptor editing, receptor down-modulation and lymphocyte sequestration. The number of antigen presenting cells, the number and activity of regulatory T cells and regulatory B cells, the nature and amount of antigenic peptides generated and the presence of co-stimulatory signals in a particular tissue are also important. Depending on the site and the level of antigen expression, different states of peripheral B-cell and T-cell tolerance can be reached. In certain situations, they could act in an additive manner. In humans, induction of immunological tolerance is an important issue in both transplantation biology and autoimmune diseases, and present research aims at designing novel strategies to induce specific tolerance.

- Tolerance induction is easier in animals with an immature immune system or with a mature immune system that has been compromised by irradiation, drugs or thoracic drug drainage.
- In general, when a given antigen is used over a wide range of concentrations, intermediate doses induce immunity, and low and high doses induce tolerance.
- The introduction route is a key variable in tolerance induction, particularly in adult animals, presumably by determining the accessibility of the antigen to professional antigen presenting cells.
- The tolerant state is not absolute and tolerance is rarely complete. With time, it gradually wanes and eventually disappears, but it can be deliberately terminated by several means.
- Persistence of the tolerogen in the periphery and its accessibility to the immune system are generally required to maintain tolerance, which continuously inactivates newly emerging T and B cells that develop in lymphoid organs.
- T lymphocytes specific for self-peptides bound to major histocompatibility complex peptides are eliminated by clonal deletion, a process known as negative selection. Similarly, self-reactive B cells are purged from the functional repertoire during the transition from the pre-B to mature B-cell stage in the bone marrow.

- Anergy is a functionally silent state induced in B cells and T cells, allowing them to persist functionally inactivated in tolerant animals.
- Receptor editing is a form of receptor processing that markedly alters the variable region genes expressed by B cells and, consequently, changes the specificity of the surface immunoglobulin and maintains B-cell tolerance to self.
- Two functional lymphocyte subsets, called regulatory T cells and regulatory B cells, have recently been found to contribute to the maintenance of the fine equilibrium required for immune tolerance.
- In humans, induction of immunological tolerance in the adult is an important issue in both transplantation biology and autoimmune diseases, and present research aims at designing novel strategies to induce specific tolerance.

Immunodeficiency disorders

- **Severe combined immunodeficiency (SCID).** This is an example of an immune deficiency that is present at birth. Children are in constant danger of infections from bacteria, viruses, and fungi. This disorder is sometimes called "bubble boy disease." In the 1970s, a boy had to live in a sterile environment inside a plastic bubble. Children with SCID are missing important white blood cells.
- **Temporary acquired immune deficiencies.** Your immune system can be weakened by certain drugs, for example. This can happen to people on chemotherapy or other drugs used to treat cancer, or to people following organ transplants who take medication to prevent organ rejection. Also, infections like the flu virus, mononucleosis (mono), and measles can weaken the immune system for a brief time. Your immune system can also be weakened by smoking, alcohol, and poor nutrition.
- **AIDS.** HIV, which causes AIDS, is an acquired viral infection that destroys important white blood cells and weakens the immune system. People with HIV/AIDS become seriously ill with infections that most people can fight off. These infections are called "opportunistic infections" because they take advantage of weak immune systems.

Overactive immune system

If you are born with certain genes, your immune system may react to substances in the environment that are normally harmless. These substances are called allergens. Having an allergic reaction is the most common example of an overactive immune system. Dust, mold, pollen, and foods are examples of allergens.

Some conditions caused by an overactive immune system are:

- **Asthma.** The response in your lungs can cause coughing, wheezing, and trouble breathing. Asthma can be triggered by a common allergen like dust or pollen or by an irritant like tobacco smoke.
- **Eczema.** An allergen causes an itchy rash known as atopic dermatitis.
- **Allergic rhinitis.** Sneezing, a runny nose, sniffling, and swelling of your nasal passages from indoor allergens like dust and pets or outdoor allergens like pollens or molds.

Autoimmune disease

In autoimmune diseases, the body attacks normal, healthy tissues. The cause is unknown. It is probably a combination of a person's genes and something in the environment that triggers those genes.

Three common autoimmune diseases are:

- **Type 1 diabetes.** In this type of diabetes, the immune system attacks the cells in the pancreas that make insulin. Insulin removes sugar from the blood to use as energy. .
- **Rheumatoid arthritis.** This type of arthritis causes swelling and deformities of the joints. An auto-antibody called rheumatoid factor is in the blood of some people with rheumatoid arthritis.
- **Lupus.** Systemic lupus erythematosis is an autoimmune disease that attacks body tissues, including the lungs, kidneys, and skin. Many types of auto-antibodies are found in the blood of people with lupus.

No one knows exactly what causes autoimmune diseases, but many factors seem to be involved. If you have an immune system disorder, learn as much as you can about it and work closely with your health care providers to manage it.

Primary immunodeficiency syndromes

- Mostly these are inherited single-gene disorders that present in infancy or early childhood with the exception of common variable immunodeficiency which usually occurs in adults.[1]
- Mutations/deletions of genes governing stem cell differentiation have been identified and over 150 disorders have been described.[2]
- Once thought to be rare, symptomatic primary immunodeficiencies are now considered to range from 1:500 to 1:500,000 in the general population in the USA and Europe.[3]
- The age of presentation varies widely.[4] 70% occur in males due to X-linked inheritance in many syndromes.[5]
- B-cell defects account for 50% of primary immunodeficiency.
- T-cell defects account for 30%, phagocytic deficiencies 18% and complement deficiencies 2%. Knowledge about the function and diversity of B cells in health and disease has now become quite detailed but there is still much to learn.[6]

The conditions are sometimes classified according to which component is faulty (T cells, B cells, phagocytic cells or complement) or according to individual clinical syndromes. Primary Immunodeficiency has identified main categories as follows:

- Combined immunodeficiencies.
- Combined immunodeficiencies with associated or syndromic features.
- Predominantly antibody deficiencies.
- Complement deficiencies.
- Congenital defects of phagocyte number, function, or both.
- Defects in innate immunity.
- Autoinflammatory disorders.

- Phenocopies of primary immunodeficiencies (presenting as inherited disorders but arising from acquired mechanisms).

- Antibody deficiency syndromes: this is a group of conditions characterised by an inability to produce antibodies in sufficient quantity or of sufficient quality.
    - Common variable immunodeficiency: this is a heterogeneous syndrome characterised by various degrees of hypogammaglobulinaemia, commonly associated with autoimmunity.[7] See the separate Common Variable Immunodeficiency article for more details.
    - Thymoma and hypogammaglobulinaemia: this is characterised by low numbers of B cells and a distinctive T-cell type.[8]
    - X-linked (Bruton's agammoglobulinaemia): the agammaglobulinaemia is an X-linked immunodeficiency in which there is a failure to produce mature B-lymphocyte cells. The defect in this disorder is a fault in the enzyme in Bruton's tyrosine kinase, a key regulator in B-cell development.[9] Novel genetic defects have been found.[10]
    - Selective IgAD occurs in about 1/400 people.[7] There is a selective severe deficiency or total absence of IgA in serum and body secretions.
- Cell-mediated immunity can be subject to a number of genetic defects affecting the function of the T cells:[11]
    - Thymic aplasia (DiGeorge syndrome): there are genetic defects of the thymus and often the parathyroid glands and heart, associated with T-cell dysfunction and significant immune deficiency.[12]
    - Severe combined immunodeficiency disease: this is in fact a group of rare congenital diseases in which there is severe and usually fatal immune deficiency. It has gained the attention of the media in the past and has been known as 'bubble boy disease'.[13]
    - Inherited syndromes associated with immunodeficiency: a wide range of inherited immunodeficiency conditions has been identified, many involving a single gene.[14]

Secondary immunodeficiencies

There are many possible causes and so it is difficult to obtain exact epidemiological data. It is known that the current epidemics of AIDs and tuberculosis have caused global increases in the condition.

Secondary immunodeficiency is common in people who are hospitalised for:

- Lymphoreticular malignancy.
- Drugs - particularly cytotoxic drugs and immunosuppressants.
- Viruses - eg, HIV.
- Malnutrition - the most common cause worldwide.
- Metabolic disorders - eg, renal disease requiring peritoneal dialysis.
- Trauma or major surgery.
- Protein loss - for example, due to nephrotic syndrome.

Autoimmune disease

An **autoimmune disease** is a condition arising from an abnormal immune response to a normal body part. There are at least 80 types of autoimmune diseases. Nearly any body part can be involved. Commons symptoms include low grade fever and feeling tired. Often symptoms come and go.

Some autoimmune diseases run in families such as lupus and certain cases may be triggered by infections or other environmental factors. Some common autoimmune disease include celiac disease, diabetes mellitus type 1, Graves disease, inflammatory bowel disease, multiple sclerosis, psoriasis, rheumatoid arthritis, and systemic lupus erythematosus. The diagnosis can be difficult to determine.

Treatment depends on the type and severity of the condition. Nonsteroidal anti-inflammatory drugs (NSAIDs) and immunosuppressants are often used. Intravenous Immunoglobulin may also occasionally be used. While treatment usually improves symptoms they do not typically cure the disease.

Coeliac disease

Coeliac disease, also spelled **celiac disease**, is an autoimmune disorder affecting primarily the small intestine that occurs in people who are genetically predisposed. Classic symptoms include gastrointestinal problems such as chronic diarrhoea, abdominal distention, malabsorption, loss of appetite, and among children failure to grow normally. This often begins between six months and two years of age. Non-classic symptoms are the most common, especially in people older than two years. There may be mild or absent gastrointestinal symptoms, a wide number of symptoms involving any part of the body, or no obvious symptoms. Coeliac disease was first described in childhood; however, it may develop at any age. It is associated with other autoimmune diseases, such as diabetes mellitus type 1 and thyroiditis, among others.

