Basic Concepts in Applied Immunology

Basic Concepts in Applied Immunology

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Contents

Welcome to Basic Concepts in Applied Immunology. This resource aims to provide an overview of the principles of immunology and their applications in a simplified format suitable for allied health professionals.

This resource consists of seven chapters and one appendix:

Acknowledgements

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Adaptions

Several Open Education resources were adapted and included in this resource. Where indicated, these resources are in their original format or an abbreviated version. The following resources are used and noted at the appropriate part of the text in the adaption statement at the bottom of the page.

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Chapter 1: Overview of the Human Immune System

Learning Objectives

By the end of this chapter you will be able to:

- Define Immunology
- Describe the functions of the immune system
- Describe the features of the immune system, including:
  - The innate and adaptive responses
  - The three lines of immunological defense
- Explain the role of the lymphatic tissues in both returning fluid to the circulatory system and in monitoring for infection
- Define hematopoiesis and name the two major lineages of immune cells
- Explain how the immune system exists in a balance between immune response and immune tolerance

- Describe how an imbalance in immune homeostasis lead to immunological disorders

Case Study

JD is 45 years old and is considered a heavy smoker, having consumed an average 30 cigarettes per day for 20 years. JD was diagnosed with chronic obstructive pulmonary disease (COPD) and presents with respiratory symptoms, reduced exercise tolerance, and frequent respiratory infections. Frequent infection resulted in chronic bronchitis, a condition where the airway epithelial tissue has eroded and become inflamed. Sputum analysis of coughed up phlegm identified large numbers of immune cells called neutrophils within the airway space. A blood test revealed elevated antibody levels but also identified antibodies that specifically attach to collagen and elastin, proteins on the surface of human airway epithelial cells.

1. *What examples of immune defense impairment are described in this case study?*
2. *Why does the respiratory airway produce **mucus** when mucus plugs could obstruct the airway and impair normal breathing?*
3. *Why is it concerning that JD is producing antibodies against human tissues?*

Answers to these questions are at the end of the chapter.

1.1 The Immune System and its Functions

We exist in a world of microorganisms that are invisible to the human eye but impact our health in remarkable ways. Most microorganisms are harmless to a healthy person. However some, classified as **pathogens**, have evolved alongside us for million of years to adapt ways of overcoming our defenses, evading our immune system and causing debilitating or even life threatening infection.

> ***What is Immunology?***
>
> **Immunology** is the study of immune barriers and responses that form the immune system to protect us from infection by microorganisms. Immunology also involves the study of dysfunction that may occur in the immune response as well as medical methodologies that aim to use or modulate components of our immune system to promote health.

Since pathogens are diverse and have independently developed many ways to infect the human body, the immune system has adapted a layered approach that uses a series of strategies to prevent infection.

> ***Some of these strategies include:***
>
> **Surface Barriers:** These barriers may include physical barriers, like the skin and secreted **mucus**, or chemical barriers, like sebum (oil), enzymes, salty or acidic body

fluids and antimicrobial substances. Even the harmless microbes that inhabit our body surface and form the microbiome create a protective barrier from infection.

Immune Sensing and Communication: Most immune cells as well as epithelial cells, which line the surface of the body, have special receptor proteins on the cell surface that allow them to recognize characteristic patterns associated with infection. Some patterns, called **pathogen associated molecular patterns (PAMPs)** include molecules that are typically found on the surface of pathogens but are not normally in the human body. Other patterns, called damage associated molecular patterns (DAMPs) indicate cell damage that might be resulting from infection or may place a person at greater risk for infection. On sensing **PAMPs** and/or DAMPs, immune cells communicate the risk of infection to other cells by secreting cytokines.

Fever Grading

- Normal temperature: 36.5-37.5
- Mild low-grade fever: 37.7-38.3
- Moderate fever: 38.8-39.4
- High

Inflammation and Fever: Large numbers of immune cells are found in the bloodstream. When a pathogen is found in the body tissues, the affected cells secrete **cytokines** to temporarily remodel the local blood vessel through inflammation. Specifically, during inflammation, blood vessels swell and become leaky, allowing more immune cells to flow into the affected tissue and combat the infection. Cytokines released into the bloodstream can spread the inflammation to other parts of the body. Some of these cytokines will also migrate to the brain and increase the body

temperature to induce a fever. Higher body temperatures will slow down the growth of bacteria and viruses as the environment become unfavourable for growth.

fever: >40

Phagocytosis: Many immune cells will sense **PAMPs** or DAMPs and, upon detecting these foreign patterns, consume the target through the process of phagocytosis. During phagocytosis, the surface of the immune cell wraps around the foreign material to create a bubble inside the cell, called a phagosome. Within the phagosome, the foreign cell or substance is degraded with the aid of acid and digestive enzymes.

Adaptive Response: The adaptive immune response is highly specific to the offending pathogen. Typical responses may induce cells that directly engage and eliminate pathogens and infected cells as well as indirect elimination using antibodies. Adaptive responses typically require a period of time to initiate, following exposure to the pathogen or a vaccine antigen. However, after the initial adaptive response, immunological memory may be established to ensure a rapid and effective response on each subsequent future exposure.

1.2 Features of the Immune System

The immune response is most easily imagined at the level of a single tissue, but in reality the immune response spans the entire body and consists of integrated and cooperating tissues and organs. Central components of the immune system include the **lymphatic system**, which drains fluid form tissues and monitors this fluid for infection, and the **hematopoietic cells** that produce immune cells and regulates the balance between **immune response** and **immune tolerance**.

Lymphatic System

The human heart pumps blood through the body under high pressure. This causes blood fluid to leak into tissues, where it is then described as *interstitial fluid*. While most of the interstitial fluid is reabsorbed into the bloodstream, approximately 3L of fluid accumulates in the tissues and is absorbed by lymphatic vessels. The fluid is then filtered within lymph nodes and other lymphastic tissues before returning to the bloodstream.

The lymphatic tissues are a major site for the production and storage of immune cells, as well as monitoring for evidence of infection in the interstitial fluid.

Hematopoietic Cells

After birth, most of our immune cells are produced through the process of hematopoiesis that primarily occurs in the red bone marrow. In children, this process occurs in long bones. Hematopoiesis is mostly restricted to the cranial and pelvic bones in adults.

Hematopoiesis starts with a hematopoietic stem cell, which is called a pluripotent cell because it can produce red blood cells (*erythrocytes*), platelets (*thrombocytes*) and white blood cells (*leukocytes*). Leukocytes are the primary immune cells and they become specialized to form two specific stem cell populations, *myeloid stem cells* and *lymphoid stem cells*.

> **Myeloid Cells:** Stem cells in these lineages produce red blood cells and platelets, as well as white blood cells that perform phagocytosis and mediate inflammation.
>
> **Lymphoid Cells:** Stem cells in these lineages form the lymphocytes (T-cells and B-cells) and natural killer (NK)

> cells, which are largely associated with the development of immunity.

More details on hematopoiesis are available in Chapter 2.

1.3 Classification of immune defenses

Innate and adaptive immune systems

The immune system is often divided into two branches: (1) The **innate** immune system and (2) the **adaptive** immune system. The innate immune system is not specific to any particular pathogen and is largely developed at birth. The adaptive immune system involves responses that are develop *after* exposure to a pathogen and generate a stronger and more tailored response upon future exposures. This enhanced subsequent response involves a phenomenon called **immunological memory** and is responsible of the state that we usually refer to as "**immunity**" following infection or vaccine immunization.

Table 1.1 – Features of Innate and Adaptive Immune systems

Innate Immunity	Adaptive Immunity
Includes body's barriers, such as skin, **mucus**, sebum oil ("*First line of defense*").	Involves cellular and antibody mediated immune responses ("*Third line of defense*")
Includes rapid induced chemical and cellular responses ("*Second line of defense*").	Initially triggered by the innate immune response. The adaptive response may be delayed by days/weeks.
Immune response is largely the same for every pathogen encountered (*non-specific*).	Immune response is specifically enhanced toward a particular pathogen (*specific*) after initial exposure to that pathogen (*secondary response*).
Immune response is the same each time a pathogen is encountered (*no immunological memory*).	After first exposure to pathogen, the immune response is stronger against subsequent exposures (*immunological memory*).

The three lines of defense

The immune system is also frequently described as having **three lines of defense**, where some immunological defenses are **ubiquitous**, meaning they are always present, while others are **induced** only when the immune response is triggered by infection or another source.

> The **first line of defense** involves surface barriers, listed above. Skin, sebum, **mucus** and other barriers are formed before infection and are **ubiquitously** present. However, these barriers may be fortified during infection. For example, a respiratory infection may result in enhanced **mucus** secretion that is coughed out as phlegm.
>
> The **second line of defense** involves cells and chemicals that are **ubiquitously** present but may also be rapidly **induced** during infection. The ubiquitous components

make up the primary defense during the first four hours of infection. The induced response becomes more prominent after the first few hours. An example of this transition is seen in immune cells. Tissues often have *resident* immune cells that are already present and patrol for infectious agents. However, following infection these resident cells will become exhausted and a large number of new immune cells will be recruited from the bloodstream.

The **third line of defense** coincides with the adaptive immune response. If an infection becomes serious enough that it cannot be overcome by the innate defense mechanisms, specialized immune cells (e.g. monocytes, macrophages, dendritic cells) will interact with T-cells to trigger a stronger and more tailored response against the pathogen. The third line of defense involves enhanced stimulation of immune cells and production of antibodies or both. Helper T-cells secrete cytokines to induce and coordinate the adaptive immune response. B-cells secrete antibodies that mark a foreign cell or agent for immune destruction. Cytotoxic T-cells perform surveillance of all cells and eliminate cells that are infected or become tumor cells that might cause cancer.

