Host Defense Mechanisms – Innate Immunity

Microorganisms are abundant in the environment, and though most of them are not pathogenic, some of them are or potentially so depending on circumstances. Fortunately, the human body is well equipped to defend itself against a wide variety of potential pathogens. Despite the "hype" proliferated on television and radio, normally functioning humans, even young ones, do not require that disinfectants and antiseptics be used regularly to "protect" them from the multitude of "germs" lurking in the environment. In fact, raising children in ultra-clean environments is now recognized as having detrimental consequences, e.g., increasing the incidence of allergic reactions. Humans have been living with microorganisms for thousands of years, and we are well adapted to their presence.

Immunity – Immunity can be defined as resistance to or the ability to resist infection and disease. Although immunity can be though of as being maintained by an **immune system**, in actuality, it is dependent on a variety of structures and mechanisms involving many different types of cells, tissues and organs. Humans vary considerably with respect to their ability to resist disease, so for this section, assume the information presented applies to a normal, healthy individual with a fully functional immune system. For convenience, immune mechanisms are often divided into two categories, **innate** (non-specific) and **adaptive or acquired** (specific immunity) as described below.

Innate immunity:

Innate immunity is resistance to or ability to resist infection and disease that is **built in**, or **present at birth**. All immune mechanisms involve cells and tissues that are present when an individual is born, but adaptive or acquired immunity typically involves some type of interaction with an external agent; an **antigen**, while innate immunity does not. Innate immunity is generally **non-specific**, i.e., it provides defense against multiple different types of pathogens, while adaptive or acquired immunity ends to be quite specific. The cells, tissues and immune substances involved in innate immunity are more varied and wide spread than are those associated with adaptive or acquired immune responses (at lease superficially), and often not considered part of the immune system proper (though they are here). Some important structures and defense mechanisms associated with innate immunity are described below.

1. **Skin and Mucous Membranes** – Dry skin and mucous membranes (mucosa) cover all external surfaces of the body and form an effective barrier between our cells/tissues and the external environment. These structures provide our **first line of defense** against infectious agents or potential pathogens. **Dry skin** covers the **external surfaces** of our bodies and connects with mucous membranes in specific regions, e.g., the oral and anal openings of the gastrointestinal tract, urethral openings, vaginal openings, eyes, nostrils, etc., while **mucous membrane** cover the **internal surfaces**. Although the concept may seem foreign, the gastrointestinal system is actually a tube running through the body, and can be thought of as a long, complex donut hole. Material in the stomach or intestine (in the lumen of the tract) is technically outside the body. Skin

and mucous membranes provide both **mechanical** (**physical**) and **chemical barriers** against potential pathogens.

Mechanical aspects of dry skin include the following:
a) The skin is **multilayered**, i.e., it includes an **epidermis** composed of epithelial cells supported by an underlying **dermis**, a layer of dense connective tissue. The **epidermis** is composed of **stratified squamous epithelium**, so is itself a multilayered structure, with flattened surface cells.
b) Cells at the skin surface are highly **keratinized**, i.e., contain high levels of **keratin proteins**. These are tough, insoluble, fibrous proteins that interconnect to help make skin surfaces effective mechanical barriers.
c) Cells at the skin surface are **dead and constantly being shed**, taking microorganisms with them. Many of the bacteria commonly found on air plates are inhabitants of human skin shed regularly by their human hosts.
d) The **dermis** is composed of dense connective tissue, and forms a **tough, leather-like** barrier that is difficult to penetrate. **Collagen**, a long, fibrous protein with great tensile strength is one of the primary components. Note – Leather is typically made from animal hides (dermis layers) so accurately represents dermis structure.

Chemical aspects of dry skin include the following:
a) The skin surface is **salty**, due to the evaporation of water at the skin surface during thermoregulation and the natural salt content of sweat (perspiration). Salt in association with keratin makes the skin surface **hypertonic**, and inhospitable for many types of microorganisms.
b) The skin surface is often **acidic** (pH around 5.5) and this also tends to inhibit microbial growth, as most bacteria prefer a pH around 7. This acidity is due primarily to the keratinization of epithelial cells as they move toward the skin surface.
c) **Oils and waxes** produced by the sebaceous glands help waterproof the skin and prevent it from drying and cracking. Some of these also inhibit microbial growth.