Coeliac disease is caused by a reaction to gluten, which are various proteins found in wheat and in other grains such as barley, and rye. Moderate quantities of oats, free of contamination with other gluten-containing grains, are usually tolerated but problems may depend on the type consumed. Upon exposure to gluten, an abnormal immune response may lead to the production of several different autoantibodies that can affect a number of different organs. In the small-bowel this causes an inflammatory reaction and may produce shortening of the villi lining the small intestine (villous atrophy). This affects the absorption of nutrients, frequently leading to anaemia.

Diagnosis is typically made by a combination of blood antibody tests and intestinal biopsies, helped by specific genetic testing. Making the diagnosis is not always straightforward.[14] Frequently, the autoantibodies in the blood are negative and many people have only minor intestinal changes with normal villi. People may have severe symptoms and be investigated for years before a diagnosis is achieved. Increasingly, the diagnosis is being made in people without symptoms as a result of screening. While the disease is caused by a permanent intolerance to wheat proteins, it is usually classified as different from the other forms of wheat allergy.

The only known effective treatment is a strict lifelong gluten-free diet, which leads to recovery of the intestinal mucosa, improves symptoms, and reduced risk of developing complications in most people. If untreated it may result in cancers such as intestinal lymphoma and a slight

increased risk of early death. In developed countries, it is estimated that five out of six cases (83%) remain undiagnosed, usually because of non-classic, minimal, or absent complaints. Coeliac disease is slightly more common in women than in men. The term "coeliac" is from the Greek κοιλιακός (*koiliakós*, "abdominal") and was introduced in the 19th century in a translation of what is generally regarded as an ancient Greek description of the disease by Aretaeus of Cappadocia.

Diabetes mellitus type 1

Diabetes mellitus type 1 (also known as **type 1 diabetes**) is a form of diabetes mellitus in which not enough insulin is produced. The lack of insulin results in high blood sugar levels.[1] The classical symptoms are frequent urination, increased thirst, increased hunger, and weight loss.[4] Additional symptoms may include blurry vision, feeling tired, and poor healing.[2] Symptoms typically develop over a short period of time.

The cause of type 1 diabetes is unknown. It however is believed to involve a combination of genetic and environmental factors. Risk factors include having a family member with the condition. The underlying mechanism involves an autoimmune destruction of the insulin-producing beta cells in the pancreas. Diabetes is diagnosed by testing the level of sugar or A1C in the blood. Type 1 diabetes may be distinguished from type 2 by autoantibody testing.

There is no way to prevent type 1 diabetes. Treatment with insulin is typically required for survival. Insulin therapy is usually given by injection just under the skin but can also be delivered by an insulin pump. A diabetic diet and exercise are an important part of management. Untreated, diabetes can cause many complications. Complications of relatively rapid onset include diabetic ketoacidosis and nonketotic hyperosmolar coma. Long-term complications include heart disease, stroke, kidney failure, foot ulcers and damage to the eyes. Furthermore, complications may arise from low blood sugar caused by excessive insulin treatment.

Graves' disease, also known as **toxic diffuse goiter**, is an autoimmune disease that affects the thyroid.[1] It frequently results in and is the most common cause of hyperthyroidism.[2] It also often results in an enlarged thyroid. Signs and symptoms of hyperthyroidism may include

irritability, muscle weakness, sleeping problems, a fast heartbeat, poor tolerance of heat, diarrhea, and weight loss. Other symptoms may include thickening of the skin on the shins, known as pretibial myxedema, and eye bulging, a condition caused by Graves' ophthalmopathy. The exact cause is unclear; however, it is believed to involve a combination of genetic and environmental factors. A person is more likely to be affected if they have a family member with the disease. If one twin is affected there is a 30% chance the other twin will also have the disease. The onset of disease may be triggered by stress, infection, or giving birth. Those with other autoimmune diseases such as type 1 diabetes and rheumatoid arthritis are more likely to be affected. Smoking increases the risk of disease and may worsen eye problems. The disorder results from an antibody, called thyroid stimulating immunoglobulin (TSI), that has a similar effect to thyroid stimulating hormone (TSH). These TSI antibodies cause the thyroid gland to produce excess thyroid hormone. The diagnosis may be suspected based on symptoms and confirmed with blood tests and radioiodine uptake. Typically blood tests show a raised T_3 and T_4, low TSH, increased radioiodine uptake in all areas of the thyroid, and TSI antibodies

There are three treatment options: radioiodine therapy, medications, and thyroid surgery. Radioiodine therapy involves taking iodine-131 by mouth which is then concentrated in and destroys the thyroid over weeks to months. The resulting hypothyroidism is treated with synthetic thyroid hormone. Medications such as beta blockers may control some of the symptoms and anti-thyroid medications such as methimazole may temporarily help people while other treatments are having effect. Surgery to remove the thyroid is another option. Eye problems may require additional treatments.

Rheumatoid arthritis

Rheumatoid arthritis (**RA**) is a long-lasting autoimmune disorder that primarily affects joints. It typically results in warm, swollen, and painful joints. Pain and stiffness often worsen following rest. Most commonly, the wrist and hands are involved, with the same joints typically involved on both sides of the body. The disease may also affect other parts of the body. This may result in a low red blood cell count, inflammation around the lungs, and inflammation around the heart. Fever and low energy may also be present. Often, symptoms come on gradually over weeks to months.

While the cause of rheumatoid arthritis is not clear, it is believed to involve a combination of genetic and environmental factors. The underlying mechanism involves the body's immune system attacking the joints. This results in inflammation and thickening of the joint capsule. It also affects the underlying bone and cartilage. The diagnosis is made mostly on the basis of a person's signs and symptoms. X-rays and laboratory testing may support a diagnosis or exclude other diseases with similar symptoms. Other diseases that may present similarly include systemic lupus erythematosus, psoriatic arthritis, and fibromyalgia among others.

The goal of treatment is to reduce pain, decrease inflammation, and improve a person's overall functioning. This may be helped by balancing rest and exercise, the use of splints and braces, or the use of assistive devices. Pain medications, steroids, and NSAIDs are frequently used to help with symptoms. A group of medications called disease-modifying antirheumatic drugs (DMARDs) may be used to try to slow the progression of disease. They include the medications hydroxychloroquine and methotrexate. Biological DMARDs may be used when disease does not respond to other treatments. However, they may have a greater rate of adverse effects. Surgery to repair, replace, or fusion joints may help in certain situations. Most alternative medicine treatments are not supported by evidence.

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE), also known simply as **lupus**, is an autoimmune disease in which the body’s immune system mistakenly attacks healthy tissue in many parts of the body.[1] Symptoms vary between people and may be mild to severe. Common symptoms include painful and swollen joints, fever, chest pain, hair loss, mouth ulcers, swollen lymph nodes, feeling tired, and a red rash which is most commonly on the face. Often there are periods of illness, called flares, and periods of remission when there are few symptoms.

The cause is not entirely clear. It is believed to involve hormonal, environmental, and genetic factors. Among identical twins, if one is affected there is a 24% chance the other one will be as well. Female sex hormones, sunlight, smoking, vitamin D deficiency, and certain infections, are also believed to increase the risk. The mechanism involves an immune response by autoantibodies against a person's own tissues. These are most commonly anti-nuclear antibodies

and they result in inflammation. Diagnosis can be difficult and is based on a combination of symptoms and laboratory tests. There are a number of other kinds of lupus erythematosus including discoid lupus erythematosus, neonatal lupus, and subacute cutaneous lupus erythematosus.

There is no cure for SLE. Treatments may include NSAIDs, corticosteroids, immunosuppressants, hydroxychloroquine, and methotrexate. Alternative medicine has not been shown to affect the disease. Life expectancy is lower among people with SLE. SLE significantly increases the risk of cardiovascular disease with this being the most common cause of death. With modern treatment about 80% of those affected survive more than 15 years. Women with lupus have pregnancies that are higher risk but are mostly successful.

Hypersensitivity

Hypersensitivity: Hypersensitivity describes an abnormal or pathologic immune reaction that is caused by an immune response to repeated exposure to an antigen. Hypersensitivity diseases include autoimmune diseases, in which immune responses are directed against self-antigens, AND diseases that result from uncontrolled or excessive responses to foreign antigens. Because these reactions tend to occur against antigens that cannot be escaped (i.e. self-antigens) and because of positive feedback systems intrinsic to various aspects of the immune response, hypersensitivity diseases tend to manifest as chronic problems.

4 major types of hypersensitivity

Although the immune system performs some amazing and vital functions, its mechanisms can also go awry, resulting in human disease.

Autoimmunity is the response of the adaptive immune system to self-antigens that occurs when mechanisms of self-tolerance fail. Autoimmunity is one cause of hypersensitivity. In order to further understand autoimmunity, we should describe in more detail what happens when self-tolerance fails:

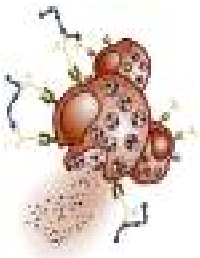
The failure of self-tolerance: It's pretty amazing that the immune system can produce 10^7 different cell types that can each respond to a different foreign antigen; and it's even more amazing that, in most people, none of these randomly generated cell types reacts against self antigens. How does this happen? Normally, as the immune system is randomly generating some 10^7 different clones, it is constantly culling the self-reacting ones from the final pool as they are created. If this process of getting rid of self-reacting lymphocytes fails or is incomplete, then B or T cells that may become activated against self-antigens will be produced. These rogue cells or their products (antibodies, mediators…) can then react against self-antigens. This is the basis of autoimmune disease. We do not know why self-tolerance fails in any human autoimmune disease, though many hypothesize the failure to arise from a combination of susceptibility genes and tissue damage. For example, tissue damage may lead to the release of antigens that wouldn't otherwise be found outside of damaged tissue; in genetically susceptible individuals, this may initiate a lymphocyte response against these cellular components and be the trigger for an autoimmune phenotype. But, again, no one knows for sure.