Table 1.2 – Three Lines of Immune Defense

Line of Defense	Pathogen-Specific?	Innate/ Acquired	Mechanism and Outcomes
First	No	Innate	Surface barriers provide ubiquitous defense. – Skin or scabs on wounds – **mucus** or wax – Sebum oil
Second	No	Innate	Ubiquitous and induced defenses that include phagocytes, inflammation, fever, complement activation – Secreted chemicals like defensins that damage bacterial cell membrane – Phagocytes and innate immune cells – Cytokines that induce fever and inflammation – Complement proteins
Third	Yes	Adaptive	Induced response that involves T-lymphocytes, B-lymphocytes and antibodies. Produces stronger and pathogen-specific response as well as immunological memory to establish future immunity.

Immune Response and Immune Tolerance

The immune system needs to balance two opposing challenges, which are associated with inducing a rapid and effective response to infection while simultaneously minimize the immune response to human tissues and the harmless microbes of our microbiome.

A rapid immune response can be achieved through pre-formed immune components in their inactive forms. Examples

of pre-formed components include leukocytes stored within lymph nodes and immune proteins that circulate in an inactive form. Infected cells and immune cells also release chemicals called *cytokines* that can rapidly accumulate in the tissues or bloodstream. Cytokines are potent inducers of the immune response.

In direct opposition to the immune response is the process of immune tolerance or immune homeostasis. Immune tolerance involves the suppression of the immune response. Immune tolerance is critical in suppressing immune cells following the resolution of an infection, preventing immune responses against human cells and tissues, and suppressing immune responses against harmless **commensal** microbes of the human microbiome. Immune tolerance is achieved by directly killing lymphocytes that react to human tissues during their development. In addition, specialized cells called *regulatory* T-cells detect and suppress the local immune responses to commensal microbes and ones own cells and tissue. Immune tolerance involves *immune checkpoints* where immune cells are inactivated through physical binding and the secretion of immunosuppressive cytokines, such as interleukin 10 (IL-10).

Disorders of the Immune System

The immune system is complex and multifaceted. Any disruption to the immune barriers and immune responses resulting in **immunodeficiency** increases the risk for infection. For example, broken skin resulting from trauma or a burn injury can contribute to infection. Malnutrition that affects immune cell production can impair a person's ability to mount a productive immune response. An individuals immune response fluctuates based on various factors including genetics, age, chronic stress and the environment.

An overactive immune response (**hypersensitivity**) can also

contribute to immune disorders. Immune response to harmless substances in our environment results in allergies, while immune responses to harmless microbes on the body surface create inflammatory conditions. In some cases, human tissues may be inadvertently or even specifically targeted by the immune system, resulting in an autoimmune condition. Finally, an inability to control the intensity of the immune response during an infection or to resolve the immune response after infection can contribute to persistent and potentially lethal impairment of health.

Summary

- The immune system functions to defend against pathogens by
    - Forming barriers to infection along the body surface
    - Sensing an infection and communicating between cells and tissues to develop a response
    - Inducing inflammation and fever as responses to infection in order to recruit cells to the site of infection and impair the growth and dispersal of microbes
    - Engaging cells that consume foreign particles and microbes by phagocytosis
    - Inducing an adaptive immune response that produces a pathogen-specific response as well as a long-term immunological memory
- The immune system is body-wide and the lymphatic system is an important component of the immune system
    - The lymphatic system drains fluid from the tissues

    - Lymph nodes and lymphatic tissues filter and monitor lymphatic fluid
    - Immune cells of the myeloid and lymphoid cell lineages are produced by hematopoiesis in the bone marrow
- The immune system forms a balance between immune response and immune tolerance
    - The immune response aims to rapidly eliminate pathogens from the body
    - Immune tolerance aims to prevent immune responses to *self* tissues and harmless microbes within the microbiome
    - This balance is established by killing self-reactive lymphocytes during development as well as having cytokine signals that indicate whether an immune response should be enhanced or suppressed
- Immune disorders can result from either under-activity or over-activity of the immune response
    - Under-activity of the immune response is called immunodeficiency and can result in more frequent or more severe infection
    - Over-activity of the immune response is called hypersensitivity, resulting in persistent inflammation or autoimmune disease

Chapter Review

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://pressbooks.bccampus.ca/appliedimmunology/?p=5#h5p-2

Case Study Review

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https://pressbooks.bccampus.ca/appliedimmunology/?p=5#h5p-3

Chapter 2: Innate Immune Barriers and Components

Learning Objectives

By the end of this chapter you will be able to:

- Outline physical and chemical defenses of the immune system
- Identify and assign a function to each cell of the innate immune system

Case Study

Sarah B. is 28 years old and has no history of urinary issues. Recently however, she has presented with frequent urination, pain and burning during urination, cloudy urine. Sarah has been taking antibiotics that cleared up a respiratory infection, which contributed to the death and imbalance of protective bacteria within her urinary tract. This microbial imbalance allowed *Escherichia coli* to attach to the bladder lining and multiply, causing irritation and inflammation. Inspection of her

cloudy urine shows the presence of bacteria in urine but also mucus and white blood cells (*leukocytes*). Sarah's physician prescribes her a new antibiotic that is more specific to *E. coli* and that concentrates within urine. The new prescription helps Sarah recover from the urinary tract infection and she progressively re-establishes a healthy population of bacteria within her urinary tract.

- *How do you think some bacteria protect us from infection?*
- *What characteristic of urine would protect us against excessive bacterial growth?*
- *Why do you think it would be beneficial for Sarah to drink more water and increase her frequency of urination?*
- *What are the roles of mucus and white blood cells in the urine of a person with urinary tract infection?*

Answers to these questions are at the end of the chapter.
The text is pdf-only

2.1 Physical Immune Defenses

The human immune system takes a multi-barrier approach, where layers of physical and chemical challenges prevent microbes from invading deeper into tissues.

The **skin** is a superficial barrier, composed of dead cells that are tightly connected by a dense layer of keratin. Our skin is dry, salty and acidic, and is constantly being shed. These characteristics collectively make the skin difficult to penetrate.

Where the skin is replaced by more fragile but functional tissues, such as the eyes, respiratory tract, urinary tract and digestive tract, **mucus** acts as a physical barrier. Mucus is

secreted by specialized epithelial cells called **mucous membranes**. Secreted mucus traps microbes in a thick and sticky matrix, which contains antimicrobial compounds. Mucus, along with the entrapped microbes, is then be flushed out of the body. The **mucocilliary escalator** of the upper respiratory tract is an example of this process, where epithelial cells with hair-like appendages called cilia transports mucus up and out of the airway.

Physical Barriers

Epithelial cells of the body surface (e.g. skin) and the tracts (e.g. digestive, respiratory) are tightly associated to create a barrier and have additional specialized functions based on their location.

> Specialized epithelial cells include:
>
> - **Endothelial cells** that line the blood and lymph vessels. Endothelial cells are especially tightly packed around the brain and spinal cord, forming the **blood-brain barrier**.
> - **Mucociliary cells** that sweep mucus out of the airway.
> - **Sebum (oil)** is a secretion from the sebaceous glands that locks moisture in the skin and protects against ultraviolet damage. This oily layer also represents a physical barrier to infection and promotes the growth of protective commensal microbes.

Mechanical Barriers

The body actively expels pathogens with mechanical barriers, such as the eyelids that protect the sensitive tissues of the eye. Secreted **tears** and **sweat** contain antimicrobial chemicals. The secretion of body fluids as well as the shedding of skin limits the time that microbes can occupy the body surface.

Goblet cells in the respiratory tract secrete **mucus**, which entraps microbes, while cilia extension on the epithelial surface sweeps this mucus out of the airway. A cough reflex can then eliminate these microbes from the body.

The body generally **flushes** microbes from the body through defecation or urination. The kidney supports this process through filtration and processing of blood plasma fluid for excretion.

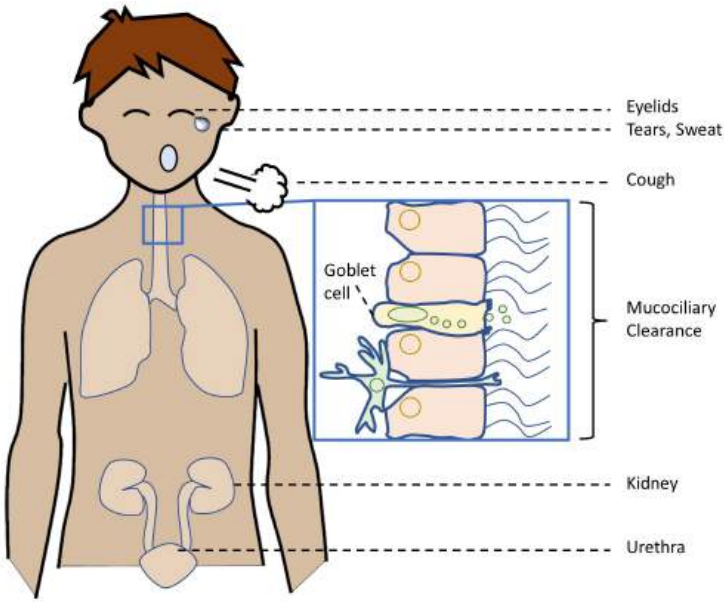

Figure 2-1 Mechanical Barriers of the Immune System.

Different body systems coordinate their actions to eliminate microbes from the body. [Image Description]

Microbial Barriers

The human **microbiome** is the collection of microbes on the surface and within the tracts of the human body. While these microbes do not formally form part of the human immune system, they contribute to immune defense in the same way as our own physical and chemical barriers.