Mechanical aspects of mucous membranes include the following:
a) Mucous membranes are **multilayered**, i.e., like dry skin they always include an **epithelial** layer supported by an underlying layer of **connective tissue**. The type of epithelium is variable.
b) Mucous membranes are often covered with a thick, sticky material called **mucus**. Within the respiratory tract, **mucus traps dust and microorganisms** entering with inspired air and prevents them from reaching the lungs. Mucus moistens and lubricates the mouth and esophagus, allowing food materials to be readily masticated and swallowed. Within the stomach, mucus provides a protective layer preventing infection and damage to epithelial cells potentially caused by the acidic environment present. Cervical mucus also helps prevent infection.
c) Within the respiratory system the epithelium is **ciliated** and the **cilia** sweep potential pathogens trapped in mucus, up and out of the airways. Since smoking causes damage to cilia, smokers are more likely to experience lung infections as bacteria are more likely to enter their lungs.

Chemical aspects of mucous membranes include the following:
a) Mucus and other secretions often associated with moist surfaces, e.g., tears and saliva, contain **lysozyme enzymes**. Lysozyme kills bacteria (especially Gram-

positive cells) by causing the **hydrolysis of peptidoglycan**, i.e., by breaking the covalent bonds linking N-acetyl muramic acid with N-acetylglucosamine. Lysozyme also acts as an **opsonin**, i.e., a substance causing **opsonization** (making particles more attractive to phagocytes). It can bind to bacteria, making them more readily engulfed by phagocytic WBCs.

b) Mucous membranes and their secretions are commonly equipped with **antibodies** (immunoglobulins) in the **isotype IgA**, capable of binding with and sometimes **immobilizing** pathogens, causing some **opsonization** or **neutralizing** toxic bacterial products (IgA does not activate compliment proteins).

c) The **pH** in specific regions lined by mucous membranes is sometimes quite low (acidic). For example, within the stomach it may 1 or 2, while within the vagina it is commonly 3.8-4.5. These acidic conditions tend to inhibit bacterial growth because most bacteria prefer a more neutral environment.

2. **Phagocytic white blood cells (leukocytes):**

Though many types of eukaryotic cells are capable of taking in materials through **endocytosis**, certain white blood cells or **leukocytes** found within the human body are particularly adept at this activity. These cells are commonly referred to as **phagocytic white blood cells** and include primarily **monocytes** and **neutrophils**.

a) **Monocytes** – Monocytes are large, agranular leukocytes (agranulocytes) forming between 3 to 8% of the WBCs circulating in the bloodstream. They are **phagocytes** produced by bone marrow stem cells called **monoblasts**. They typically stay within the bloodstream for one to three days and then pass through the vessel walls by means of a process called **diapedesis.** When they have entered the tissues, monocytes may be generically referred to as **histiocytes** or **macrophages**, but are commonly given specific different names depending on their anatomical location. For example, those entering the liver are called **Kupffer** cells and those entering lymphatic tissues are called **dendritic macrophages**.

Although monocytes typically consume or ingest cells and particles coated with **opsonizing proteins** such as **compliment factors** or **antibiodies**, they are also capable of recognizing certain pathogens by means of **pattern-recognition receptors** (toll-like receptors or TLRs) on their cell surfaces. Following phagocytosis, ingested materials are digested within **phagosomes** or **food vacules** by enzymes supplied by **lysosomes**. Monocytes observed in prepared slides of blood smears are recognized by their large size, bilobed (horseshoe or kidney-shaped) nuclei and by the presence of phagosomes within their blue-colored cytoplasm (clearly visible as white spots).

Monocytosis, the condition of having higher than normal numbers of monocytes in the peripheral bloodstream is often indicative of a disease state since monocytes tend to **increase in number during chronic infections** and stress responses.

b) **Neutrophils** – Neutrophils (also called **polymorphonuclear leukocytes**) are medium-sized granular leukocytes (granulocytes) forming around 70% of the circulating white blood cells. They are the most commonly observed WBCs in a blood smear and are easily recognized by their dark-staining, polymorphic nuclei and pale, lavender-colored cytoplasm. Since their cytoplasmic granules are not stained by commonly used reagents, they are not readily visible.

Although neutrophils act as phagocytes within the bloodstream, they also undergo **diapedesis** and enter into tissue spaces, especially during inflammation associated with bacterial infections. Within tissues, they display **positive chemotaxis**, being attracted by chemicals including **interleukin-8** (IL-8), **gamma-interferon** (interferon gamma or INF-γ) and **compliment** (C5a). Neutrophils attracted to infected tissues not only consume bacteria and damaged cells, but also secrete substances that trap and kill bacteria extracellularly. Neutrophils are short-lived cells, with a half-life of 4-10 hours in the bloodstream, and surviving only 1-2 days after entering tissue spaces.

