

Host Defense Mechanisms – Adaptive or Acquired Immunity

While innate immune mechanisms allow the body to defend itself against a wide variety of foreign agents more or less equally, i.e., is non-specific, the **adaptive or acquired immune system** allows the body to mount an effective defense against **specific pathogens** individually, i.e., **it is specific**. This type of immunity is **acquired** in that it requires some type of interaction with a specific pathogen to be activated, and this can occur at various times within the life of the individual. It is **adaptive** in that the body modifies the system in a unique way to meet the demands presented by the environment. If the same pathogen enters the body a second time, the system is better able to deal with it. The cells and immune substances involved in adaptive or acquired immunity are present within the body at birth, but their ability to mount an effective immune response against specific pathogens is acquired.

Given this, it is important to consider the various ways specific immunity can be acquired. Although it involves interaction with specific pathogens, this interaction is not necessarily restricted to those occurring within a single individual. In some cases (**active immunity**) the body is stimulated to produce its own immune cells and substances, while in other cases (**passive immunity**) it is not. Some types of acquisition (**natural immunity**) involve natural mechanisms available to humans and other organisms because of the ways their populations have evolved over time, while in other cases (**artificial immunity**) resistance to disease is administered artificially and is dependent upon the technology available to the individual. Adaptive or acquired immunity can be acquired in the following ways.

1. The individual can encounter a virulent pathogen, **become infected** and **experience disease symptoms**. If the individual is fortunate enough to survive and recover from this illness, she/he can become immune to the pathogen responsible. The immunity is acquired naturally and involves activation of immune cells and production of immune substances, i.e., is **natural and active**.
2. The individual can **receive immune substances** (antibodies and cytokines) through the **placenta** prior to birth, and through **breast milk** after being born. Under normal circumstances this type of immunity is available to all young mammals, and provides them with protection during the first few months of their lives. In this case, immunity is acquired indirectly because the individual receiving the immunity is dependent on interactions between potential pathogens and his/her mother's immune system. This type of immunity occurs naturally, but does not require the individual to produce immune cells or substances, so is passive, i.e., is **natural and passive**.
3. The individual can **receive a vaccination** and can then produce immune cells and substances in large quantities as a defense against the pathogen or microbial product introduced. In this case immunity is artificially induced, but requires the body to actively defend itself, i.e., is **artificial and active**.
4. The individual can **receive immune substances**, e.g., immune serum (antiserum), or cytokines produced by some other individual or by cells maintained in vitro. Again immunity is indirectly acquired because the immune substances are being produced by some other individual (or by their cells maintained in vitro). This type of immunity is dependent on the technology available to the individual, and is passive, i.e., is **artificial and passive**.

Adaptive or acquired immunity involves complex mechanisms still under investigation, but certain features of the system appear consistent, and will be explained here. For simplicity, the system involved in specific immunity will be referred to as the **adaptive immune system**, and the immunity acquired will be termed **adaptive immunity**, from this point on.

The primary cells involved in adaptive immunity are agranular leukocytes (white blood cells) called **lymphocytes**. These arise from haematopoietic stem cells found in the **red bone marrow** (bone marrow stem cells). All lymphocytes can be formed from the same multipotent stem cells, but not all lymphocytes are alike. They are typically divided into three categories, **B-lymphocytes** (B-cells), **T-lymphocytes** (T-cells), and **null-lymphocytes** (null-cells), on the basis of specific chemical markers found on their membrane surfaces. Null-cells are neither T-cells, nor B-cells, but they are not all alike. The best known of these cells are a group called **natural kill** or NK-cells, that kill other types of cells. Natural killer cells contain granules in their cytoplasm rich in proteins including **perforin** and proteases called **granzymes**. When perforin is released in the vicinity of infected cells, it forms holes (pores) in cell membranes allowing granzymes to enter. The granzymes then degrade proteins within the target cell. Cells killed by NK-cells experience **apoptosis**, a phenomenon unlike the lysis caused by complement proteins in that during apoptosis, all proteins within the target cell are degraded. If infected cells containing fully formed virions are killed by NK-cells, the virions are destroyed, but if the same cells are killed by complement factors, the virions are released into the environment and can infect other cells. Some references consider NK-cells part of the innate immune system because their activation is typically stimulated by cytokines released from macrophages or by interferons. However, since the ability of NK-cells to recognize specific infected cells probably involves MHC-proteins (and because they were not included among the innate immune mechanisms described earlier), they will remain here. Some references consider NK-cells to be T-cells.