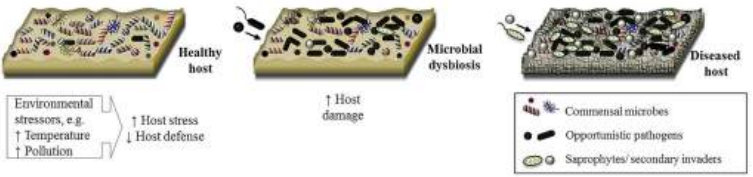

Figure 2-2 Dysbiosis. The surface of the human body is typically co-inhabited by microbes that support healthy tissues. External stressors provide an opportunity for outgrowth of opportunistic pathogens. As the opportunistic pathogens dominate the tissue, they contribute to pathology. [Image Description]

Image Source: Adapted from Suhelen Egan & Melissa Gardiner, CC BY-SA 4.0, via Wikimedia Commons via Wikimedia Commons

Genetic Barriers

A person's risk for infectious disease may depend on their unique genetic makeup. Some pathogens are specific to a species and are not transmitted from animals to humans. For

viruses, this genetic barrier may be the requirement of a specific protein to bind cells. When the receptor protein is not available on human cells the cell can not be infected. Bacteriophages, viruses specific to bacteria, an example of this. Large numbers of bacteriophage are found in the human intestine, where they infect bacteria of the microbiome but do not infect human cells.

The best-described example of genetic barriers to infection in humans relates to infection by human immunodeficiency virus (HIV-1). HIV-1 requires two proteins to enter human immune cells. The first protein is called CD4 and serves as the viral receptor that attaches it to the cell surface. The second protein is a co-receptor consisting of either CCR5 early in the infection process or CXCR4 during acceleration of the disease. As many as 10% of people have a mutation in CCR5 called CCR5-delta-32 (CCR5-Δ32). This mutation makes the protein non-functional, preventing HIV-1 from infecting human cells. This premise was used to provide the first-ever cure for HIV-1 infection to a person who was known as the *Berlin patient* to preserve their anonymity. Bone marrow from a person with CCR5-Δ32 mutation was grafted into the *Berlin patient* with HIV-1 infection. Stem cells within the bone marrow produced immune cells with the mutation, which then could not be infected by the virus. The patient thereafter maintained a low viral load without needing to take antiretroviral medication.

2.2 Chemical Immune Defenses

Chemistry underlies how biological cells work and our body leverages this fact to produce chemicals aimed at preventing infection. Chemical defenses can work in variety of ways, including:

- **Antimicrobial chemicals** that are inhibitory or toxic to microbial pathogens.
- **Chemical mediators** that participate in cellular communication in order to coordinate complex responses, such as inflammation.

Antimicrobial Chemicals of the Microbiome

Microbes that inhabit the human body may cooperate with the immune system to prevent colonization by potential pathogens. Our body secretes chemicals that promote the growth and function of these beneficial commensal microbes.

- *Propionibacterium acnes* consumes oily **sebum** that is secreted by the skin and produces oleic acid as a byproduct of metabolism. The resultant mildly acidic skin restricts the growth of potential pathogens.
- *Lactobacillus* species consume glycogen that is secreted into the vagina. These bacteria produce lactate as a byproduct of metabolism that acidifies these tissues.

Secreted Antimicrobial Chemicals

Secreted factors throughout the human body can prevent colonization by potential pathogens.

- **Body Surface**
  - Secreted tears contain **lysozyme** enzyme, which degrades the bacterial cell wall, as well as lactoferrin, which competes with microbes for iron uptake.
- **Digestive Tract**
  - Saliva contains mucus that contains **lysozyme**, lactoferrin and lactoperoxidase enzyme, which speeds up the degradation of both bacteria and viruses.
  - Stomach **acid and digestive enzymes** are destructive toward microbes.
- **Respiratory Tract**
  - Contains **mucus**, with a similar makeup to the **mucus** in saliva.
- **Urogenital Tract**
  - The inherent **acidity** of urine restricts microbial growth.

Antimicrobial peptides (AMP) is a classification of secreted peptides produced throughout the body that have antimicrobial properties. For example, defensin peptides are secreted by epithelial cells and disrupt the

membranes surrounding bacterial and fungal cells, as well as some viruses.

2.3 Cells of the Immune System

In additions to physical and chemical barriers, an intricate combination of cellular responses and cell communication plays an important role in developing an appropriate and coordinated response to infection. This process involves (1) detection of infection by cells at the site of infection, (2) an immediate response to prevent the invasion of tissue by the pathogen, (3) cellular communication to recruit more immune cells to the site of infection, and (4) develop a body-wide systemic response if the infection cannot be contained.

The main cells of the immune system are white blood cells, called *leukocytes*, and these cells along with red blood cells and platelets are all produced from a shared stem cell, called a *hematopoietic stem cell*.

Hematopoiesis

The hematopoietic stem cells are typically found in the bone marrow and produce the non-fluid component of the blood, which is collectively called the *formed elements*.

The primary formed elements are:

- **Erythrocytes** (Red blood cells) – make up the vast

majority of the formed elements produced by hematopoiesis. Function to carry oxygen to tissues.

- **Leukocytes** (White blood cells) – Often subdivided into cells with visible granules (granulocytes) and those without granules (agranulocytes). These cells are primarily responsible for the immune response.
- **Thrombocytes** (Platelets) – Fragments produced from a larger cell that are active in blood clotting and tissue repair.

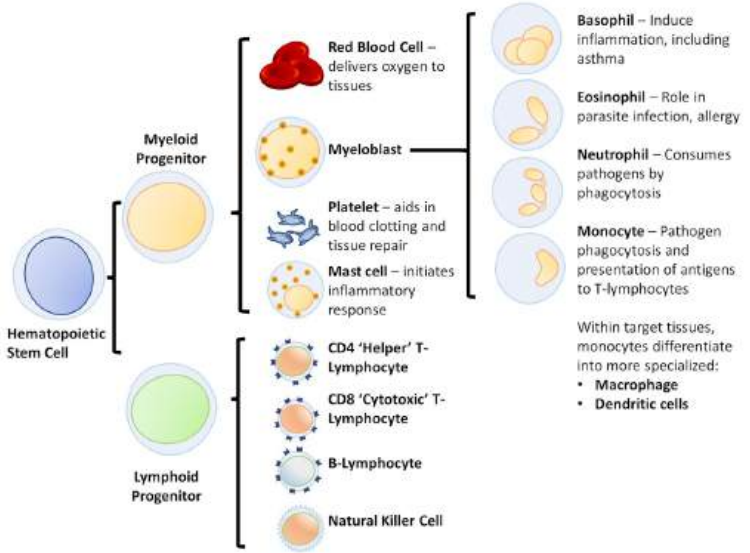

Figure 2-3 Hematopoiesis. Most immune cells generated through the process of hematopoiesis. Hematopoiesis starts with the hematopoietic stem cell, which is a pluripotent cell because it can produce red blood cells (*erythrocytes*), platelets (*thrombocytes*) and white blood cells (*leukocytes*). Leukocytes arise from two specialized stem cells the *myeloid stem*

cells and *lymphoid stem cells*. All myeloid cells and natural killer cells participate in the innate immune response. T and B lymphocytes are involved in the adaptive immune response. [Image Description]

Innate Immune Cell Types

Different immune cells perform different but often overlapping functions.

> Cells relevant in the immune defense include:
>
> **Neutrophils**: These granulocytes are sometimes called polymorphonuclear (PMN) cells due to the fact that the cell nucleus forms into multiple connected lobes. Neutrophils are often involved in elimination of bacterial infection in the space between cells. The main way that neutrophils eliminate pathogens is by phagocytosis *(process of consuming pathogens*). However, neutrophils can also release their DNA as a neutrophil extracellular trap (NET). The NETs form a lattice to restrict the spread of pathogens and are also decorated with proteins that have antimicrobial properties. When neutrophils build up around a site of infection, their numbers may be so great that we can see them as purulent drainage or 'pus'.
>
> **Eosinophils**: These granulocytes specialize in defense against parasites, both single cell (protozoa) and parasitic worms (helminth). They also regulate inflammation and are involved in allergies.
>
> **Basophils and Mast Cells**: These granulocytes regulate inflammation and are involved in allergies. While functionally similar these cells are quite different.

Basophils reside in the bloodstream while mast cells reside in tissues.

Monocytes, Macrophages and Dendritic Cells: Monocytes are agranulocytes that circulate in the bloodstream. When the monocyte enters a body tissue it differentiates into macrophage or dendritic cell. These cells collectively form the *mononuclear phagocyte system* and actively consume pathogens by phagocytosis *(described* below). These cells also release cytokines to coordinate the immune response and engage in antigen presentation, a mechanism by which to alert the adaptive immune system to the presence of a particular pathogen.

Natural Killer (NK) Cells: These agranulocytes are unique because they are the only lymphoid cells involved in the innate response. NK cells look for abnormalities in human cells, such as might arise in virus-infected or cancer cells. Human cells use a molecular display called MHC in order to present abnormal viral or cancer proteins on their surface. Some viruses try to bypass this system by preventing MHC from reaching the cell surface. As a response to viral suppression of MHC, NK cells will kill human cells that *fail* to present MHC on their surface.

Other Cell Types

Platelets are also known as *Thrombocytes* and these formed elements are cell fragments that participate in tissue repair and blood clotting.

T- and B-Lymphocytes are cells of the adaptive immune system and will be discussed in detail in Chapter 3. These

cells provide a targeted immune response to a specific pathogen and can produce memory cells that contribute to longterm immunity to infectious disease.

Blood Cell Counts

Certain immune cells accumulate in the bloodstream as needed to overcome specific infections. Conversely, reduced numbers of specific immune cell types can place a person at increased risk for a particular infection.

As such, a complete blood cell (CBC) count can be performed to aid in disease diagnosis. If a CBC test reveal an increased number of leukocytes, this may indicate an infection. CBC with *differential* involves specifically counting the different types of white blood cells to get even further information about the infectious disease. The suffixes *–philia* or *–cytosis* are used to represent overabundance and *-penia* means deficiency of cells.

For example:

- *Leukocytosis* – a high number of white blood cells indicates potential response to infection.
- *Leukopenia* – a low number of white blood cells may indicate an immunodeficiency that puts a person at risk for infection.
- *Neutrophila* frequently occurs in response to bacterial infection, especially those involving pus formation (*pyogenic* infection).