Inflammation: Inflammation is a vascular and cellular reaction to a wide variety of injurious and dangerous stimuli. Many immunological diseases manifest as chronic inflammation. Diseases that are caused by abnormal immune responses and that also involve significant inflammation are called immune-mediated inflammatory diseases.

Hypersensitivity diseases have been grouped into four major categories based upon their underlying causes. These groups are:

1.) Immediate (type I) hypersensitivity
2.) Antibody-mediated (type II) hypersensitivity
3.) Immune complex-mediated (type III) hypersensitivity
4.) Cell-mediated (type IV) hypersensitivity

The four types of hypersensitivity are outlined in the image below



Immediate (type I) hypersensitivity

Immediate (type I) hypersensitivity is a rapid IgE and mast cell-mediated vascular and smooth muscle response that occurs in genetically susceptible people. This type of reaction results from an excessive Th-2 response; we know these responses as "allergies."

Antibody-mediated (type II) hypersensitivity

Antibody-mediated (type II) reactions result when antibodies are directed against antigens on the surface of cells or other tissue components. The deposition of the antibody can have a variety of detrimental effects, including inflammation, opsonization and phagocytosis, or functional derangements.

Immune complex-mediated (type III) hypersensitivity

Immune complex-mediated (type III) hypersensitivity results when complexes of antibodies and antigens deposit in vascular walls or other tissues and cause an inflammatory response. This type of pathology is commonly implicated in vascolitis and arthritis.

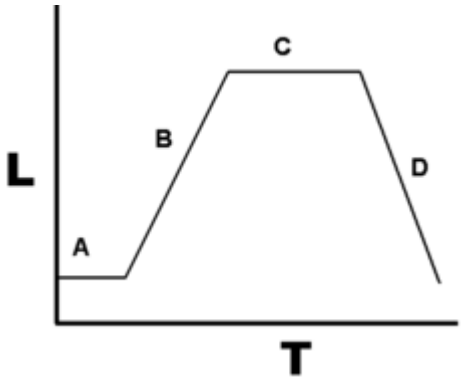
Cell-mediated (type IV) hypersensitivity

Cell-mediated (type IV) hypersensitivity results from an inappropriate or excessive immune reaction that is mediated by a specific subsets of CD4+ helper T cells (Th-1 and Th-17 cells) or by CD8+ cytotoxic T cells. These reactions are the basis for diseases such as Crohn's disease and multiple sclerosis.

Reset Show all

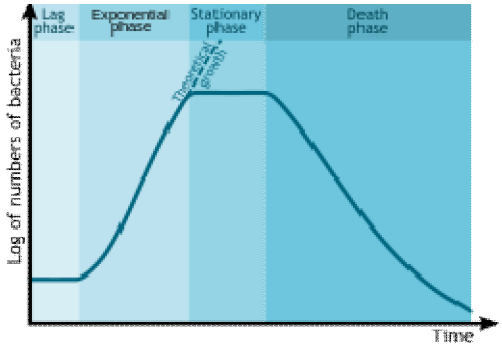
CHAPTER 3

Bacterial growth is the asexual reproduction, or cell division, of a bacterium into two daughter cells, in a process called binary fission. Providing no mutational event occurs the resulting daughter cells are genetically identical to the original cell. Hence, "local doubling" of the bacterial population occurs. Both daughter cells from the division do not necessarily survive. However, if the number surviving exceeds unity on average, the bacterial population undergoes exponential growth. The measurement of an exponential bacterial growth curve in batch culture was traditionally a part of the training of all microbiologists; the basic means requires bacterial enumeration (cell counting) by direct and individual (microscopic, flow cytometry, direct and bulk (biomass), indirect and individual (colony counting), or indirect and bulk (most probable number, turbidity, nutrient uptake) methods. Models reconcile theory with the measurements.



Growth is shown as *L* = log(numbers) where numbers is the number of colony forming units per ml, versus *T* (time.)

Phases



Bacterial growth curve\Kinetic Curve

The growth of bacteria (or other microorganisms, in batch culture can be modeled with four different phases: **lag phase** (A), **log phase** or **exponential phase** (B), **stationary phase** (C), and **death phase**(D).

1. During **lag phase**, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs.
2. The **log phase** (sometimes called the logarithmic phase or the *exponential phase*) is a period characterized by cell doubling.[4] The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period. For this type of exponential growth, plotting the natural logarithm of cell number against time produces a straight line. The slope of this line is the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time.[4] The actual rate of this growth (i.e. the slope of the line in the figure) depends upon the growth conditions, which affect the frequency of cell division events and the probability of both daughter cells surviving. Under controlled conditions, cyanobacteria can double their population four times a day.[5]Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes.
3. The **stationary phase** is often due to a growth-limiting factor such as the depletion of an essential nutrient, and/or the formation of an inhibitory product such as an organic acid.

Stationary phase results from a situation in which growth rate and death rate are equal. The number of new cells created is limited by the growth factor and as a result the rate of cell growth matches the rate of cell death. The result is a "smooth," horizontal linear part of the curve during the stationary phase.

4. At **death phase** (decline phase), bacteria die. This could be caused by lack of nutrients, environmental temperature above or below the tolerance band for the species, or other injurious conditions.

Batch culture is the most common laboratory growth method in which bacterial growth is studied, but it is only one of many. It is ideally spatially unstructured and temporally structured. The bacterial culture is incubated in a closed vessel with a single batch of medium. In some experimental regimes, some of the bacterial culture is periodically removed and added to fresh sterile medium. In the extreme case, this leads to the continual renewal of the nutrients. This is a chemostat, also known as continuous culture. It is ideally spatially unstructured and temporally unstructured, in a steady state defined by the rates of nutrient supply and bacterial growth. In comparison to batch culture, bacteria are maintained in exponential growth phase, and the growth rate of the bacteria is known. Related devices include turbidostats and auxostats.

Bacterial growth can be suppressed with bacteriostats, without necessarily killing the bacteria. In a synecological, true-to-nature situation in which more than one bacterial species is present, the growth of microbes is more dynamic and continual.

Liquid is not the only laboratory environment for bacterial growth. Spatially structured environments such as biofilms or agar surfaces present additional complex growth models.

Environmental Conditions

Environmental factors influence rate of bacterial growth such as acidity (pH), temperature, water activity, macro and micro nutrients, oxygen levels, and toxins. Conditions tend to be relatively consistent between bacteria with the exception of extremophiles. Bacterium have optimal growth conditions under which they thrive, but once outside of those conditions the stress can result in either reduced or stalled growth, dormancy (such as formation spores), or death. Maintaining sub-optimal growth conditions is a key principle tofood preservation.

Temperature

Low temperatures tend to reduce growth rates which has led to refrigeration being instrumental in food preservation.

Acidity

Optimal acidity for bacteria tends to be around pH 6.5 to 7.0 with the exception of acidophiles. Some bacteria can change the pH such as by excreting acid resulting in sub-optimal conditions.

Water activity

Bacteria can be aerobes or anaerobes.

Micronutrients

Toxins

Toxins such as ethanol can hinder or kill bacterial growth. This is used beneficially for disinfection and in food preservation.

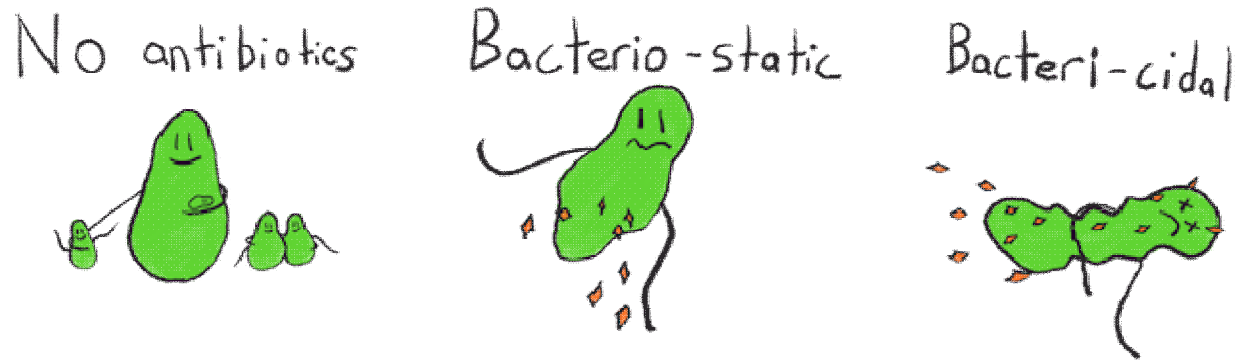
Antibiotics

Antibiotics are a type of medicine which are used to treat bacterial infections.To help the immune system, we sometimes use antibiotics, which are chemicals (specifically a swarm of small molecules) that enter and stick to important parts (think of targets) of the bacterial cell, and interfere with its ability to survive and multiply. If the bacteria are susceptible to the antibiotic, then they will stop growing or simply die.

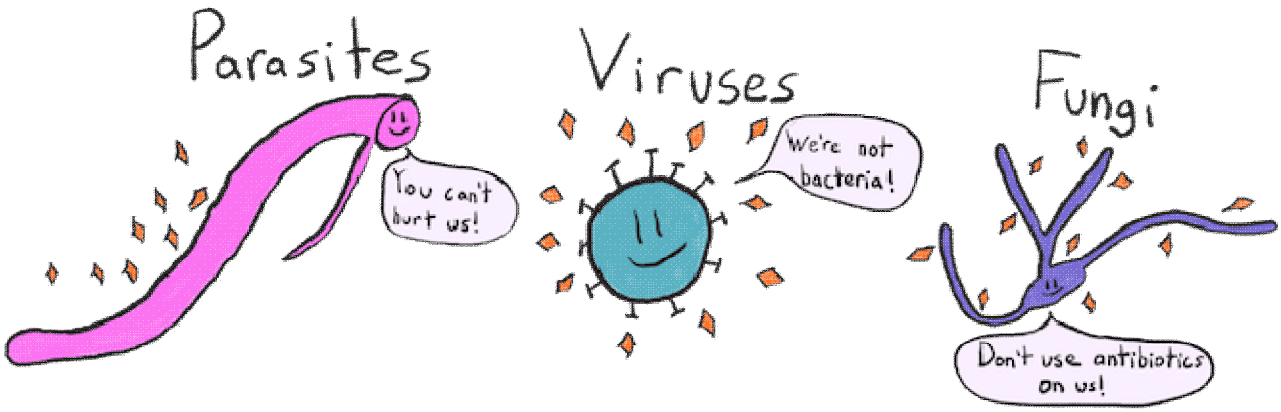
These important parts include:

- Proteins/sugars in the bacterial wall
- Important enzymes that make new bacterial DNA or proteins

When an antibiotic molecule sticks to its target, it will disable or destroy that protein or enzyme. If enough of the antibiotic is present, the bacterial cell is crippled and either stops growing (bacterio-static effect) or simply dies (bacteri-cidal effect).