Neutrophilia, an increase in the number of neutrophils within the bloodstream can occur in association with acute bacterial infections and acute inflammatory responses, e.g., resulting from a heart attack or other infarct.

c. **Reticuloendothelial tissues** – The reticuloendothelial tissues (also called reticular connective tissues) are sponge-like tissues composed of **reticular fibers** surrounded by **endothelial cells**. These are commonly inhabited by lymphocytes and phagocytic white blood cells (monocytes) that have left the circulation and form an important part of the immune system. Reticuloendothelial tissues include the liver, spleen, lymph nodes, thymus gland, and a variety of cell accumulations associated with the gut (Peyer's patches) and respiratory tract.

Reticuloendothelial tissues have two major functions:
1) They **filter body fluids** (blood and lymph), removing dead cells, pathogens and debris that are then readily consumed by phagocytes.
2) They **allow for rapid communication** between the phagocytes of the innate immune system and the lymphocytes of the adaptive or acquired immune system. As we shall see later, this communication often plays an essential role in the initiation of humoral immune responses.

3. **Inflammation (inflammatory response):**

Inflammation (an **inflammatory response**) is usually the immune system's first response to trauma, and is characterized by an **increase in redness** (rubor), **swelling** (tumor) and **temperature** (calor) within an area of traumatized tissue, typically accompanied by **pain** (dolor). Although inflammation is a complex process involving a variety of different cells and chemical substances, it can be summarized as follows:

a) Traumatized tissue cells (including **mast cells**) release **inflammatory substances** into the surrounding area. Among these are **histamine** and **prostaglandins**. Histamine is a powerful **vasodilatory substance**, i.e., it causes relaxation of precapillary sphinctors allowing increased blood flow into capillary beds (resulting in increased redness and heat within the area), it also increases the permeability of capillary walls. Prostaglandins are lipids with a variety of functions, but in this case responsible for vasoconstriction beyond (downstream from) the area of trauma and increased capillary permeability.
b) **Increased capillary permeability** coupled with an increase in hydrostatic pressure due to vessel dilation upstream from and vasoconstriction downstream from the traumatized area, causes fluid to move from the capillary into the tissue spaces

resulting in swelling. Increased capillary permeability also allows proteins including **antibodies, complement factors** and **fibrin** to exit the bloodstream and accumulate in the traumatized area. This also changes capillary membrane dynamics resulting in fluid movement into the tissue and additional swelling. The swelling puts pressure on nerve endings, causing pain.

c) Substances called **leukotrienes** are also released by mast cells in the area of trauma, and have a variety of functions. They cause vasoconstriction (especially in venules downstream from the trauma site), increase capillary permeability, and **attract phagocytic white blood cells** to the area.

d) Phagocytes (including **neutrophils** and **monocytes**) attracted to the area by leukotrienes pass readily through the vessel walls (due to their increased permeability) and begin to consume bacteria and dead/damaged body cells. Neutrophils also release lysozyme enzymes that kill bacteria.

e) **Antibodies** entering the area (IgG and IgM), with blood can immobilize pathogens, neutralize their toxins, cause opsonization and activate complement factors.

f) **Complement factors** (proteins) entering the area with blood and produced by monocytes cause opsonization and make holes in the cell membranes of pathogens (bacteria, fungi, protozoa, etc.) and sometimes in host cells as well. Complement factors also attract phagocytes and stimulate mast cells to release more histamine.

g) **Pyrogens** from various sources stimulate an **increase in temperature** (fever). **Exogenous pyrogens** are substances associated with pathogens, e.g., the lipopolysaccharide material from bacterial cell walls or various toxins produced by cells. **Endogenous pyrogen**, also known as **interleukin 1**, is a protein produced by monocytes (and by macrophages in reticuloendothelial tissues). It causes fever by acting on the thermoregulatory center within the hypothalamus. Raising the body temperature often inhibits microbial growth, and sometimes causes cell death.

When functioning as a protective response, inflammation increases blood flow to the traumatized area and improves access for phagocytes, antibodies and complement factors. The phagocytes then consume pathogens, kill them with lysozyme, and release endogenous pyrogen, while antibodies and complement factors interact to immobilize, neutralize, opsonize and/or blow holes in pathogens. Fibrin serves to wall off the area, preventing potential pathogens from spreading into other regions of the body, and the infection remains localized. If the initial trauma involves the entry of foreign objects, e.g., splinters of wood, rock fragments, etc., the inflammatory response may lead to an accumulation of **puss** (dead bacteria and neutrophils) around the offending object. If interleukin 1 is released in sufficient quantity, fever results, and this also kills some types of pathogens.