- *Eosinophila* may indicate parasite infection or allergy response

Summary

- Physical defenses prevent pathogens from entering the body
    - Physical barriers such as the skin and mucous block access to sensitive cells and tissues
    - Mechanical barriers such as urination and shedding are utilized to actively expel pathogens from the body
    - The unique genetic makeup of an individual may protect them from infection acting as a genetic barrier
- Chemical defenses include antimicrobial chemicals that act directly on pathogens and chemical mediators (such as cytokines), which are utilized to induce immune responses
- All myeloid cells participate in the innate immune response
    - Neutrophils, monocytes, macrophages, dendritic cells are professional phagocytes
    - Monocytes, macrophages and dendritic cells are antigen presenting cells that bridge innate and adaptive responses
    - Mast cells (in tissue) and basophils (in blood) are associated with inflammation including allergies

    - Eosinophils are pro-inflammatory cells and are most effective against parasites

- Natural killer cells are innate immune cells that recognize and target cells that are infected or damaged by injecting toxins. They originate from the lymphoid progenitor cell.

Chapter Review

An interactive H5P element has been excluded from this version of the text. You can view it online here:
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Case Study Review

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Image Descriptions

Figure 2-1 Mechanical Barriers of the Immune System image description:

A diagram depicts a stylized human figure showing various body parts and their functions related to innate immune defense. On the head, the "Eyelids" are labeled, with a droplet near the eye representing "Tears, Sweat." The mouth area has a visual representation of a "Cough." On the right of the diagram, there's a magnified view of a respiratory tract section. Within this section, "Goblet cells" are depicted as orange oval entities, producing green mucus. Surrounding these goblet cells, hair-like structures called cilia, which are involved in the process of "Mucociliary Clearance." Below the torso, the "Kidney" is outlined, and further down, the "Urethra" is indicated. Each body part or function is clearly marked with labels. [Return to Figure 2-1]

Figure 2-2 Dysbiosis image description:

The image presents a visual representation of the transition of a host's microbial environment from healthy to diseased under various conditions. There are three primary sections:

1. "Healthy host" shows a section of tissue with equal numbers of striped rods, spiked circles and filamentous patches as well as a small number of black oval-shapes, indicating a balanced microbial environment with a suppressed population of opportunistic pathogens.
2. "Microbial dysbiosis" displays the tissue after being subjected to stressors, resulting in a diminished number of "commensal" striped rods, spiked circles and filamentous patches and a larger number of "opportunistic pathogen" black oval-shapes. There are also a small number of dotted ellipses and spheres, representing "secondary invaders."
3. "Diseased host" shows the tissue in a highly compromised

state, where the hue of the tissue has been darkened. There are more “opportunistic pathogen” black oval-shapes as well as with the appearance of more pathogenic microbes, including elongated ones and those “secondary invader” dotted ellipses and spheres.

At the bottom of the image, a legend indicates the types of microbes:

- “Commensal microbes” are represented by striped rods, spiked circles and filamentous patches.
- “Opportunistic pathogens” are shown as black oval-shapes
- “Saprophytes/secondary invaders” are depicted by dotted ellipses and spheres.

To the left, there’s a box indicating “Environmental stressors, e.g., ↑Temperature and ↑Pollution” which lead to “Host stress” and reduced “Host defense”, eventually resulting in “Host damage”. This suggests that environmental factors can impact the microbial balance in a host, leading to disease. [Return to Figure 2-2]

Figure 2-3 Hematopoiesis image description:

The image illustrates the differentiation pathway of hematopoietic stem cells into various blood cells. Starting from a “Hematopoietic Stem Cell”, there are two main pathways:

1. Myeloid Progenitor Pathway:

- “Red Blood Cell” that delivers oxygen to tissues.
- “Platelet” that aids in blood clotting and tissue repair.
- “Mast cell” that initiates the inflammatory response.
- “Myeloblast” which further differentiates into:

- “Basophil” which induces inflammation, including asthma.
- “Eosinophil” which has a role in parasite infection and allergy.
- “Neutrophil” that consumes pathogens by phagocytosis.
- “Monocyte” involved in pathogen phagocytosis and antigen presentation to T-Lymphocytes.

The note mentions that within target tissues, monocytes differentiate into more specialized macrophages and dendritic cells.

2. Lymphoid Progenitor Pathway:

- “CD4 ‘Helper’ T-Lymphocyte”
- “CD8 ‘Cytotoxic’ T-Lymphocyte”
- “B-Lymphocyte”
- “Natural Killer Cell”

Each cell type is represented with a unique color and shape to distinguish it from others. Lines and arrows indicate the progression and differentiation of cells from their progenitors [Return to Figure 2-3]

Chapter 3 Induced Innate Immune Responses

Learning Objectives

By the end of this chapter you will be able to:

- Describe how innate immune cells identify pathogens and engage in eliminating pathogens by phagocytosis.
- Define cytokine signalling and identify relevant cytokines involved in immune responses.
- Describe the physiological events and immune cell recruitment associated with localized inflammation.
- Explain the potential outcomes of systemic inflammation and fever.
- Describe the actions of plasma proteins such as complement.

Case Study

Emily is 48 years old and identifies as a female. Her medical history is unknown and she does not appear to have underlying conditions. Emily presented with signs of COVID-19 and tested positive for the virus that causes this infectious disease (SARS-CoV-2). The viral infection centered within the respiratory tract. Emily was admitted to the hospital on day 10 of infection after experiencing worsening localized respiratory inflammation, including dry cough for 10 days and shortness of breath for 5 days. However, Emily also experienced a systemic response involving a fever of 38.7°C and general malaise. As the infection progressed, Emily's signs and symptoms became more severe. By day 13, anti-pyretic medication was used to manage the persistent fever and on day 18 a blood test revealed elevated cytokines IL-6, IL-8, and TNF-α. Emily's conditions began to worsen rapidly and further blood tests revealed excessive accumulation of these same cytokines. Aggressive therapy was used to support breathing and tissue oxygenation and immunomodulatory drugs were administered to suppress the apparent cytokine storm. Following treatment, Emily progressively overcame the infection and recovered.

1. *Reflect on what happens to tissue when it is injured and inflamed. Why do you think that inflammation of the respiratory tract contributes to difficulty in breathing?*
2. *Cytokines are chemicals used by immune cells to communicate and trigger a specific immune response. What do you think is the relevance of the cytokines identified in Emily's blood test?*
3. *Why was it important to use immunomodulatory drugs to suppress the immune response in this case of COVID-19?*

Answers to these questions are at the end of the chapter.

3.1 Phagocytosis

Many human cells, including epithelial cells lining the body surface and endothelial cells around the blood vessels, are capable of consuming dead human cells and foreign microbes through a process called **phagocytosis**. However, these cells would be regarded as *non-professional* phagocyte cells. Immune cells, including neutrophils, macrophages and dendritic cells, are **professional phagocytes**, also simply referred to as *phagocytes*. These cells differ from *non-professional phagocytes* because they have specific cell receptors that allow them to efficient detect and eliminate pathogens.

Phagocytes detect the target cell using pattern recognition receptors (PRRs), which include toll-like receptors (TLRs). These receptors do not identify specific pathogens but rather detect molecular patterns that are commonly found on the surface of pathogens. These pathogen-associated molecular patterns are abbreviated as **PAMPs**. Examples of PAMPs include components of the bacterial cell wall, such as peptidoglycan and lipopolysaccharide (LPS).

Upon binding a microbial cell, the phagocyte cell surface undergoes structural changes that wrap the phagocyte cell membrane around to the microbe. When fully encapsulated, the phagocyte surface fuses to fully contain the microbe inside a *bubble* within the cell, called a **phagosome**. Specialized organelles, called lysosomes, contain chemicals and enzymes that are capable of killing and digesting the microbial cell. These lysosomes fuse with the phagosome to produce a structure called the **phagolysosome**.

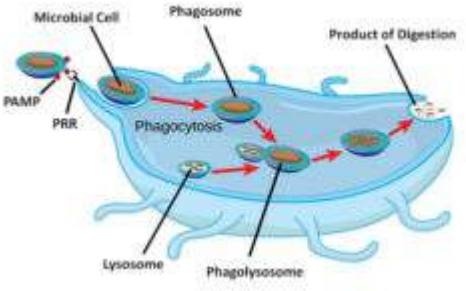

Figure 3-1 Phagocytosis. Phagocytes have specialized pattern recognition receptors (PRR) to bind distinguishing patterns (PAMPs) on the surface of microbial cells. The membrane surface of the cell folds and encapsulates the microbe into a vacuole called a phagosome. Lysosomes concentrate within them digestive chemicals and enzymes, and these lysosomes fuse with the phagosome to produce a phagolysosome. The microbial cell is killed within this vacuole and the digested material is expelled from the cell.

Image Source: Modified image from OpenStax CC BY 4.0, via Wikimedia Commons

Two methods are used to kill the microbial cell within the phagolysosome:

1. **Oxygen-dependent mechanisms** can be used to destroy entrapped cells. This mechanism is also known as a *respiratory burst* or *oxidative burst*. Metabolism of oxygen produces molecular byproducts that are toxic, called **reactive oxygen species (ROS).**

Normally reactive oxygen species are detoxified within the cell, however, they can also be repurposed to digest captured microbes. Relevant ROS include hydrogen peroxide (H_2O_2), hydroxyl free radicals (HO^-), superoxide anion (O_2^-), singlet oxygen (O^-) or hypochlorite (OCl^-).

2. **Oxygen independent mechanisms** include other chemicals and proteins (e.g. enzymes) stored within the lysosome. Lactic acid and nitric oxide (NO) are harmful chemicals. Cationic proteins can damage the bacterial cell membrane. Enzymes include proteases, phospholipases, nucleases and lysozymes.