B-cells were first identified in birds during the 1960s, and were found to develop within a specific organ called the bursa of Fabricius. The "B" in B-cell meant **bursa-derived**. Human B-cells were given the same name in anticipation of finding a bursa equivalent in humans, but no such organ was ever found. Instead, researchers discovered that human B-cells undergo development and differentiation with the bone marrow. Conveniently, the "B" in B-cell is now explained as referring to bone marrow, i.e., B-cells are **bone marrow-derived**. B-cells go through several stages of development during which their genetic content is modified at specific loci associated with antibody production. Multiple different sub-populations are formed, each with a different combination of nucleotide sequences in these regions. The process of genetic recombination is similar to post-transcriptional modification, but involves DNA rather than RNA. Different types of B-cells are formed on a more or less continuous basis, and released into the circulation. Each one carries membrane-bound receptors capable of binding with a specific type of antigen (these receptors are actually immunoglobulins or antibodies and are described in greater detail later). Some references refer to these B-cells as **immunocompetent B-cells**. When immunocompetent B-cells encounter specific antigens, they can bind with them; then, under the influence of helper-T cells, the B-cells can become either **plasma cells** or **B-memory cells**. Plasma cells are basically antibody factories, producing and releasing antibodies in large quantities. B-memory cells are involved in anamnestic responses

(described later). Other options available to B-cells exist, but are beyond the scope of this presentation.

T-cells are **thymus-derived** lymphocytes, and after exiting the bone marrow must spend some time within the thymus gland for additional development. The **thymus gland** is located in the upper chest or lower throat region, and is typically larger in young individuals; it must not be confused with the thyroid gland. Hormones within the thymus gland including **thymosin** and **thymopoietin** participate in T-cell development, which is quite complex. Immature T-cells entering the thymus reproduce there, and pass through several stages of development involving genetic recombination similar to that experienced by B-cells. They are also subjected to rigorous selection processes during which most (98%) are eliminated through apoptosis. Mature T-cells exiting the thymus are called **immunocompetent T-cells** and occur as a variety of sub-populations described later.

Adaptive immunity can be divided into two categories based on the types of cells and immune substances involved, although considerable overlap occurs. **B-cells** are involved in **humoral immunity**, while **T-cells** are involved in **cellular immunity**. Although these two categories are not entirely separate from one another, they are often presented as separate types of immune responses as described below.

- 1) **Humoral immunity** – **Humoral immunity**, also called **antibody-mediated immunity** involves B-lymphocytes (plasma cells and/or memory cells) and **antibodies** produced by these cells.
- 2) **Cellular immunity** – **Cellular immunity**, also called **cell-mediated immunity** involves T-cells of various types and the substances they produce, generally referred to as **cytokines**.

Humoral Immunity:

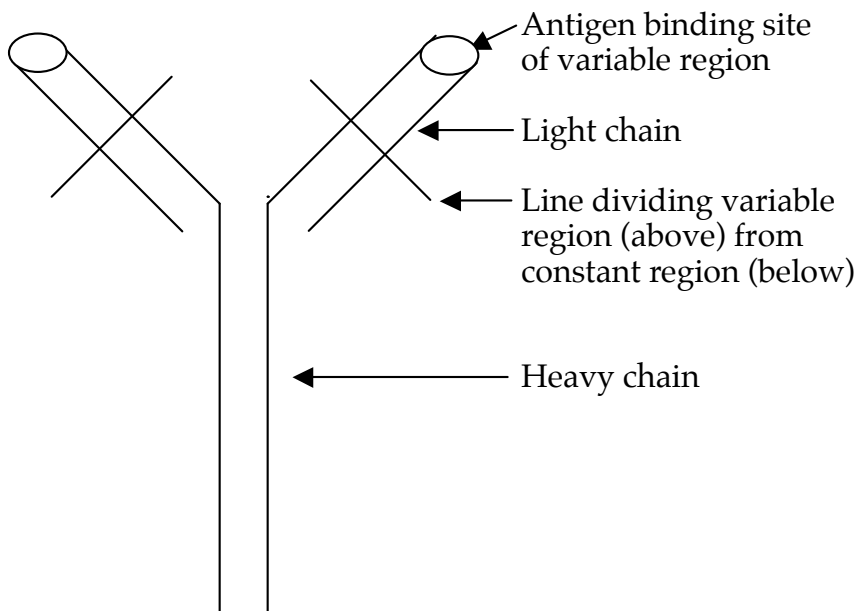
In order to understand how adaptive immune responses are initiated, it is essential to gain a basic understanding of the components involved. In the case of humoral immunity these include antibodies and antigens. **Please note** – Antibodies are **not** antibiotics, and although the two terms look similar and are often (unfortunately) abbreviated in the same manner (Ab), they have entirely different functions. The terms are **not** synonymous.

1. **Antibodies** – **Antibodies**, also called **immunoglobulins** are globular proteins (glycoproteins) with quaternary structure. They are produced primarily by **B-lymphocytes** and occur on the surfaces of these cells bound to their cell membranes. They are also produced and released by **plasma cells**, so occur within body fluids, e.g., blood, lymph, tears, saliva, breast milk, etc. as free proteins (some are bound to mucous membranes). The human body typically produces large quantities of specific antibodies when challenged by a specific type of **foreign agent** or **antigen**, e.g., bacteria, protozoa, fungi, microbial toxins, etc. These antibodies are antigen-specific and able to bind with specific antigens through hydrogen bonding.

Antibodies can be divided into five categories or classes called **isotypes** (iso = same) on the basis of their structure and physiological properties. **Please note** – isotypes are **not** isotopes, and have no relationship to them. The five antibody **isotypes** are **IgA**, **IgM**, **IgG**, **IgD** and **IgE** (**Ig stands for immunoglobulin**) are labeled according to the first five letters in the Greek alphabet (α , β , γ , δ , and ϵ) except that β was replaced by M for mega because IgM antibodies are very large. Antibodies are assigned to specific

isotypes on the basis of the amino acid sequences present within the constant domains of their heavy chains (see diagram below). In humans, IgG and IgA are the most abundantly produced antibodies, and occur as two different sub-types. Most **IgA antibodies bind to mucous membranes** and occur in body fluids associated with these, e.g., saliva, nasal secretions, tears, and mucous associated with the gut. IgA also occurs in breast milk. **IgG and IgM** are the most abundant antibodies within the general circulation (blood and lymph), and **provide our primary defense against invading pathogens**. **IgE** antibodies are involved in **type 1 hypersensitivity reactions** (described later), but the role of free IgD has not yet been determined.

The diagram below is a simplified representation of immunoglobulin gamma (IgG).



The "Y" shape often attributed to antibodies is based on the shape of IgG, as shown, but not all antibodies have this shape. IgA antibodies have four light chains rather than two and have four antigen-binding sites. IgM antibodies have five heavy chains and ten light chains forming ten antigen-binding sites. Though the light and heavy chains are shown here as straight lines, the proteins

involved are actually highly folded and have a much more globular form. All antibodies categorized within the isotype IgG have the same amino acid sequences within the constant regions of their light and heavy chains.