Just to be clear, antibiotics **don't** affect **viruses**, **fungi**, or **parasites** - they only bind to bacterial cell targets so they only affect bacterial cells. In fact, they specifically target bacteria rather than human cells.



Mechanism of Action

Penicillin and Azithromycin.

Penicillin

Penicillin is a fabulous antibiotic because it isn't toxic to humans at concentrations that can kill bacteria and it can kill a lot of different types of bacteria.

So how does it work? Penicillin weakens the bacterial wall by:

- Deactivating a bacterial enzyme (transpeptidase) that builds and repairs the bacteria wall.

- Activating a bacterial enzyme (autolysin) that cuts open parts of the bacterial wall, an enzyme normally only activated when the bacteria is multiplying.

In short, penicillin causes the bacteria to weaken its own cell wall (imagine being forced to punch yourself!), and prevents the bacteria from being able to repair itself. With a weak wall, water seeps in, and the bacteria swells up and explodes.

Azithromycin

Azithromycin is a broad spectrum antibiotic which is often used to treat a wide variety of infections; everything from pneumonia to sexually transmitted diseases.

So how does it work?

Azithromycin prevents the bacteria from multiplying by:

- Blocking the cell's ability to create proteins by attaching to ribosomes in the cell.

In short, azithromycin prevents bacteria from multiplying, making it much easier for the immune system to handle the infection.

Antibiotic development

Over the years, a number of antibiotics have been discovered in nature or synthesized in the lab. Some antibiotics target only specific bacteria and are called “narrow spectrum” antibiotics, whereas other antibiotics target many types of bacteria and are called “broad spectrum” antibiotics.

Developing completely new classes of antibiotics (as opposed to variations on existing antibiotics) is very difficult. It’s easy to find chemicals that kill bacteria, but not so easy to find substances that could be used as medicines, even if researchers were given infinite resources! Researchers are basically shooting in the dark. In fact, the most recent discovery of a novel antibiotic class was in 1987, almost 30 years ago (Silver, L., 2011)! While there are a few new

antibiotics currently in development, researchers don't know if they'll ever become usable as medicine.

INTRODUCTION

Disinfection and sterilization are essential for ensuring that medical and surgical instruments do not transmit infectious pathogens to patients. Because sterilization of all patient-care items is not necessary, health-care policies must identify, primarily on the basis of the items' intended use, whether cleaning, disinfection, or sterilization is indicated.

Sterilization: Sterilization describes a process that destroys or eliminates all forms of microbial life and is carried out in health-care facilities by physical or chemical methods.

Disinfection: Disinfection describes a process that eliminates many or all pathogenic microorganisms, except bacterial spores, on inanimate objects.

Cleaning: Cleaning is removal of visible soil (e.g., organic and inorganic material) from objects and surfaces. It is normally accomplished manually or mechanically using water with detergents or enzymatic products.

Decontamination: Decontamination removes pathogenic microorganisms from objects so they are safe to handle, use, or discard.

METHODS OF STERILIZATION

The various methods of sterilization are:

1. Physical Method

(a) Thermal (Heat) methods

(b) Radiation method

(c) Filtration method

2. Chemical Method

3. Gaseous method

Heat Sterilization

Heat sterilization is the most widely used and reliable method of sterilization, involving destruction of enzymes and other essential cell constituents. The process is more effective in hydrated state where under conditions of high humidity, hydrolysis and denaturation occur, thus lower heat input is required. Under dry state, oxidative changes take place, and higher heat input is required.

This method of sterilization can be applied only to the thermostable products, but it can be used for moisture-sensitive materials for which dry heat (160- 180°C) sterilization, and for moisture-resistant materials for which moist heat (121-134°C) sterilization is used.

The efficiency with which heat is able to inactivate microorganisms is dependent upon the degree of heat, the exposure time and the presence of water. The action of heat will be due to induction of lethal chemical events mediated through the action of water and oxygen. In the presence of water much lower temperature,time exposures are required to kill microbe than in the absence of water. In this processes both dry and moist heat are used for sterilization.

Dry Heat Sterilization: Examples of Dry heat sterilization are:

1. Incineration
2. Red heat
3. Flaming
4. Hot air oven

It employs higher temperatures in the range of 160-180°C and requires exposures time up to 2 hours, depending upon the temperature employed. The benefit of dry heat includes good penetrability and non-corrosive nature which makes it applicable for sterilizing glass-wares and metal surgical instruments. It is also used for sterilizing non-aqueous thermo-stable liquids and thermo- stable powders. Dry heat destroys bacterial endotoxins (or pyrogens) which are difficult to eliminate by other means and this property makes it applicable for sterilizing glass bottles which are to be filled aseptically.

Hot-air oven

Dry heat sterilization is usually carried out in a hot air oven, which consists of the following:

(i) An insulated chamber surrounded by an outer case containing electric heaters.

(ii) A fan (iii) Shelves

(iv) Thermocouples

(v) Temperature sensor

(vi) Door locking controls.

1. Dry saturated steam – Autoclaving
2. Boiling water/ steam at atmospheric pressure
3. Hot water below boiling point

Moist heat sterilization involves the use of steam in the range of 121-134°C. Steam under pressure is used to generate high temperature needed for sterilization. Saturated steam acts as an effective sterilizing agent. Steam for sterilization can be either wet saturated steam (containing entrained water droplets) or dry saturated steam (no entrained water droplets).

An Autoclave

Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable systems available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents. For efficient heat transfer, steam must flush the air out of the autoclave chamber. Before using the autoclave, check the drain screen at the bottom of the chamber and clean if blocked. If the sieve is blocked with debris, a layer of air may form at the bottom of the autoclave, preventing efficient operation. Autoclaves should be tested periodically with biological indicators like spores of Bacillus stearothermophilus to ensure proper function. This method of sterilization works well for many metal and glass items but is not acceptable for rubber, plastics, and equipment that would be damaged by high temperatures (Figure 4.1). Autoclaves, or steam sterilizers essentially consist of following: 1. A cylindrical or rectangular chamber, with capacities ranging from 400 to 800 litres.

Gaseous Sterilization

The chemically reactive gases such as formaldehyde, (methanol, H.CHO) and ethylene oxide (CH2)2O possess biocidal activity. Ethylene oxide is a colorless, odorless, and flammable gas.

The mechanism of antimicrobial action of the two gases is assumed to be through alkylations of sulphydryl, amino, hydroxyl and carboxyl groups on proteins and amino groups of nucleic acids. The concentration ranges (weight of gas per unit chamber volume) are usually in range of 800-1200 mg/L for ethylene oxide and 15-100 mg/L for formaldehyde with operating temperatures of 45-63°C and 70- 75°C respectively.

Both of these gases being alkylating agents are potentially mutagenic and carcinogenic. They also produce acute toxicity including irritation of the skin, conjunctiva and nasal mucosa.

(a) Ethylene oxide sterilizer: An ethylene oxide sterilizer consists of a chamber of 100-300-Litre capacity and surrounded by a water jacket. Air is removed from sterilizer by evacuation, humidification and conditioning of the load is done by passing sub-atmospheric pressure steam, then evacuation is done again and preheated vaporized ethylene oxide is passed. After treatment, the gases are evacuated either directly to the outside atmosphere or through a special exhaust system.

Ethylene oxide gas has been used widely to process heat-sensitive devices, but the aeration times needed at the end of the cycle to eliminate the gas made this method slow.

(b) Low temperature steam formaldehyde (LTSF) sterilizer: An LTSF sterilizer operates with sub atmospheric pressure steam. At first, air is removed by evacuation and steam is admitted to the chamber.

Liquid Sterilization

(a) Peracetic Acid liquid sterilization: Peracetic acid was found to be sporicidal at low concentrations. It was also found to be water soluble, and left no residue after rinsing. It was also shown to have no harmful health or environmental effects. It disrupts bonds in proteins and enzymes and may also interfere with cell membrane transportation through the rupture of cell walls and may oxidize essential enzymes and impair vital biochemical pathways.

In a low-temperature liquid chemical sterile processing system, several steps must be followed for effective sterilization:

1. Pre-cleaning of the devices is necessary because many devices have small connected lumens.
2. Leak testing is done to ensure there are no leaks that could allow fluid to enter/leak the ampoules/vials and cause damage.
3. The appropriate tray/container must then be selected, and if the device has lumens, the appropriate connector attached.
4. The sterilant concentrate is provided in a sealed single- use cup and requires no pre-mixing or dilution.

Classification of microorganisms

Why study diversity?

Taxonomy - the science of biological classification; the grouping of organisms according to their mutual similarities (i.e., establishing relationships between one group of organisms and another; to differentiate one group of organisms from another).

Systematics - The study of biodiversity in an evolutionary context (i.e., the study of the evolutionary history of organisms)

Principles of classification

- organisms exist as real, separate groups
- natural ordering into the groups
- reflect genetic relationships
- established by evolutionary processes (phylogeny - evolutionary history = evolutionary relatedness of organisms)

Components of taxonomy

Classification

- Ordering organisms with like characteristics into groups or taxa (singular - taxon)
- Based on established procedures and rules
- Describes groups of organisms, their interrelationships and boundaries between groups.