3.2 Cytokine Signaling

Complex cellular responses, such as inflammation and fever, are coordinated by an equally complex system of chemical communication. This communication is accomplished by secreting small proteins called **cytokines**. Cytokines are secreted by immune cells as well as other cells that are stressed or damaged. Cytokines bind receptor proteins on the surface of other immune cells to induce a specific response in that target cell. Cytokines are involved in a range of immunological responses, including (but not limited to) an inflammatory response to infection, an anti-inflammatory response to return to normal after infection, as well as induction of fever.

Cytokines may be classified into various groups of signalling molecules:

- **Tumor Necrosis Factors (TNF)** are multifunctional cytokines that regulate fever and inflammation, and inhibit cancer cell growth and viral replication.
- **Interleukins (IL)** were originally isolated from leukocytes but are now known to be secreted by other cells as well.

Many cytokines are produced in response to injury and infection but two signalling functions are particularly important:

1. **Chemotactic cytokines** – These chemicals are released by both immune cells and other cells in the tissue to direct immune cells to the site of infection. Immune cells follow the *chemotactic gradient* from regions of low cytokine concentration to regions of higher concentration. The immune cells will ultimately swarm the tissue region where these chemotactic agents were produced and secreted.
    - ***Examples of chemoattractants and chemotactic cytokines include***:
        - **Fibrin** and **Platelet activating factors (PAF)** – proteins involved in blood clotting attract leukocytes to the site of injury.
        - **Collagen** – tissue repair protein attracts leukocytes.
        - **Mast cell chemotactic factors** – specific proteins secreted by mast cells to recruit leukocytes when they encounter pathogens.

2. **Vasoactive cytokines** – These chemicals alter the structure of the blood vessels, or *vasculature*. For example, vasoactive cytokines are responsible for vasodilation that enables white blood cells to extravasate from the bloodstream into tissues.

    - ***Examples of vasoactive cytokines include***:

        - **Histamine** – stored in granules within mast cells to rapidly induce vasodilation at the early stages of inflammation.
        - **Bradykinin** – produced found in tissues and blood, this protein acts on endothelium to produce vasodilation.
        - **Serotonin** – often known for its function as a neurotransmitter in the nervous system, serotonin is also released by mast cells and platelets, causing both vasodilation and vasocontriction
        - **Prostaglandins** – complex chemical mediators that can induce vasoconstriction or vasodilation, depending on the biological context.

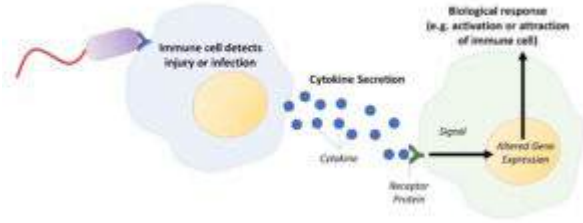

Figure 3-2 Cytokine Secretion. Immune cells communicate by secretion of cytokine proteins, where the secretion of these cytokines by one cell induces a biological response in another cell. The specific biological response depends on both the type and intensity of secreted cytokine.

3.3 Localized Inflammation in Response to Infection

Inflammation is a physiological response induced by cytokine signalling. These inflammatory cytokines may be produced and released by damaged, infected or stressed cells or these cytokines may be secreted by leukocyte immune cells upon detection of infection or injury. The immediate consequence of inflammation is to alter blood vessels in the local tissues so that leukocytes can be recruited to the site for infection control, removal of dead cells and tissue repair. However, excessive or prolonged inflammation can result in tissue damage, dysfunction or even death.

Acute Inflammation

Immediately following an injury, blood vessels briefly constrict (*vasoconstriction*) to stop the flow of blood and to minimize blood loss. However, as platelets help form a blood clot, the constriction of blood vessels is no longer required. Once the clot forms, inflammation drives a series of events that lead to (1) expansion or *vasodilation* of blood vessels to cause more blood cells to collect around the site of injury/infection and (2) increased blood vessel (*vascular*) permeability to allow blood plasma fluid and leukocytes to enter into the tissues.

> **Vasodilation** increases blood flow into vasculature around the site of infection. In this context, blood is actually flowing *slowly* through the blood vessels and the increase in blood flow means that blood is *collecting* in these tissues. The increased volume of fluid in blood

vessels and tissues dilutes potential toxins produced by the pathogen. Also, leukocytes in the blood will flow closer to the walls of the blood vessels in a process called *margination*. Once at the site of injury, these immune cells may undergo *diapedesis* to extravasate from the bloodstream into the inflamed tissues.

Edema occurs as the blood vessels become more permeable and blood plasma fluid leaks into the tissue. This fluid may include extravasated leukocytes, clotting factors, defensive chemicals such as complement proteins and many other factors to support an efficient immune defense. When the fluid is rich in leukocytes, it may appear white and is commonly called **pus**.

Manifestation of localized inflammation stems from both these processes of vasodilation and edema. Vasodilation increases the flow of blood to the site of injury or infection. The tissue becomes red (erythema) and warm, owing to these characteristics of blood. Edema causes fluid to enter the tissue and cause swelling and altered function. An additional outcome of inflammation may be pain as swollen tissues impact on sensitive nerve endings.

Five **cardinal signs** of inflammation have been described, including rubor (redness), dolor (pain), calor (warmth at the site), tumor (swelling) and loss of function. These signs are directly related to the effects of vasodilation and edema, as illustrated in Figure 3-3.

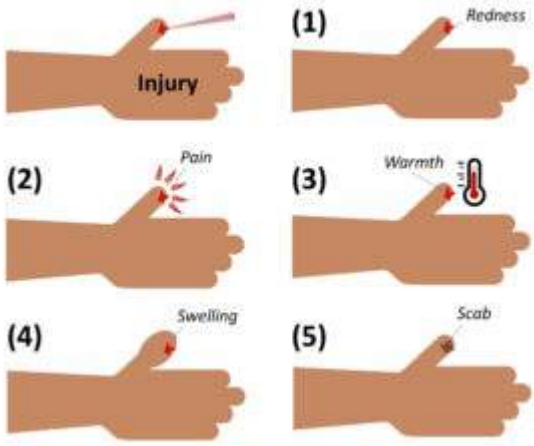

Figure 3-3 Cardinal Signs of Inflammation. In response to infection, injury or damage, inflammation may produce (1) **Rubor** – Redness as a consequence of increased blood flow to the site; (2) **Dolor** – Pain due to activation of nerve endings; (3) **Calor** – Heat due to accumulation of blood; (4) **Tumor** – where tissue swells as a consequence of edema; (5) **Loss of function** – where pain, swelling and/or rigid scab formation reduces the mobility of the tissue in order to prevent further injury.

Acute Inflammation in Recruitment of Immune Cells

A primary purpose of the inflammatory process is to respond to infection and injury by recruiting immune cells and components to the inflamed tissue. Cells are attracted to the inflamed site by specific proteins that are expressed on the surface of endothelial cells in the blood vessels around the affected site. Leukocytes are then able to deform and migrate into the local tissue, through the pores formed during vasodilation of the blood vessel. Once inside the tissue,

leukocytes follow chemotactic cytokine gradients to the origin of the inflammation signals.

The process by which leukocytes migrate out from the blood vessels into the tissues involves a series of steps:

1. **Margination**: The leukocytes form temporary adhesions to proteins on the blood vessel endothelial wall. These proteins are called **selectins**. The endothelial cells near the site of inflammation express additional proteins called **intercellular adhesion molecules (ICAMs).** The leukocytes have **integrin** proteins on their surface that specifically bind ICAMs and form **tight adhesion** in the vicinity of the inflammatory site.
2. **Extravasation:** The leukocytes flatten and pass through gaps between endothelial cells. This process is sometimes also called *diapedesis*.
3. **Chemotaxis:** Once the leukocytes cross from the blood vessel into the tissue, the cell will follow a cytokine concentration from the distant region of low concentration to the site of inflammation, where the cytokine concentration is much higher.

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https://pressbooks.bccampus.ca/appliedimmunology/?p=360#h5p-4

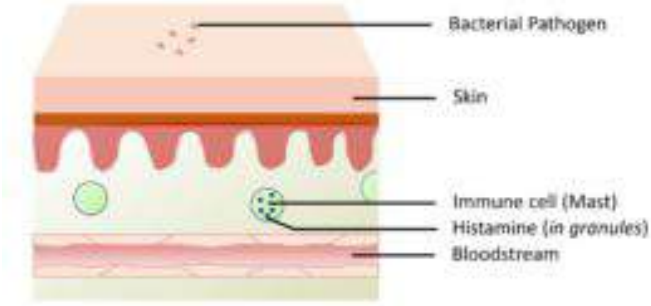

Figure 3-3A. Ubiquitous State. Even prior to infection or injury, mast cells can be found deposited throughout the body. These mast cells have granules with pre-formed histamine that is capable of rapidly inducing an inflammatory response.

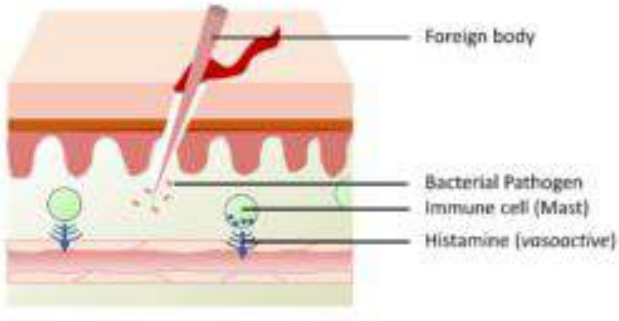

Figure 3-3B. Mast cell degranulation. On detecting a pathogen, mast cells *degranulate* to secrete the histamine from internal granules into the surrounding tissue.