2. **Antigens – Antigens** (antibody generators) are foreign agents (usually) that enter the body and stimulate the production of antibodies in large quantity. Antigens are often cellular pathogens such as bacteria, fungi, protozoa or multicellular parasites, but they can also be viruses or large molecules. Cellular antigens have multiple chemically defined sites on their surfaces called **antigenic determinant groups** or **epitopes**. It is these sites antibodies bind with. Although the term antigen and epitope are sometimes used interchangeably, epitopes are much smaller and more specific. Many epitopes are peptides (short amino acid chains), but some are glycolipids or other groups. In order to be an effective antigen, a foreign agent must have at least two epitopes, but if more are present, the immune response generated tends to be greater. The term **hapten** applies to a small molecule capable of binding specifically with an antibody, but not, by itself, capable of stimulating antibody production. These are sometimes referred to as **incomplete antigens**.

Antigens associated with microorganisms entering the body through various means are called **exogenous antigens**. Human cells also have chemically defined groups associated with their surfaces, and many of these can act as antigens if cells from one individual are transferred to another. These would also be exogenous antigens. Tumor cells and infected body cells can be recognized as antigens if their surfaces carry unique chemical groups recognized as foreign. These antigens, generated within the body, are called **endogenous antigens**. Although antigens are named for their ability to stimulate antibody production, the epitopes associated with antigens can also bind with T-cell receptors and stimulate T-cell activity. As we shall see, this is often essential to the initiation of humoral immune responses.

Initiation of a Humoral Immune Response:

Humoral immune responses are described as involving B-lymphocytes and antibodies; however, they are often dependent on other types of cells as well. In fact, humoral immunity is often dependent on both innate immune structures/cells and cells associated with cellular immunity. Although separating the immune system into component parts is useful for descriptive purposes, effective immune function is dependent on all components of the system working together. This can be demonstrated by describing the initiation of a **T-cell dependent** humoral immune response as outlined below.

1. A pathogen such as a virus capable of causing measles (Rubeola) enters the body. The virus has managed to pass barriers present within the respiratory system (first line of defense), and has gained access to the circulation where it is infecting lymphocytes.
2. Virus particles and infected cells are recognized by **macrophages** residing within reticuloendothelial tissues, and are consumed. Within the macrophages, viral components are taken apart (digested) within lysosomes.
3. Viral epitopes are presented on the surfaces of macrophages (antigen-presenting cells) in combination with **major histocompatibility complex** (MHC) proteins. MHC proteins are also called human leukocyte antigens, and are proteins common to eukaryotic cells.
4. **Helper-T lymphocytes** recognize the measles epitopes presented on the surfaces of macrophages in combination with MHC proteins and are **activated** or **primed**.
5. **B-lymphocytes** carrying antibodies bound to measles virus epitopes also present these to helper-T cells, and the T-cells are stimulated to release cytokines called **interleukins**.
6. Interleukins cause the B-cells to reproduce (proliferate) forming **clones** of antibody-secreting **plasma cells**, and **B-memory cells**.
7. Antibody titer increases within the body and the offending pathogen is eliminated.
8. If the same pathogen enters the body a second or subsequent time, the B-memory cells can launch an **anamnestic response**.

In this example, the initiation of a humoral immune response involved the interaction of macrophages, T-lymphocytes and B-lymphocytes, and took place within reticuloendothelial tissues, i.e., involved components associated with innate, cellular and humoral immunity. Note – Although the antigen used in this example was the virus responsible for causing measles, this is actually a poor example because elimination of measles-causing viruses is primarily accomplished by T-cells.

Anamnestic response:

The term **anamnesis** (an = without, amnesia = memory loss) means to recall or to remember, and when applied to the immune system (**anamnestic response**) involves a **rapid increase in antibody titer** following a second or subsequent exposure to the same antigen. The body "remembers" interacting with specific antigens by maintaining populations of **B-memory cells**. These cells (sometimes acting in combination with T-memory cells) can bring about the production of antibodies much faster, and maintain high antibody titers within the body longer, than do initial exposures to antigens. The "memory" function of the immune system is unique to adaptive immunity, and not available with innate mechanisms alone.