Nomenclature

- Assignment of names for purposes of communication and identification
- Use a binomial systems of nomenclature

Identification

- Application of classification & nomenclature to assign proper name to unknown organism and place it in its proper position within classification system.

Taxonomic Hierarchies

Ideally

- Represent a coherent degree of homology - genetic and evolutionary similarity
- Members of each taxa should be monophyletic - same evolutionary history. (i.e., members of a genus had a common ancestor)

Taxa

Domain

Kingdom

Phylum (Divisions)

Class

Order

Family

Genus

Species

Subspecies

Subspecies

Describes a specific clone of cells that differs from others within the same species

• physiologically • morphologically • antigenically • pathogenically

strain - a population of cells that descended from a single.

Criteria for classification of microorganisms

i) Phenetic Classification

• Classification according to phenotypic characteristics

• Group analogously similar organisms

The Phenetic approach is problematic

• taxa are often polyphyletic, i.e., contain organisms with different evolutionary histories (i.e., homologously dissimilar organisms are grouped together)

Phenetic Classification Parameters

a. Morphology

• cell shape and size, arrangement of cells, arrangement of flagella, capsule, endospores, mechanism of motility

• staining properties – e.g., Gram stain reaction and acid-fast stain reaction

b. Nutrition and physiology

• Modes of metabolism (phototroph, chemoorganotroph, chemolithotroph); energy sources, carbon, nitrogen and sulfur sources, fermentation products, growth factor requirements; Temperature range and optima, pH tolerance range, osmotic tolerance, salt requirements and tolerance, secondary metabolites formed, storage inclusions.

• Many different biochemical tests are used to assess a microbes nutrition and physiology

• Serotyping – Identifying a microorganism based on its reaction to particular antibodies. The antibodies are used to identify microorganisms carrying particular antigens. Techniques like the Western blot or Enzyme Linked Immunosorbent Assay (ELISA)

• Phage typing – determines the susceptibility of a bacterium to a particular phage type. Highly specialized and usually restricted to the species level and lower.

c. Ecological Characteristics

• The ability of a microorganism to colonize a particular environment

• Life cycle patterns, the nature of symbiotic relationships, the ability to cause disease in a particular host, habitat preferences (e.g., requirements for temperature, pH, oxygen, osmotic concentration)

d. Genetic analysis – the study of chromosomal gene exchange through transformation, conjugation and transduction is sometimes useful for classification.

CHAPTER 4

Human microbiota

The **human microbiota** is the aggregate of microorganisms, a microbiome that resides on or within a number of tissues and biofluids, including the skin, mammary glands, placenta, seminal fluid, uterus, ovarian follicles, lung, saliva, oral mucosa, conjunctiva, and gastrointestinal tracts. They include bacteria, fungi, and archaea. Micro-animals which live on the human body are excluded. The human microbiome refers to their genomes.

Though widely known as *flora or microflora*, this is a misnomer in technical terms, since the word root *flora* pertains to plants, and *biota* refers to the total collection of organisms in a particular ecosystem. Recently, the more appropriate term *microbiota* is applied, though its use has not eclipsed the entrenched use and recognition of *flora* with regard to bacteria and other microorganisms. Both terms are being used in different literature.[6]

Types

Bacteria

Populations of microbes (such as bacteria and yeasts) inhabit the skin and mucosal surfaces in various parts of the body. Their role forms part of normal, healthy human physiology, however if microbe numbers grow beyond their typical ranges (often due to a compromised immune system) or if microbes populate (such as through poor hygiene or injury) areas of the body normally not colonized or sterile (such as the blood, or the lower respiratory tract, or the abdominal cavity), disease can result (causing, respectively, bacteremia/sepsis, pneumonia, and peritonitis).

A number of types of bacteria, such as *Actinomyces viscosus* and *A. naeslundii*, live in the mouth, where they are part of a sticky substance called plaque. If this is not removed by brushing, it hardens into calculus (also called tartar). The same bacteria also secrete acids that dissolve tooth enamel, causing tooth decay.

The vaginal microflora consist mostly of various lactobacillus species. It was long thought that the most common of these species was *Lactobacillus acidophilus*, but it has later been shown that the most common one is *L. iners* followed by *L. crispatus*. Other lactobacilli found in the

vagina are *L. jensenii*, *L. delbruekii* and *L. gasseri*. Disturbance of the vaginal flora can lead to infections such as bacterial vaginosis or candidiasis ("yeast infection").

Archaea

Archaea are present in the human gut, but, in contrast to the enormous variety of bacteria in this organ, the numbers of archaeal species are much more limited. The dominant group are the methanogens, particularly *Methanobrevibacter smithii* and *Methanosphaera stadtmanae*. However, colonization by methanogens is variable, and only about 50% of humans have easily detectable populations of these organisms.

Fungi

Fungi, in particular yeasts, are present in the human gut.[26][27][28][29] The best-studied of these are *Candida* species due to their ability to become pathogenic inimmunocompromised and even in healthy hosts. Yeasts are also present on the skin, such as *Malassezia* species, where they consume oils secreted from thesebaceous glands.[30][31]

Viruses

Viruses, especially bacterial viruses (bacteriophages), colonize various body sites. These colonized sites include the skin, gut, lungs, and oral cavity. Virus communities have been associated with some diseases, and do not simply reflect the bacterial communities.

Skin

The skin acts as a barrier to deter the invasion of pathogenic microbes. The human skin contains microbes that reside either in or on the skin and can be residential or transient. Resident microorganism types vary in relation to skin type on the human body. A majority of microbes reside on superficial cells on the skin or prefer to associate with glands. These glands such as oil or sweat glands provide the microbes with water, amino acids, and fatty acids. In addition, resident bacteria that associated with oil glands are often Gram-positive and can be pathogenic.

Conjunctiva

A small number of bacteria and fungi are normally present in the conjunctiva. Classes of bacteria include Gram-positive cocci (e.g., *Staphylococcus* and Streptococcus) and Gram-negative rods and cocci (e.g., *Haemophilus* and *Neisseria*) are present.[40] Fungal genera

include *Candida*, *Aspergillus*, and *Penicillium*.[26] The lachrymal glandscontinuously secrete, keeping the conjunctiva moist, while intermittent blinking lubricates the conjunctiva and washes away foreign material. Tears contain bactericides such aslysozyme, so that microorganisms have difficulty in surviving the lysozyme and settling on the epithelial surfaces.

Gut

The gut flora has the largest numbers of bacteria and the greatest number of species compared to other areas of the body. In humans the gut flora is established at one to two years after birth, and by that time the intestinal epithelium and the intestinal mucosal barrier that it secretes have co-developed in a way that is tolerant to, and even supportive of, the gut flora and that also provides a barrier to pathogenic organisms.

Vagina

Vaginal microbiota refers to those species and genera that colonize the lower reproductive tract of women. These organisms play an important role in protecting against infections and maintaining vaginal health. The most abundant vaginal microorganisms found in premenopausal women are from the genus *Lactobacillus*, which suppress pathogens by producing hydrogen peroxide and lactic acid. Bacterial species composition and ratios vary depending on the stage of the menstrual cycle. Ethnicity also influences vaginal flora. The occurrence of hydrogen peroxide-producing lactobacilli is lower in African American women and vaginal pH is higher. Other influential factors such as sexual intercourse and antibiotics have been linked to the loss of lactobacilli. Moreover, studies have found that sexual intercourse with a condom does appear to change lactobacilli levels, and does increase the level of *Escherichia coli* within the vaginal flora. Changes in the normal, healthy vaginal microbiota is an indication of infections, such as candidiasis or bacterial vaginosis.[50] *Candida albicans* inhibits the growth of *Lactobacillus* species, while *Lactobacillus* species which produce hydrogen peroxide inhibit the growth and virulence of *Candida albicans* in both the vagina and the gut.

Placenta

Until recently the placenta was considered to be a sterile organ but commensal, nonpathogenic bacterial species and genera have been identified that reside in the placental tissue.

Uterus

Until recently, the upper reproductive tract of women was considered to be a sterile environment. A variety of microorganisms inhabit the uterus of healthy, asymptomatic women of reproductive age. The microbiome of the uterus differs significantly from that of the vagina and gastrointestinal tract.

Oral cavity

The environment present in the human mouth allows the growth of characteristic microorganisms found there. It provides a source of water and nutrients, as well as a moderate temperature. Resident microbes of the mouth adhere to the teeth and gums to resist mechanical flushing from the mouth to stomach where acid-sensitive microbes are destroyed by hydrochloric acid.

Lung

Much like the oral cavity, the upper and lower respiratory system possess mechanical deterrents to remove microbes. Goblet cells produce mucous which traps microbes and moves them out of the respiratory system via continuously moving ciliated epithelial cells. In addition, a bactericidal effect is generated by nasal mucus which contains the enzyme lysozyme.

Nonetheless, the upper and lower respiratory tract appears to have their own set of microbiota. Pulmonary bacterial microbiota belong to 9 major bacterial genera: *Prevotella*,*Sphingomonas*, *Pseudomonas*, *Acinetobacter*, *Fusobacterium*, *Megasphaera*, *Veillonella*, *Staphylococcus*, and *Streptococcus*. Some of the bacteria considered "normal biota" in the respiratory tract can cause serious disease especially in immunocompromised individuals; these include *Streptococcus pyogenes*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Staphylococcus aureus*.

Fungal genera that compose the pulmonary mycobiome include *Candida*, *Malassezia*, *Neosartorya*, *Saccharomyces*, and *Aspergillus*, among others.

Unusual distributions of bacterial and fungal genera in the respiratory tract is observed in patients with cystic fibrosis. The bacterial flora found in the lungs of patients with cystic fibrosis often contains antibiotic-resistant and slow-growing bacteria, and the frequency of these pathogens changes in relation to age.