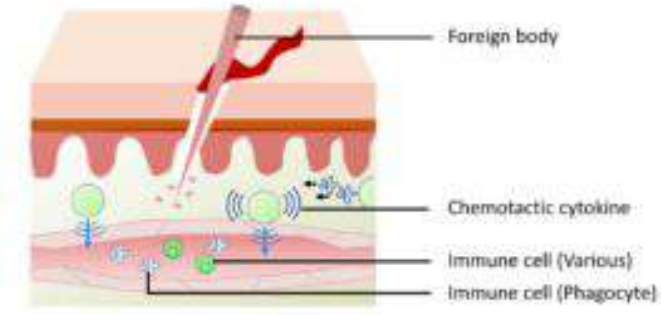

Figure 3-3C. Cytokine Signalling. Mast cells secrete "mast cell chemotactic factors" that attract local leukocytes. The secreted histamine induces vasodilation which results in accumulation of blood and blood cells in the region of inflammation.

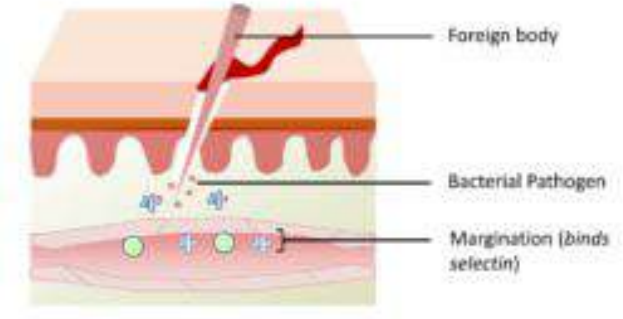

Figure 3-3D. Margination. Leukocytes in the blood stick to the endothelial wall because of temporary binding to selectin proteins.

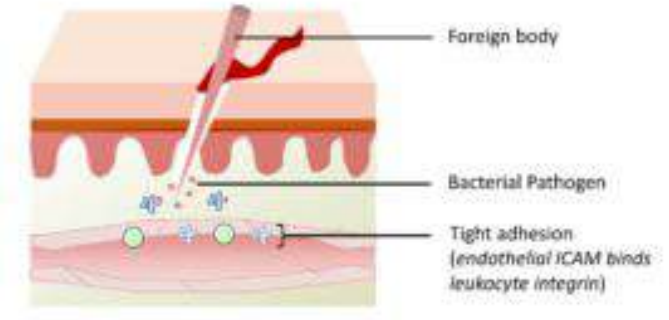

Figure 3-3E. Tight Adhesion. Endothelial cells around the site of inflammation express ICAM proteins. Integrin proteins on the surface of leukocytes bind tightly to ICAM, causing the leukocyte to bind tightly to the blood vessel wall.

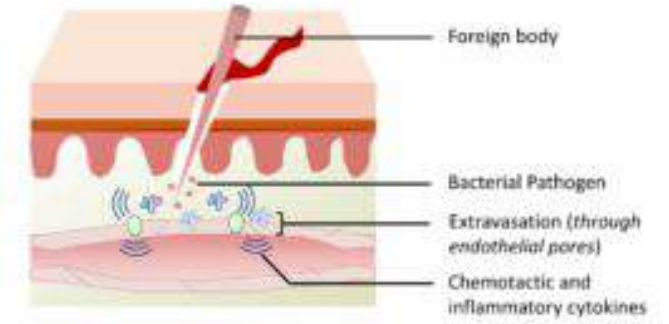

Figure 3-3F. Extravasation. Leukocytes flatten and migrate through pores that form between the endothelial cells. Within the tissues, these cells follow a chemotactic gradient back to the site of infection. These newly-arrived leukocytes also secrete cytokines that make the inflammatory response even stronger.

Chronic Inflammation

Chronic inflammation involves the same biological mechanisms as acute inflammation but the tissue is affected for a longer period of time that persists for weeks, months and even years. Chronic inflammation may occur if the body is continuously exposed to irritants or if a person is affected by autoimmune disease. In chronic inflammation, immune cells like lymphocytes and macrophages continuously release inflammatory cytokines, leading to tissue damage and fibrosis. Unlike acute inflammation, chronic inflammation is often characterized by tissue destruction and repair attempts occurring simultaneously, which can eventually lead to organ dysfunction and long-term health complications. Managing chronic inflammation is essential to prevent further tissue damage and maintain overall health.

3.4 Systemic Inflammation

In contrast to acute and chronic localized inflammation, where a specific tissue becomes red and swollen, systemic events affect the entire body. This may occur if cytokines or activated immune cells migrate from the inflamed tissue into the bloodstream. Systemic responses are particularly likely in the context of a **septicemia**, which describes a circumstance where a pathogen gains entry and spreads within the bloodstream. Once in the bloodstream, immune cells and cytokines are dispersed throughout the body. Systemic cytokines may stimulate immune cells throughout the body to release inflammatory cytokines of their own This produces a feed-forward loop called **hypercytokinemia** where excessive amounts of cytokines accumulate in the bloodstream. The dysregulated release of cytokines may be considered a

cytokine storm and may lead to **sepsis**, which is an overwhelming and potentially life-threatening response to infection.

Systemic inflammation may pose an immediate health threat because vasodilation and edema occur throughout the body, resulting in a sudden and dramatic reduction of the fluid volume in the bloodstream. Without sufficient fluid, the **blood pressure becomes too low** to transport oxygen- and nutrient-rich blood to tissues and organs that need it, which may result in multi-organ failure and possibly even death.

3.5 Fever

Fever is a physiological response triggered by the immune system in response to infection or inflammation. Specifically, fever is triggered by molecules shed from pathogens or by cytokines, which are collectively called **pyrogens**. Pyrogens act on the hypothalamus, raising the body's temperature set-point, which leads to an elevation in body temperature.

A higher body temperature may be beneficial as it makes it more difficult for certain pathogens to replicate and survive. Additionally, fever stimulates the production of interferons, which are proteins that help combat viral infections. Elevated body temperature also increases the efficiency of certain immune cells, such as T cells and macrophages, enhancing their ability to target and destroy pathogens.

However, fever can also cause discomfort and fatigue. In more severe cases, very high fevers can lead to dehydration and electrolyte (salt) imbalances, especially in young children or individuals with medical conditions that make them sensitive to electrolyte imbalance. Therefore, while fever is a crucial defense mechanism, managing it with appropriate fever-reducing medications **(antipyretic medications)** and ensuring

adequate fluid and electrolyte intake is important to strike a balance between its beneficial effects and potential risks.

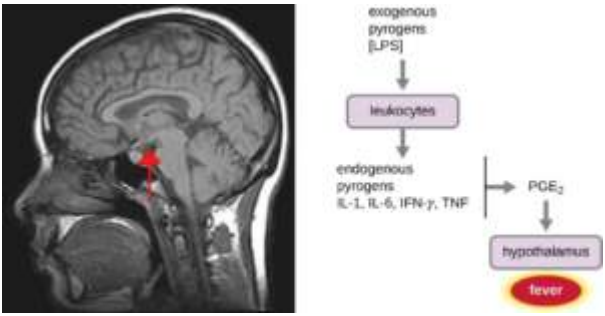

Figure 3-4 Mechanism of Fever. Leukocytes detect exogenous (*non-human source*) pyrogens, such as bacterial lipopolysaccharide (LPS). These leukocytes then secrete cytokines that serves as endogenous pyrogens in order to signal the hypothalamus of the brain to increase the body temperature set point.

Image Source: By OpenStax CC BY 3.0, via OpenStax Microbiology

3.6 Plasma Protein Inflammatory Mediators

Whole blood is composed of cells and formed elements, as well as a fluid called **plasma**. Within the blood plasma are electrolytes, nutrients and proteins involved in blood clotting and immune response.

Some plasma proteins, called **acute phase proteins (APPs),** are produced in the liver and become concentrated in the bloodstream during the acute inflammation stage of the immune response. APPs can be analyzed using blood tests for detecting inflammation.

Examples include:

- **C-reactive protein** – binds the surface of bacteria and promotes their phagocytosis.
- **Ferratin and Transferrin** – sequester free iron so that it cannot be absorbed and utilized by microbial pathogens.
- **Fibrinogen** – a protein that, when activated, forms blood clots.
- **Mannose-binding lectin** – binds sugars on the surface of pathogen cells and induces a complement cascade.
- **Complement proteins** – Potent immune mediators that both damage target cells as well as induce potent inflammation.

3.7 Complement Proteins

The complement system consists of more than 30 proteins that circulate in the bloodstream. Complement proteins are considered part of the innate immune response because they are always found in the bloodstream and tissues, and can target pathogens in a non-specific manner. However, complement proteins also play a major role in the function of antibodies, in the adaptive immune response.

There are three conditions that can initiate a complement cascade, which are referred to as the three

complement pathways.

1. **Classical complement pathway** – The complement protein C1 binds to antibodies, initiating a series of events involving activation of C2 and C4, followed by C3.
2. **Lectin complement pathway** – Mannose-binding lectin accumulates on the surface of microbes, where it activates C2 and C4, and subsequently C3.
3. **Alternative pathway** – spontaneously activates C3.

As all three pathways converge on C3 activation, a selection of other complement proteins are engaged to enact the functions of complement.

1. **Cell lysis** – C6, C7, C8 and C9 cooperate to form a pore through the cell membrane. This pore is called the **membrane attack complex (MAC)**. If enough pores form, the target cell lyses, or "bursts".
2. **Opsonization** – C3b and C4b remain attached to the cell surface, where they enhance the likelihood the cell will be phagocytized.
3. **Induce inflammation** – C3a and C5a are potent inducers of inflammation.
4. **Chemotaxis** – C5a is a potent attractant for neutrophils, causing them to move toward the source of these chemicals through the process of chemotaxis.