4. **Innate immune proteins:**

Proteins involved in defending the body against a variety of different pathogens and potentially present in the body from the time of birth are generally considered as innate immune proteins. Several different proteins fall into this category including **interferons**, **interleukins** and **complement factors**.

a) **Interferons** – Interferons were initially named for their ability to interfere with the life cycles of cytolytic viruses, but have a variety of other functions within the body. They are produced by several different types of cells including lymphocytes, monocytes, macrophages, endothelial cells and others, usually in response to some

type of infection. Type I interferons (INF-α and INF-β) have antiviral action and also act against tumor cells. Type II interferon (INF-γ) attracts phagocytes to areas of infection and increases their activity (ingestion and digestion of bacteria). Recombinant-DNA technology allowing researchers to transfer genes encoding interferons into **E. coli** cells has made it possible to mass produce these proteins. They are currently being used in the treatment of several different types of cancer. Interferon-alpha (INF-α) is also used in the treatment of hepatitis C, and interferon-beta (INF-β) is used to treat patients with multiple sclerosis (an autoimmune disease).

b) **Interleukins** – Interleukins are cytokines released by lymphocytes, macrophages and a variety of other cells. They occur as multiple different types (IL-1 through IL-23 so far), and have a variety of functions. **Interleukin 1** (IL-1), sometimes called endogenous pyrogen acts on the hypothalamus to elevate body temperature (induce fever) among other actions. Other interleukins, IL-2, 4, and 5 stimulate the growth and proliferation of immune cells including B and T-lymphocytes, attract neutrophils (IL-8), or induce the production and release of other cytokines and/or interferons.

c) **Complement factors** – Complement factors are **serum proteins** produced constitutively by macrophages and released into the circulation as inactive proteins. When activated, they become **proteases** that break peptide bonds within other complement proteins, thus activating them, i.e., they act on one another in a sequence. Since each active complement factor can act on multiple others, the initiation of complement activity typically results in a massive reaction called a **complement cascade**.

Complement activity is commonly initiated by the binding of antibodies (IgG or IgM) with antigens, but can also be initiated by certain molecules (such as lipopolysaccharides) on the surfaces of bacteria and indirectly by cytokines. In the classical pathway, C3 is cleaved to form C3a and C3b, C3a then causes **opsonization**, i.e., binds to cells or other particles making them more attractive to phagocytes, while C3b interacts with other complement factors to bring about the formation of **membrane attack complexes** (C7,8, and multiple C9 units that insert into cellular membranes forming holes). These cause cell lysis and death. Although human cells are generally protected from the potentially destructive activity of C7,8 and 9, this is not always the case, and these factors sometimes cause considerable damage within the human body.

The Role of Normal Flora in Host resistance:

Microorganisms commonly referred to as **normal flora** include a variety of different bacteria, fungi, protozoa and other organisms living on and within the human body. They are not all present at birth, but gain access to the body shortly thereafter (many passed from mother to infant) and colonize the various regions or habitats available. Although we typically view ourselves as autonomous beings, we are actually walking ecosystems supporting a plethora of prokaryotic cells (there are more of them than there are eukaryotic cells within all our various tissues, organs and systems). Fortunately, most of the bacteria living on our skin and within our gastrointestinal, urinary, reproductive and

respiratory tracts are not pathogenic. Instead, these organisms help to defend us against other organisms that are. Organisms living as normal flora associated with skin and mucous membranes protect our cells and tissues in a variety of ways as described below.

1) They provide protection by competing against potential pathogens for available nutrients. When non-pathogenic bacteria are abundant, they use up most of the nutrients and limit the growth of pathogens.

2) They provide protection by competing against potential pathogens for binding sites on cell surfaces. Most pathogens must bind with cellular surfaces in order to infect or cause damage. If the binding sites available on cellular surfaces are occupied by normal flora, pathogens can't bind.

3) They provide protection by producing chemical substances called **bacteriocins** that kill other closely related cells. Humans typically have *Escherichia coli* cells living within their guts, but do not normally carry highly pathogenic strains. The *E. coli* comprising the normal flora of most individuals form toxins called **colicins** that kill other, more pathogenic strains such as the *E. coli* O157:H7 responsible for causing hemolytic uremic syndrome.

Be nice to your normal flora, they are helping you survive, and most of us need all the help we can get.