Virulence vs Pathogenicity

Pathogenicity is a organism's ability to cause disease. Some organisms are harmless and can live on you or in you without you even noticing. But, if they do cause some sort of disease process, then they are called "pathogens". Some pathogens are less pathogenic than others. For example, E. coli is pathogenic depending on the strain. Others are pathogenic all the time, like HIV, where you will progress to AIDS almost 100% of the time.

Virulence is a measure of the degree of disease that a pathogen causes. For example, there are some very virulent influenza viruses out there that will knock you out and might even kill you. On the other hand, you might catch a strain that infects you, causes disease, but the disease isn't so bad. In that case, the organism is infectious, pathogenic, but not very virulent. Ebola, on the other hand, is very infectious, very pathogenic (because most people who are infected develop disease), and very virulent (because it causes a severe, often fatal disease).

Toxins

A **toxin** is a poisonous substance produced within living cells or organisms; synthetic toxicants created by artificial processes are thus excluded. Toxins can be small molecules, peptides, or proteins that are capable of causing disease on contact with or absorption by body tissues interacting with biological macromoleculessuch as enzymes or cellular receptors. Toxins vary greatly in their toxicity, ranging from usually minor (such as a bee sting) to almost immediately deadly (such as botulinum toxin).

Toxins are often distinguished from other chemical agents by their method of production—the word toxin does not specify method of delivery (compare with venom and the narrower meaning of poison—all substances that can also cause disturbances to organisms). It simply means it is a biologically produced poison.

A rather informal terminology of individual toxins relates them to the anatomical location where their effects are most notable:

- Hemotoxin, causes destruction of red blood cells (hemolysis)
- Phototoxin, causes dangerous photosensitivity

On a broader scale, toxins may be classified as either exotoxins, being excreted by an organism, or endotoxins, that are released mainly when bacteria are lysed.

Related terms are:

Toxoid, weakened or suppressed toxin

Venom, toxins in the sense of use by certain types of animals

Biotoxins

The term "biotoxin" is sometimes used to explicitly confirm the biological origin.[6][7] Biotoxins are further classified into fungal biotoxins, or short mycotoxins, microbial biotoxins, plant biotoxins, short phytotoxins and animal biotoxins.

Toxins produced by microorganisms are important virulence determinants responsible for microbial pathogenicity and/or evasion of the host immune response.[8]

Biotoxins vary greatly in purpose and mechanism, and can be highly complex (the venom of the cone snail contains dozens of small proteins, each targeting a specific nerve channel or receptor), or relatively small protein.

Biotoxins in nature have two primary functions:

- Predation in the spider, snake, scorpion, jellyfish, wasp
- Defense in the bee, ant, termite, honeybee, wasp, poison dart frog

Some of the more well known types of biotoxins include:

- Cyanotoxins, produced by cyanobacteria
- Dinotoxins, produced by Dinoflagellates
- Necrotoxins cause necrosis (i.e., death) in the cells they encounter and destroy all types of tissue[*citation needed*]. Necrotoxins spread through the bloodstream[*citation needed*]. In humans, skin and muscle tissues are most sensitive to necrotoxins[*citation needed*].
- Myotoxins are small, basic peptides found in snake and lizard venoms,They cause muscle tissue damage by a non enzymatic receptor based mechanism. Organisms that possess myotoxins include:
  - rattlesnakes
  - eastern bearded dragon

- Cytotoxins are toxic at the level of individual cells, either in a non-specific fashion or only in certain types of living cells:
  - Ricin, from castor beans
  - Apitoxin, from honey bees
  - T-2 mycotoxin, from certain toxic mushrooms

Environmental toxins

The term "environmental toxin" can sometimes explicitly include synthetic contaminants such as industrial pollutants and other artificially made toxic substances. As this contradicts most formal definitions of the term "toxin", it is important to confirm what the researcher means when encountering the term outside of microbiological contexts.

Modes of action of toxic agents

The principal modes of action of toxic agents are discussed in relation to the type of chemical bond formed between the poison and the target constituent of tissues. Alteration of enzyme activity, interference with the binding of poisonous chemicals to proteins, intercalation with nucleic acids, disturbances in electrolyte balance and the disorganization of cellular water and membrane lipids are illustrated as toxic processes involving ionic or van der Waals forces. The reactions of heavy metals with tissue nucleophiles and of exogenous nucleophiles with tissue metals are given brief attention in connection with coordinate-covalent binding. Covalent binding of poisons can arise from the incorporation of an antimetabolite into a larger molecule or reactions of electrophiles or free radicals with tissue constituents. These modes of action are illustrated by chemicals that produce necrosis, allergy or cancer.

Soil microbiology

Soil microbiology is the study of organisms in soil, their functions, and how they affect soil properties. It is believed that between two and four billion years ago, the first ancientbacteria and microorganisms came about in Earth's oceans. These bacteria could fix nitrogen, in time multiplied and as a result released oxygen into the atmosphere. This led to more advanced microorganisms. Microorganisms in soil are important because they affect soil structure and fertility. Soil microorganisms can be classified

As

bacteria,

actinomycetes,

fungi,

algae and protozoa.

Each of these groups has characteristics that define them and their functions in soil.

Bacteria

Bacteria and Archaea are the smallest organisms in soil apart from viruses. Bacteria and Archaea are prokaryotic. All of the *other* microorganisms are eukaryotic, which means they have a more advanced cell structure with internal organelles and the ability to reproduce sexually. A prokaryote has a very simple cell structure with no internal organelles.[1]Bacteria and archaea are the most abundant microorganisms in the soil, and serve many important purposes, including nitrogen fixation.

Biochemical processes

One of the most distinguished features of bacteria is their biochemical versatility. A bacterial genus called *Pseudomonas* can metabolize a wide range of chemicals and fertilizers. In contrast, another genus known as *Nitrobacter* can only derive its energy by turning nitrite into nitrate, which is also known as oxidation. The genus *Clostridium* is an example of bacterial versatility because it, unlike most species, can grow in the absence of oxygen, respiring anaerobically. Several species of *Pseudomonas*, such as *Pseudomonas aeruginosa* are able to respire both aerobically and anaerobically, using nitrate as the terminal electron acceptor.

Nitrogen fixation

Bacteria are responsible for the process of nitrogen fixation, which is the conversion of atmospheric nitrogen into nitrogen-containing compounds (such as ammonia) that can be used by plants. Autotrophic bacteria derive their energy by making their own food through oxidation, like the *Nitrobacters* species, rather than feeding on plants or other organisms. These bacteria are responsible for nitrogen fixation. The amount of autotrophic bacteria is small compared to heterotrophic bacteria (the opposite of autotrophic bacteria, heterotrophic bacteria acquire energy

by consuming plants or other microorganisms), but are very important because almost every plant and organism requires nitrogen in some way, and would have no way of obtaining it if not for nitrogen-fixing bacteria.

Actinomycetes

Actinomycetes are soil microorganisms. They are a type of bacteria, but they share some characteristics with fungi that are most likely a result of convergent evolution due to a common habitat and lifestyle.

Fungi

Fungi are abundant in soil, but bacteria are more abundant. Fungi are important in the soil as food sources for other, larger organisms, pathogens, beneficial symbiotic relationships with plants or other organisms and soil health. Fungi can be split into species based primarily on the size, shape and color of their reproductive spores, which are used to reproduce. Most of the environmental factors that influence the growth and distribution of bacteria and actinomycetes also influence fungi. The quality as well as quantity of organic matter in the soil has a direct correlation to the growth of fungi, because most fungi consume organic matter for nutrition. Fungi thrive in acidic environments, while bacteria and actinomycetes cannot survive in acid, which results in an abundance of fungi in acidic areas. Fungi also grows well in dry, arid soils because fungi are aerobic, or dependent on oxygen, and the higher the moisture content in the soil, the less oxygen is present for them.

Algae

Algae can make their own nutrients through photosynthesis. Photosynthesis converts light energy to chemical energy that can be stored as nutrients. For algae to grow, it must be exposed to light because photosynthesis requires light, so algae are typically distributed evenly wherever sunlight and moderate moisture is available. Algae, do not have to be directly exposed to the Sun, but can live below the soil surface given uniform temperature and moisture conditions. Algae are also capable of performing nitrogen fixation.

Protozoa

Protozoa are eukaryotic organisms that were some of the first microorganisms to reproduce sexually, a significant evolutionary step from duplication of spores, like those that many other soil microorganisms depend on. Protozoa can be split up into three categories: flagellates, amoebae and ciliates.

Flagellates

Flagellates are the smallest members of the protozoa group, and can be divided further based on whether they can participate in photosynthesis. Nonchlorophyll-containing flagellates are not capable of photosynthesis because chlorophyll is the green pigment that absorbs sunlight. These flagellates are found mostly in soil. Flagellates that contain chlorophyll typically occur in aquatic conditions. Flagellates can be distinguished by their flagella, which is their means of movement. Some have several flagella, while other species only have one that resembles a long branch or appendage.

Amoebae

it is larger than flagellates and move in a different way. Amoebae can be distinguished from other protozoa by their slug-like properties and pseudopodia. A pseudopodia or “false foot” is a temporary obtrusion from the body of the amoeba that helps pull it along surfaces for movement or helps to pull in food. The amoeba does not have permanent appendages and the pseudopodium is more of a slime-like consistency than a flagellum.

Ciliates

Ciliates are the largest of the protozoa group, and move by means of short, numerous cilia that produce beating movements. Cilia resemble small, short hairs. They can move in different directions to move the organism, giving it more mobility than flagellates or amoebae.

Air Microbiology

Microbes normally found in atmosphere within 300-10000 feet above from the land. Fungal spores which are found in air consist of Alternaria, Cladosporium, Penecillium and Aspergillus found above 4000 feet from the land, found in both polar and non polar air masses. Organisms found below 500 feet is mainly in overpopulated area, these include spores of Bacillus and Clostridium, ascospores of yeast and fragments of mycelium, mould, streptomycetaceae, pollen,

protozoan cysts, algae, Micrococcus and corynebacterium. Air found in school and hospital or living places of the person suffered from infectious disease usually found microbes like tubercle bacilli, streptococci and pneumococci.