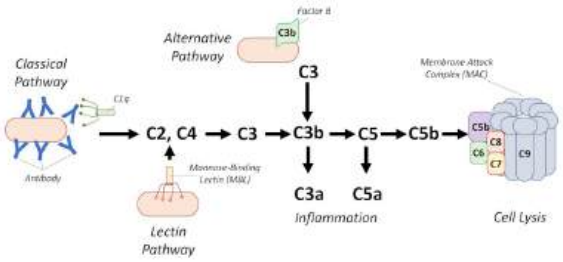

Figure 3-5 Complement Activation. Simplified diagram showing the convergence of the three complement pathways. Note that the classical pathway is unique in its requirement for C1q. Both The classical and lectin pathways use C2 and C4. The alternative pathway involves autocatalytic production of C3, with help from Factor B.

Summary

- The immune system responds to infection by:
    - **Phagocytosis:** Specific immune cells may use pattern recognition receptors (PRRs, e.g. TLR) to detect patterns (PAMPs) on a pathogen surface. The pathogen is internalized within the phagocyte, where it is degraded by oxygen-dependent and oxygen-independent mechanisms.
    - **Cytokine Signalling:** Chemical agents are secreted by immune cells to communicate and coordinate the immune response.
        - **Chemotactic cytokines** attract more immune cells to the site of infection.
        - **Vasoactive cytokines:** act on the local blood

vessels to support uptake of immune cells from the bloodstream into the infected tissue.

- **Acute Localized Inflammation:** Blood vessels dilate and blood plasma (fluid) leaks into infected tissues, causing warmth, swelling and redness of the tissues. Pain and scar formation may also be associated with localized inflammation.
- **Chronic inflammation:** Inflammation that persists over a longer period of time may damage the tissue.
- **Systemic Inflammation:** Inflammation may occur systemically throughout the body. The most severe systemic inflammation may produce sudden loss of blood pressure, which can result in death if vital organs do not receive sufficient oxygenated blood.
- **Complement proteins** contribute to each of these immune responses by causing direct cell lysis, but also by promoting phagocytosis, chemotaxis and vasodilation.

Chapter Review

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://pressbooks.bccampus.ca/appliedimmunology/?p=360#h5p-7

Case Study Review

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://pressbooks.bccampus.ca/appliedimmunology/?p=360#h5p-8

Chapter 4: Components of Adaptive Immune System

Learning Objectives

By the end of this chapter you will be able to:

- Identify the key components of the lymphatic system and describe the major functions of primary and secondary lymphoid organs/tissues
- Define and describe the clinical significance of immunogens, antigens, epitopes and haptens.
- Explain the differences between auto-antigens, allo-antigens and heterophilic/heterogenic antigens, with examples.
- Define Human Leukocyte Antigens (HLAs)/ Major Histocompatibility Complex (MHC).
- Describe the functions and tissue distribution and structure of Class I and II MHC molecules.
- Define haplotypes, isotypes, allotypes and

polymorphism, in the context of HLA.

- Explain the inheritance of MHC genes and discuss their clinical significance.

Case Study

Daniel introduces himself as a 50 year old male who has a long history of alcohol abuse, resulting in cirrhosis (scarring) of the liver. Daniel was placed on a waiting list for a liver transplant and received a transplant after seven months. The surgery was successful, and initially, his new liver functioned well. After the transplant, Daniel was able to manage his alcohol addiction but still developed dark urine, jaundice and abdominal discomfort. A blood test found circulating liver enzymes indicating liver damage. Further investigation found that Daniel was experiencing transplant liver rejection. Following a long-term treatment with immunosuppressive medications, rejection of the liver subsided and Daniel benefitted from a functional liver.

1. *Where did the transplant come from and what kind of antigen might be found on the transplanted tissue?*
2. *Which antigen is the most relevant in transplant immunology and why?*
3. *Why did it take so long to match Daniel with a donor liver?*
4. What is the purpose of immunosuppressive medication in transplant rejection?

Answers to these questions are at the end of the chapter.

4.1 Overview of Adaptive Immune Response

For a vast majority of insults to the body, the innate immune response is sufficient to prevent infectious disease. As a consequence, we can be routinely exposed to potential pathogens without ever experiencing the effects of illness and infectious disease. However if an infection persists, a more strategic response to infection is engaged. This is called the **adaptive response** (aka the **acquired response**)

The adaptive response to infection extends from the innate response. Monocytes arrive at infected tissues and differentiate into macrophages or dendritic cells. These newly arriving cells support the existing neutrophils in phagocytizing pathogens. Monocyte-derived cells have an additional characteristic that neutrophils do not possess. These cells display fragments of the consumed pathogen, called **antigens,** on their surface. Antigens are presented to T-lymphocytes, which subsequently coordinate a distinct immune response that specifically targets this pathogen.

The adaptive immune response takes two basic forms: (1) the humoral adaptive response and (2) the cell mediated adaptive response. The **humoral adaptive response** involves producing antibodies that mark pathogens and hyperstimulate the immune system to eliminate them. The **cell mediated adaptive response** involves activation of macrophages and 'cytotoxic' T-lymphocytes that collectively specialize in eliminating pathogens that reside inside human cells, like viruses or intracellular bacteria.

The final phase of the adaptive response is the formation of **memory lymphocytes**. Memory lymphocytes are long-lived

cell populations that enhances the rapidity and intensity of the immune response upon subsequent exposure to a pathogen in the future. Often, this future *secondary* immune response is extremely fast and intense that a person experiences little to no symptoms of the disease. This phenomenon is referred to as acquiring **immunity** to infectious disease.

4.2 Tissues of the Adaptive Immune Response

Leukocytes are dispersed throughout the body by the **bloodstream** and enter into the tissues by deforming through gaps between the endothelial cells that line the capillary vessels. Leukocytes then migrate through the tissue fluid, called the **interstitial fluid**, and respond to any pathogen within the tissue space.

Leukocytes are then drained, along with tissue fluid and debris, into vessels called **lymphatic vessels** (also called *lymph* vessels). Interstitial fluid is filtered through lymph nodes, distributed around the body, before being return to the bloodstream. Lymph nodes are one of the lymphoid organs and are packed with **lymphocytes**, including T-cells and B-cells.

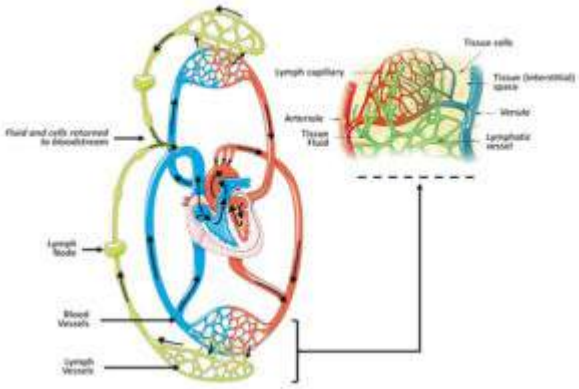

Figure 4-1 Lymphatic drainage. Fluid and leukocytes from the bloodstream flow into the interstitial space within the tissues. Fluid that does not passively return to the bloodstream is drained into local lymph capillaries. These fluids, along with tissue debris and potential pathogens, are filtered through lymphocyte-rich lymph nodes before returning to the bloodstream.

Image Source: Main image by Alicia Fagerving CC BY 3.0, via Wikimedia Commons; Inset by Lennert B CC BY 3.0, via Wikimedia Commons

Lymphatic organs and tissues are classified into two categories: (1) primary lymphoid organs where immune cells are produced and (2) secondary lymphoid organs where immune cells are stored. **Primary lymphoid organs** include bone marrow and thymus in adults, which replace the yolk sac and liver as the sites where immune cells are produced and matured after birth. **Secondary lymphoid organs** include lymph nodes, spleen, tonsils and mucosa-associated lymphoid tissues (MALT).

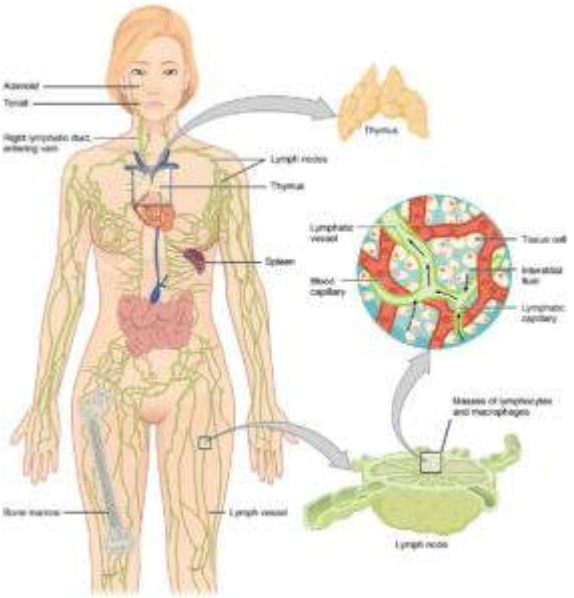

Figure 4-2 Lymphoid organs. Lymphoid organs are distributed throughout the body and play distinct roles in preventing infection within sensitive tissues.

Image Source: By OpenStax CC BY 3.0, via Wikimedia Commons

The functions of lymphoid organs

Bone marrow: Primary lymphoid organ responsible for hematopoiesis, the process of forming red blood cells, white blood cells and platelets within the red bone marrow in the core of the bone.

Thymus: Primary lymphoid organ where immature thymocytes mature into functional T-cells. Also the site of T lymphocyte selection to ensures that mature T-cells do not respond to human tissues.

Lymph node: Filters interstitial fluid before it returns to

the circulatory system. Lymph nodes are also the site where antigen is presented to B-cells and T-cells to produce an adaptive immune response when pathogens are encountered in the tissues.

Spleen: Filters blood during routine circulation. Rich in macrophages and dendritic cells that remove pathogens and dying red blood cells from the bloodstream. Infections within the bloodstream pose a serious risk, making the role of the spleen extremely important.

Tonsils: Lymphoid nodules that survey microbes in the upper digestive and respiratory tracts. Swollen tonsils can be an important sign of infection.