Serological reactions:

Serology is the study of antibody-antigen interactions in vitro and is often used in association with diagnostic immunology. The term initially applied to the study of **serum**, i.e., blood plasma with the clotting factors removed, and dealt primarily with the detection of antibodies. Modern serological reactions can be used to detect and assay either antibody or antigen. Serological reactions also indicate how antibodies interact with antigens inside the body (in vivo). Some examples of serological reactions are listed below.

1. **Precipitation** – During precipitation reactions, antibodies bind with soluble antigens and cause them to become insoluble, i.e. cause them to precipitate, or fall out of solution. When precipitation reactions occur within glass tubes or in agarose preparations, the resulting precipitate is visible to the naked eye, and is often useful in detecting the presence of antibodies within serum samples (e.g., Ouchterlony test).
2. **Agglutination** – During agglutination reactions, antibodies bind with cellular antigens and cause them to clump or agglutinate. This type of reaction is also visible to the naked eye, and has application in the identification of microorganisms (serological typing) and in blood typing (hemagglutination).
3. **Neutralization** – During neutralization reactions, antibodies bind with toxic antigens and neutralize them, or render them non-toxic. Neutralization is essential to our defense against bacterial toxins such as tetanus toxin (tetanospasmin).
4. **Immobilization** – During immobilization reactions, antibodies bind to the surfaces of antigens covering receptor sites essential for host cell attachment. If the pathogens can't bind with host cells, they cannot infect.
5. **Opsonization** – During opsonization, antibodies bind to the surfaces of antigens and make them more attractive to phagocytes. This occurs because phagocytes have receptors sensitive to the Fc-fragments of antibodies (the ends opposite the antigen binding sites in IgG) and readily bind with these.
6. **Initiation of the complement cascade** – Complement factors activated by antibody-antigen binding interact with other complement factors causing a cascade of events resulting in opsonization and the formation of holes in cell membranes.

Although antibodies sometimes control pathogens alone (through immobilization) and can detoxify microbial toxins without assistance, humoral immunity is again dependent on innate immune components (phagocytes and complement factors).

Immune Tolerance:

Immune tolerance refers to a **state of unresponsiveness** to a specific antigen, or an individual's ability to not mount an immune response against specific antigens. This is important because humans are exposed to numerous foreign agents associated with air inspired, food eaten, and materials contacting skin surfaces, and it is essential they not mount immune reactions against all of these. It is also essential that human immune systems not mount immune reactions against molecular groups normally present within their own bodies, i.e., against "self" molecules. Individuals that do, suffer from **autoimmune disorders**, some of which cause severe disease symptoms, e.g., those associated with multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus.

Immune tolerance involves a process called **clonal selection**, during which cells capable of responding to and initiating immune reactions against self are inactivated or eliminated from the immune system through apoptosis. Both B-cells and T-cells are subject to clonal selection, which occurs primarily during embryonic development. Evidence indicates that common exogenous antigens encountered early in the life of an individual are also tolerated because immune cells that would have triggered immune responses against them are eliminated. This may be why children raised in extremely clean environments (and not allowed to play in dirt) are more prone to allergies than are their grubbier counterparts.

Cellular Immunity:

Cellular immunity – Cellular immunity or **cell-mediated immunity** is that portion of the adaptive immune system involving **T-lymphocytes** and **cytokines**, but it also involves **macrophages** and **natural killer (NK) cells**. T-lymphocytes behave somewhat differently than do B-lymphocytes in that they **do not form antibodies**, and can only respond to antigens **in combination with MHC proteins** (with one exception not included here). This means T-cells cannot respond to bacteria or viruses free in the environment, i.e., not bound to host cells, because MHC proteins are only found on **eukaryotic cells**. T-cells can respond to all types of **eukaryotic pathogens** (fungi, protozoa, multicellular parasites, etc.), **infected body cells**, **tumor cells** and **transplanted cells/tissues/organs**. There are multiple sub-populations of T-cells released from the thymus gland, and these are often divided into categories as indicated below.