The characteristics of atmosphere as a habitat include extreme temperature variations, light, temperature, low amount of available water and organic water. All these characters make the atmosphere unsuitable for growth of micro organisms. Usually most of the organisms are found in the lower region of atmosphere. The air in the atmosphere is often exposed to sunlight thus it contains less moisture and higher temperature. Thus if the micro organisms are not protected from desiccation, almost most of the organisms will die.

The origin of the micro organisms takes place through various ways. Soil is one of the source to transfer micro organisms to the air. Whenever the wind blows it disturbs the micro organisms and liberate them into the air and these micro organisms remains suspended in the air for long time. Another way of transferring micro organisms to the air is by manmade actions like plugging and digging. Organisms can also be released in the form of water droplets or aerosols which are produced by wind or tidal actions. Micro organisms from plant and animal surfaces are also transferred by air currents. But the main source of micro organisms is human beings. These are discharged through human activities like coughing, sneezing, laughing and even talking. This article gives a description about the origin of micro organisms in air.

Air micro flora significance in human health: Human being inhales air every moment. Even most of the micro organisms present in air are harmless but still less than 1% of the airborne bacteria is pathogens. Outdoor air mostly does not contain disease causing pathogen whereas indoor air has more chances of infections especially in large gatherings like theaters and schools.

Food processing: Micro organisms transferred through various methods in air and are settled on various materials are involved in various fermentation products like alcoholic beverages, vinegar, dairy products etc. In Industrial processes which requires growth of any particular organisms, getting supply of sterile air free from contamination is a problem.[6].Food products are either animal or plant origin. Some bacteria cause food poison. Clostridia cause toxins in meat and meat products. Staphylococci produce infection in fish products. *Salmonella* cause infection through milk, eggs and salads. All of these are happened only when the unhygenic

handling of food stuffs. Microbial biofilms also act as a significant one, it appears in food and food contact surface, if micro organisms are not removed from the surface it create biofilms will be hazard to consumers. The microbiology of air is significant in many places such as hospitals, food processing, air conditioning and many other places.

Water Microbiology

Water microbiology is concerned with the **microorganisms** that live in **water**, or can be transported from one **habitat** to another by water.

Water can support the growth of many types of microorganisms. This can be advantageous. For example, the chemical activities of certain strains of yeasts provide us with beer and bread. As well, the growth of some **bacteria** in contaminated water can help digest the poisons from the water.

However, the presence of other **disease** causing microbes in water is unhealthy and even life threatening. For example, bacteria that live in the intestinal tracts of humans and other warm blooded animals, such as ***Escherichia coli***, *Salmonella*, *Shigella*, and *Vibrio*, can contaminate water if feces enters the water. **Contamination** of drinking water with a type of *Escherichia coli* can be fatal. The intestinal tract of warm-blooded animals also contains viruses that can contaminate water and cause disease. Examples include rotavirus, enteroviruses, and coxsackievirus.

Another group of microbes of concern in water microbiology are **protozoa**. The two protozoa of the most concern are *Giardia* and *Cryptosporidium*. They live normally in the intestinal tract of animals such as beaver and **deer**. *Giardia* and *Cryptosporidium* form dormant and hardy forms called cysts during their life cycles. The cyst forms are resistant to **chlorine**, which is the most popular form of drinking water disinfection, and can pass through the filters used in many **water treatment** plants. If ingested in drinking water they can cause debilitating and prolonged diarrhea in humans, and can be life threatening to those people with impaired immune systems. *Cryptosporidium* contamination of the drinking water of Milwaukee, Wisconsin with in 1993 sickened more than 400,000 people and killed 47 people.

Many microorganisms are found naturally in fresh and **saltwater.** These include bacteria, cyanobacteria, protozoa, **algae**, and tiny animals such as rotifers. These can be important in the food chain that forms the basis of life in the water. For example, the microbes called cyanobacteria can convert the **energy** of the **sun** into the energy it needs to live. The plentiful numbers of these organisms in turn are used as food for other life. The algae that thrive in water is also an important food source for other forms of life.

A variety of microorganisms live in fresh water. The region of a water body near the shoreline (the littoral zone) is well lighted, shallow, and warmer than other regions of the water. Photosynthetic algae and bacteria that use **light** as energy thrive in this zone. Further away from the shore is the limnitic zone. Photosynthetic microbes also live here. As the water deepens, temperatures become colder and the **oxygen** concentration and light in the water decrease. Now, microbes that require oxygen do not thrive. Instead, purple and green **sulfur** bacteria, which can grow without oxygen, dominate. Finally, at the bottom of fresh waters (the benthic zone), few microbes survive. Bacteria that can survive in the absence of oxygen and sunlight, such as methane producing bacteria, thrive.

Salt water presents a different environment to microorganisms. The higher **salt** concentration, higher **pH**, and lower **nutrients**, relative to **freshwater,** are lethal to many microorganisms. But, salt loving (halophilic) bacteria abound near the surface, and some bacteria that also live in freshwater are plentiful (i.e., *Pseudomonas*and *Vibrio*). Also, in 2001, researchers demonstrated that the ancient form of microbial life known as **archaebacteria** is one of the dominant forms of life in the **ocean**. The role of archaebacteria in the ocean food chain is not yet known, but must be of vital importance.

Another microorganism found in saltwater are a type of algae known as dinoflagellelates. The rapid growth and multiplication of dinoflagellates can turn the water red. This "red tide" depletes the water of nutrients and oxygen, which can cause many **fish** to die. As well, humans can become ill by eating contaminated fish.

Water can also be an ideal means of transporting microorganisms from one place to another. For example, the water that is carried in the hulls of ships to stabilize the vessels during their ocean

voyages is now known to be a means of transporting microorganisms around the globe. One of these organisms, a bacterium called *Vibrio cholerae*, causes life threatening diarrhea in humans.

Drinking water is usually treated to minimize the risk of microbial contamination. The importance of drinking water treatment has been known for centuries. For example, in pre-Christian times the storage of drinking water in jugs made of **metal** was practiced. Now, the anti-bacterial effect of some metals is known. Similarly, the boiling of drinking water, as a means of protection of water has long been known.

An important aspect of water microbiology, particularly for drinking water, is the testing of the water to ensure that it is safe to drink. Water quality testing can de done in several ways. One popular test measures the turbidity of the water. Turbidity gives an indication of the amount of suspended material in the water. Typically, if material such as **soil** is present in the water then microorganisms will also be present. The presence of particles even as small as bacteria and viruses can decrease the clarity of the water. Turbidity is a quick way of indicating if water quality is deteriorating, and so if action should be taken to correct the water problem.

Staphylococcal Food Poisoning

Staphylococcal food poisoning results from eating food contaminated with toxins produced by certain types of staphylococci, resulting in diarrhea and vomiting.The staphylococci bacteria grow in food, in which they produce toxins. Thus, staphylococcal food poisoning does not result from ingesting the bacteria but rather from ingesting the toxins made by the bacteria that are already present in the contaminated food. Typical contaminated foods include custard, cream-filled pastry, milk, processed meats, and fish. The risk of an outbreak is high when food workers with skin infections contaminate foods that are undercooked or left at room temperature. Despite contamination, many foods have a normal taste and odor.

Symptoms

Symptoms usually begin abruptly with severe nausea and vomiting starting about 2 to 8 hours after the contaminated food is eaten. Other symptoms may include abdominal cramping, diarrhea, and sometimes headache and fever. Severe fluid and electrolyte loss may cause

weakness and very low blood pressure (shock—see Shock). Symptoms usually last less than 12 hours, and recovery is usually complete.

Occasionally, staphylococcal food poisoning is fatal, especially in the very young, the very old, and people weakened by long-term illness.

Diagnosis

- A doctor's evaluation
- Sometimes laboratory tests of food

The symptoms are usually all a doctor needs to diagnose gastroenteritis. A more specific diagnosis of staphylococcal food poisoning may be suspected when other people who ate the same food are similarly affected and when the disorder can be traced to a single source of contamination. To confirm the diagnosis, a laboratory must identify staphylococci in the suspected food, but this testing is not usually done.

Prevention

Careful food preparation can prevent staphylococcal food poisoning. Anyone who has a skin infection should not prepare food for others until the infection heals. Food should be consumed immediately or refrigerated and not kept at room temperature.

Treatment

- Fluids
- Sometimes drugs to control nausea and vomiting
- Sometimes fluids by vein

Treatment usually consists of drinking an adequate amount of fluids. A doctor may give an antinausea drug, either as an injection or as a suppository, to help control severe nausea and vomiting. Sometimes so much fluid is lost that fluids have to be given by vein (intravenously).

Salmonellosis

An infection with *Salmonella* bacteria usually affects the gastrointestinal system (the stomach and intestines) in humans. In more severe cases, *Salmonella* can spread to the blood, the bones, or even to the fluid around the brain, but these types of infection are less common.

An American scientist named Daniel E. Salmon is credited with the discovery of the *Salmonella* family of bacteria in the late 1800s. Since then, scientists have identified more than 2,400 types of *Salmonella*bacteria. They've also figured out where *Salmonella* live, how they spread to humans, and how to reduce their spread among the general public. Even so, each year the United States has about 40,000 cases of salmonellosis. And many more cases go unreported.

Salmonella bacteria are often found in the feces (poop) of some animals, particularly reptiles. Iguanas, for example, carry *Salmonella marina*. People who have these animals as pets are at more risk of getting salmonellosis because the bacteria from a reptile's feces can get on its skin. Then, when people handle the reptiles, they get the bacteria on their hands. (Hand washing is a good way to reduce the risk of getting salmonellosis.)