1. **Helper -T cells** – Helper-T cells or T-helper cells (T_h cells) are **T4-lymphocytes**, i.e., cells expressing a membrane marker called **CD4**. Although cells with this marker were at one time divided into two categories designated as helper and inducer, T4-cells typically have both helper and inducer function. Helper-T cells cannot kill other cells, and are not phagocytic, but they do release high molecular weight, chemical substances called **cytokines**, and these can significantly influence the activity of other cells. As described earlier, Helper-T cells release cytokines called **interleukins** (several types) that stimulate the proliferation (reproduction) of other cells, as well as their differentiation and development. Certain types of Helper-T cells also release a cytokine called **gamma-interferon** or **interferon-gamma** ($INF-\gamma$) that activates phagocytic cells (macrophages). Some T-helper cells produce a cytokine called **tumor necrosis factor- β** ($TNF-\beta$), also called **lymphotoxin**, that causes tumors to die by disrupting blood flow to them. $TNF-\beta$ also influences the migration of macrophages, and is sometimes called phagocyte attracting factor.

As described earlier, Helper-T cells often play an essential role in the initiation of humoral immune responses. They also influence the reproduction, differentiation and activation of other T-cells. Since these cells are among the primary targets of the human immunodeficiency virus (HIV), infection with this virus eventually leads to immune system failure and a collection of symptoms known as **acquired immune deficiency syndrome** (AIDS). The body cannot effectively defend itself against pathogens, and is subject to multiple opportunistic infections and tumors. Despite continued research, there remains no cure for HIV infection, and currently no vaccine effective in preventing it.

2. **Cytotoxic-T cells – Cytotoxic-T lymphocytes** (also known as **Killer-T cells**) serve as the primary defensive portion of the cellular immune system. They carry membrane markers (glycoproteins) called **CD8 markers**, so are sometimes called T8-lymphocytes. Killer-T cells release cytokines called **cytotoxic factors** including **perforin** and **granzymes**, and these effectively kill other types of cells. They also destroy virus particles within infected cells.

Killer-T cells kill eukaryotic pathogens, infected body cells and tumor cells, but they do not attack and kill antigen-presenting macrophages. Like other T-lymphocytes, the killer-T cells recognize and respond to antigens in combination with MHC proteins, but not all MHC proteins are the same. Killer-T cells (and other cells carrying CD8 markers) recognize MHC class I markers, but macrophages carry MHC class II markers. It is significant that other types of blood cells carry MHC class II membrane markers, because if they didn't blood transfusions would not be possible.

Though essential to normal immune function, killer-T cells can cause problems. They are involved in delayed hypersensitivity reactions and have been strongly implicated in the destruction of transplanted tissues and organs.

3. **Suppressor-T cells – Suppressor-T lymphocytes** also called **Regulatory-T cells**, carry CD8 membrane markers, so like killer cells are T8-lymphocytes. Their role is to turn off or depress immune system activity toward the end of an immune response and prevent damage to host tissues.

Since some references state that suppressor-T cells carry CD4 markers, perhaps both T4 and T8 cells have suppressor function.

4. **Natural Killer cells** – Natural killer cells (NK cells) are generally considered null lymphocytes, but some references group them with T-cells. Their function is similar to killer-T cells, i.e., they release perforin and granzyme killing other cells, but they have no memory function. Natural killer cells were described in greater detail earlier, and might best be considered as part of the innate immune system.

T-lymphocytes, both T4 and T8 types can give rise to memory cells that persist within the body for long periods of time. These cells allow the body to launch a swift and powerful immune response against pathogens entering the body for a second or subsequent time.