Other strains of *Salmonella* can spread to people in foods that have come into contact with infected animal feces. These exposures happen when foods such as poultry, eggs, and beef are not cooked enough. Fruit and vegetables can also become contaminated from feces in the soil or water where they are grown.

People who are more likely to become ill from *Salmonella* include:

- young children and babies (their developing immune systems have a harder time fighting off the infection)
- older people
- those with weakened immune systems (such as people with HIV and those with sickle cell anemia)
- people who take cancer-treatment drugs
- people who take antacids or stomach acid suppression medication

In these higher risk groups, *Salmonella* is more likely to invade beyond the gastrointestinal tract and cause **bacteremia** (bacteria in the bloodstream). From there, the bacteria can spread deeper into the body and cause more serious diseases, like meningitis.

Symptoms

Symptoms of salmonellosis can include diarrhea that may be bloody, belly cramping and pain, and fever. These symptoms can take anywhere from 6 to 72 hours to appear after someone ingests the bacteria. Not everyone who swallows the bacteria will become ill. In most people, the illness lasts 4 to 7 days once symptoms begin.

Some strains of *Salmonella* can cause a more serious form of the disease known as typhoid or enteric fever. The symptoms of typhoid fever can include a prolonged fever, belly pain, headache, tiredness, a distinct rash, constipation or diarrhea, and a change in mental state. Typhoid fever is rare in the United States, but it can be common in developing countries.

Treatment

Antibiotics do not appear to help a healthy person whose infection is not severe — and may actually lengthen the amount of time the person will carry the bacteria. Some people with salmonellosis may have to stay in the hospital to be treated fordehydration, which can be a complication of any type of diarrhea.

A severe *Salmonella* infection will require more testing to see which specific germ is causing the illness and which antibiotics can be used to treat it.

People who are infected with *Salmonella* can still be contagious from several days to several weeks after they've been infected — even if their symptoms have disappeared or they've been treated with antibiotics. So while you recover, be sure to wash your hands often and don't share your food or drinks with anyone. And if you work in a restaurant or your work involves handling food, check with your doctor before returning to work.

Clostridium Perfringens

Clostridium perfringens are bacteria that produce toxins harmful to humans.*Clostridium perfringens* and its toxins are found everywhere in the environment, but human infection is most likely to come from eating food with *Clostridium perfringens* in it. Food poisoning from *Clostridium perfringens* fairly common, but is typically not too severe, and is often mistaken for the 24-hour flu.

SOURCE OF *CLOSTRIDIUM PERFRINGEN*

The majority of outbreaks are associated with undercooked meats, often in large quantities of food prepared for a large group of people and left to sit out for long periods of time. Because of this, it is sometimes referred to as the "food service germ." Meat products such as stews, casseroles, and gravy are the most common sources of illness from *C. perfringens*. Most outbreaks come from food whose temperature is poorly controlled. If food is kept between 70 and 140 F, it is likely to grow *Clostridium perfringens* bacteria.

SYMPTOMS OF *CLOSTRIDIUM PERFRINGENS* INFECTION

People generally experience symptoms of *Clostridium perfringens* infection 6 to 24 hours after consuming the bacteria or toxins. *Clostridium perfringens* toxins cause abdominal pain and stomach cramps, followed by diarrhea. Nausea is also a common symptom. Fever and vomiting are not normally symptoms of poisoning by *Clostridium perfringens* toxins.

Illness from Clostridium perferingens generally lasts around 24 hours, and is rarely fatal.

COMPLICATION FROM *CLOSTRIDIUM PERFRINGENS*

The Type C strain of *Clostridium perfringens* can cause a more serious condition called Pig-bel Syndrome. This syndrome can cause death of intestinal cells and can often be fatal.

PREVENTING A *CLOSTRIDIUM PERFRINGENS* INFECTION

To prevent infection by *Clostridium perfringens*, follow the these tips:

- Cook foods containing meat thoroughly
- If keeping foods out, make sure they maintain a temperature of 140 F (60 C)
- When storing food in the refrigerator, divide it into pieces with a thickness of three inches or less so that it cools faster
- Reheat foods to at least 165 F (74 C)

Poliomyelitis

Polio (also known as poliomyelitis) is a highly contagious disease caused by a virus that attacks the nervous system. Children younger than 5 years old are more likely to contract the virus than any other group. It's estimated that 95 to 99 percent of people who contract poliovirus are asymptomatic. This is known as subclinical polio. Even without symptoms, people infected with poliovirus can still spread the virus and cause infection in others.

Pathophysiology

Poliovirus is spread by the fecal-oral route and by aerosol droplets. The poliovirus is shed in oral secretions for several weeks and in the feces for several months. The poliovirus destroys the anterior horn cells in the spinal cord.

Etiology

Acute poliomyelitis is caused by small RNA viruses of the *Enterovirus* genus of the Picornaviridae family. The single-stranded RNA core is surrounded by a protein capsid without a lipid envelope, which makes poliovirus resistant to lipid solvents and makes it stable at a low pH. Three antigenically distinct strains are known, with type 1 accounting for 85% of cases of paralytic illnesses. Infection with one type does not protect from the other types; however, immunity to each of the three strains is lifelong.

History and Physical Examination

Stages of presentation

Acute stage

Poliovirus is primarily spread by fecal-hand-oral transmission from one host to another. The virus is shed in oral secretions for several weeks and in the feces for several months. It destroys the anterior horn cells in the spinal cord. Poliovirus infections can be divided into minor and major forms.

The minor associated illnesses occur 1-3 days before the onset of paralysis, with gastrointestinal complaints such as nausea and vomiting, abdominal cramps and pain, and diarrhea. There are also systemic manifestations, such as sore throat, fever, malaise, and headache. This stage lasts usually for 2-3 weeks but may extend for up to 2 months; the presence of any tenderness in the muscles is evidence that the acute stage is not over.

The major associated illnesses include all forms of central nervous system (CNS) disease caused by poliovirus, including aseptic meningitis (or nonparalytic polio), polio encephalitis, bulbar polio, and paralytic poliomyelitis, alone or in combination.

The clinical findings associated with an attack of polio are as follows:

- Fever, neck stiffness (nuchal rigidity), and a pleocytosis in the CSF
- Profound asymmetrical muscle weakness
- The initial phase is typically followed by some recovery of muscle strength, but permanent weakness results from necrosis of anterior horn cells
- Rarely, a transverse myelitis with paraparesis, urinary retention, sensory symptoms and signs, autonomic dysfunction (including hyperhidrosis or hypohidrosis), and decreased limb temperature may occur

Recovery stage

In the recovery stage, also known as the convalescent stage, the acute symptoms and muscle tenderness disappear, and the paralyzed muscles begin to recover. This stage lasts for up to 2 years after the onset of the disease. During this entire period, there is gradual recovery of the muscles; the recovery is rapid in the first 6 months but is slower during the subsequent months.

Residual-paralysis stage

The period beyond 2 years after the onset of the disease is called the residual-paralysis stage. No recovery of muscle power occurs in this stage. Deformities are liable to occur as a consequence of imbalance of muscle power and poor posture. There is also disuse atrophy of muscles and shortening of the leg from interference with growth. In neglected cases, gross fixed deformities of the hip, knee, and foot occur with severe wasting of muscles. Children with extensive paralysis and gross deformities have to crawl on all fours to move from place to place. (See the image below.)

The typical contractures of post-polio residual paralysis.

Pattern of muscle weakness and deformities

Upper-limb involvement

Late functional deterioration is common in long-term poliomyelitis patients. Whereas upper-limb pain in individual functional regions is common, its overall prevalence and pattern in long-term poliomyelitis are poorly documented.[10] There are data in support of overuse due to greater mobility and independence as a cause of increasing upper-limb pain in long-term poliomyelitis, especially among severely paralyzed polio patients.

Lower-limb involvement

Typical osseous or soft-tissue abnormalities around the knees associated with poliomyelitis include the following:

- External rotation of the tibia
- Excessive valgus alignment
- Ligamentous laxity
- Genu recurvatum (see the image below)

With localized wasting, the quadriceps can help compensate for a weak calf. With hamstring weakness, the ability to decelerate the tibia is lost, and therefore, flexion of the knee will persist throughout the stance phase. In order to prevent this, the patient may attempt to compensate with increased quadriceps activity for a longer portion of the stance phase of gait.

In the case of a weak quadriceps and hamstrings, the occurrence of an equinus contracture or a hinged ankle-foot orthosis (AFO) with a dorsiflexion block will prevent excessive ankle dorsiflexion, as well as knee flexion during the stance phase. Lengthening of the Achilles tendon should be avoided in these patients. They may require an ischial bearing, double upright locked knee orthosis, which helps prevent the knee from buckling during gait.

Common foot and ankle deformities seen include the following:

- Pes cavovarus (hindfoot cavus) due to evertor paralysis (peroneus brevis and longus)
- Pronated everted foot due to invertor paralysis (tibialis anterior and posterior)

Foot intrinsics are typically spared in poliomyelitis. Claw toes result from relative overactivity of the long toe flexors and extensors (to compensate for weakness of the triceps).

Treatment options for poliomyelitis include the following:

- Release of joint contractures
- Reestablishment of muscle balance around the joint to prevent deformities
- Muscle transplantation to replace a paralyzed muscle
- Stabilization of a relaxed or flail joint by means of (a) tenodesis, (b) fixation of ligaments, or (c) construction of artificial check ligaments
- Arthrodesis

- Limb lengthening
- Ilizarov techniques
- Joint replacement surgery

The surgeon managing the residual weakness of poliomyelitis and postpolio syndrome (PPS) must possess an understanding of the pathologic process in poliomyelitis, as well as the variations in the pattern of the disease in different parts of the body. Poliomyelitis causes a lower motor neuron disease unlike other types of neuromuscular paralysis. The neurologic problems and the pattern of paralysis following poliomyelitis are different from upper motor neuron paralysis or, indeed, lower motor neuron paralysis caused by other diseases.