Mucosa-associated lymphoid tissue (MALT): Various tissues throughout the body that protect regions that are particularly vulnerable to infection. These tissues vary in name by location (e.g. **GALT**=Gastrointestinal; **BALT**=Bronchus airway). The **Peyer's patch** is an important MALT of the small intestine. MALT can also be found within the **appendix**. Intestinal blockage may lead to inflammation of the appendix, resulting in painful *appendicitis*.

4.3 Antigen

If phagocytosis does not resolve an infection, phagocytized pathogen fragments (*antigens*) are presented to T-cells in order to induce a much stronger *adaptive* immune response.

Recall that phagocytes detected pathogens based on general patterns (**PAMPs**) commonly found on the surface of multiple microbes (e.g. a cell wall is common to most bacteria). Conversely, the adaptive immune system responds to **antigens**, which are often specific to a particular pathogen. For example, a person may be exposed to influenza antigen to stimulate immunity through vaccination. However, these antigens are no found in other viruses as such the influenza vaccine will not protect against other viruses.

Antigens were traditionally named because they were capable of inducing **anti**body **gen**eration. However, it is now known that the adaptive immune response includes more than just antibody production. According to the current understanding antigen represents specific molecules that can be bound by specific antibodies or lymphocyte receptors (T-cells and B-cells). However, binding antigen alone is not always sufficient to trigger a productive adaptive immune response. Antigens that **gen**erate an adaptive **immune** response are called **immunogens**.

Antigenicity

Another important concept related to antigens is the typical intensity of antigen binding (*antigenicity*), which often relates to the intensity of the induced response *(immunogenicity)*. Antigenicity depends largely on the three-dimensional of the molecule. Of particular importance is the precise part of the molecule where the antibody or lymphocyte receptor attaches designated the **epitope**. Epitopes are very small regions of the protein (*as small as 4-6 amino acids*) that adopt a unique three dimensional conformation. Owing to their role in antigen binding, epitopes are also called **antigenic determinants**.

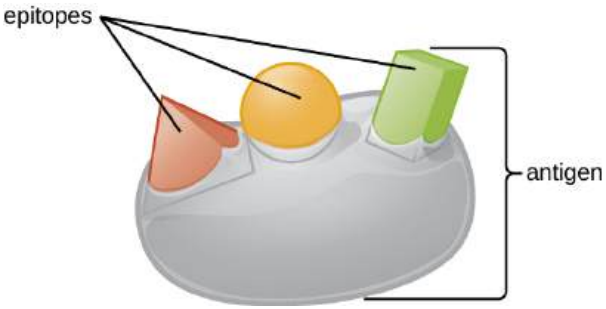

Figure 4-1 Antigen. An antigen is a specific molecule with one or more defined three-dimensional shapes, called 'epitopes'. This image illustrates one antigen with three different epitopes.

Image Source: By OpenStax CC BY 3.0, via Wikimedia Commons

Various characteristics of the antigen are important in determining its intrinsic antigenicity.

Size of the antigen is important because the substance must be large enough to physically interact with immune cells. Typical protein units (peptides) range 10-30 kDa in size. While antigenic molecules less than 10 kDa in size are only weakly immunogenic. For example, the molecules in perfumes and scented detergents are so small that they rarely trigger new immune responses. However, once a person develops an adaptive immune response toward these perfumes this response may be trigger whenever the molecules are encountered, in the form of an allergic response.

- *If small molecules are poorly immunogenic, how*

do people develop adaptive responses (including allergies) toward them? Small antigens that are antigenic but not immunogenic are called **haptens**. By attaching haptens to larger molecules, called **carrier** molecules, an immune response can be triggered against the hapten-carrier complex, which will potentially provide long term immunity. This strategy is used in development of some vaccines. For example, *Haemophilus influenzae* bacteria have a capsule coat made of sugars too small to serve as vaccine immunogens. Physically attaching (*conjugating*) these sugar molecules (*hapten*) to larger inactivated tetanus toxin molecules (*carrier*) produces a new conjugate molecule that is sufficiently immunogenic for use in vaccines.

Proteins are typically more immunogenic than polysaccharides (sugars) or lipids (oils and fats). However, the other characteristics of the molecule may enhance the immunogenicity of polysaccharides and even lipids.

Complex molecules with several distinct epitopes are more likely to be immunogenic than microbes with few epitopes or molecules that simply consist of a long chain where the same epitope structure is repeated. For example DNA and RNA are large molecules bound by proteins but their regular repeating structure ensures that they are less frequently able to trigger an adaptive response.

Accessibility of epitopes is often difficult to predict because molecules may form complex three-dimensional structures. However, immunogenic antigens typically have the relevant epitopes on the outer surface, where they are more readily detected by immune cells.

Foreignness of the molecule within the human body is a consideration in its immunogenicity. The adaptive immune system has a mechanism for *tolerance* for human cell antigens as well as antigens that frequently occur within the microbiome.

- As is the case with haptens, immune tolerance only makes *human-like* antigens less immunogenic. These molecules may still be antigenic once the adaptive response is initiated. This is exemplified in the context of **molecular mimicry**, where a microbe may have antigen structures that mimic human antigen. The outcome of molecular mimicry is that the immune response toward a pathogen may **cross-react** to bind unrelated proteins on human tissues. *Streptococci* bacteria have a surface protein called the M-protein, which has similar epitope structure to human mysoin/tropomysin found in heart muscle. While these epitopes are poorly immunogenic, during a severe infection the adaptive response may be triggered to react toward them. Once the adaptive response is triggered, antibodies will continue to be produced against the M-protein and cross-react to also bind proteins in heart muscle. The outcome is that the streptococcal infection will be effectively cleared but the antibodies will produce chronic illness, presenting as carditis and rheumatic fever.

Classification of Antigens

Autoantigens represent the body's self-recognition system. These antigens emerge from an individual's own tissues and cells, reflecting the inherent distinction between the body's components and potential threats. As such, cells of the adaptive immune system undergo a strict selection process to ensure that they do not react significantly to these 'self' antigens. In many cases, self-reactive cells are killed and deleted during maturation. Alternatively, the lymphocyte receptor that dictates the specificity antigen recognition may be modified to no longer detect self-antigen, or the cells may be regulated to reduce the amount of this receptor protein on the cell surface.

However, in certain circumstances, the immune system can erroneously trigger adaptive responses against autoantigens. The example of streptococcal M-protein and human myosin cross-reactivity, described above, is an example of such an erroneous event. The outcome of adaptive response to self-antigen is **autoimmune disease**, which can manifest in different ways depending on the localization of the autoantigen in the body. For example, autoimmune reactions against the human pancreas can lead to destruction of the tissues that produce and secrete insulin, resulting in type 1 diabetes.

Alloantigens reflect the diversity of each person's unique genetic identity. Alloantigens are differences in antigen structure between individuals within the same species. While variation may occur in virtually every protein that is expressed by our cells, the most significant variation is found in the protein structures that make up the major histocompatibility complex (MHC). The MHC plays a pivotal role in immune detection of pathogens (*described later*). To prevent microbes from adapting to overcome detection, the MHC has evolved extensive variation such that identical matches between any

two people by chance alone is highly unlikely. The significance of alloantigens lies in their involvement in transplantation and transfusion reactions. When the immune system encounters foreign MHC molecules in transplanted organs or transfused blood, they might be perceived as non-self evoking a rigorous immune response. The result of this immune response is that the transplanted tissue is *rejected* and destroyed by the recipients immune system.

Heterophilic antigens bridge the gap between species. Heterophilic antigens occupy a unique niche in immunology by fostering connections between different species. The heterophilic antigen has at least one epitope that is found in one species but can induce an immune response when encountered by the immune system of another species through **cross-reactivity**. This effect is directly exemplified by the streptococcal M-protein and human myosin cross-reactivity, described above. In medical laboratory testing, heterophilic antigens can lead to false diagnoses that might profoundly impact clinical decision and the welfare of people who rely on them. For example, testing for the syphilis, a sexually transmitted infection (STI), may rely on detecting antibodies against cardiolipin, a component of the bacterial membrane. However, cardiolipin is also found in the mitochondria of human cells and may be externalized in some severe conditions, such as lupus. As many as 50% of lupus patient will make antibodies against the heterophilic cardiolipin antigen and will test positive for syphilis even though they do not have the STI.

4.4 Lymphocytes and Lymphocyte Receptors

Lymphocytes are a subset of the leukocytes (white blood cells)

that include **B-cells**, **T-cells** and **natural killer (NK) cells**. NK cells are similar to cytotoxic T-cells, but kill target cells following detection of PAMPs and DAMPs, making NK cells components of the innate immune response. This chapter will focus on the T-cells and B-cells as lymphocytes responsible for the adaptive immune response. T-cells and B-cells have cell surface proteins called **lymphocyte receptors** that are structurally different but function similarly in determining the *specific* antigen that these cells will respond toward.

Each B-cell and T-cell develops from the common **hematopoietic stem cell** and mature into functional lymphocytes. During maturation, they develop **lymphocyte receptors** that are unique in shape from one cell to another. The mechanism by which this occurs will be described in Chapter 5. The outcome of this maturation process is that populations of T-cells and B-cells emerge, called **clones**, each containing a unique receptor that will bind to only a single epitope. B-cells have the ability to secrete their lymphocyte receptor (**B-cell receptor, or BCR**) as an immunoglobulin that acts as an antibody in the immune response. Immunoglobulins and antibodies will be discussed in greater detail in Chapter 5. In Figure 4-2, we observe that three clones of B-cell have independently produced antibodies. Since the BCR of the originating clone was different, the shape of the secreted antibodies are also sufficiently different that they bind only to their matching epitope.

Since antigens almost invariably have more than a single epitope, the antibodies produced against them are a mixture of antibodies from multiple different B-cell clones. As a consequence, the antibodies produced in a natural immune response are called **polyclonal antibodies**. In the laboratory, it is possible to isolate single B-cells and to form a clonal population of cells , which produce the same antibody that all bind to only a single epitope. These laboratory-derived antibodies are called **monoclonal antibodies**.

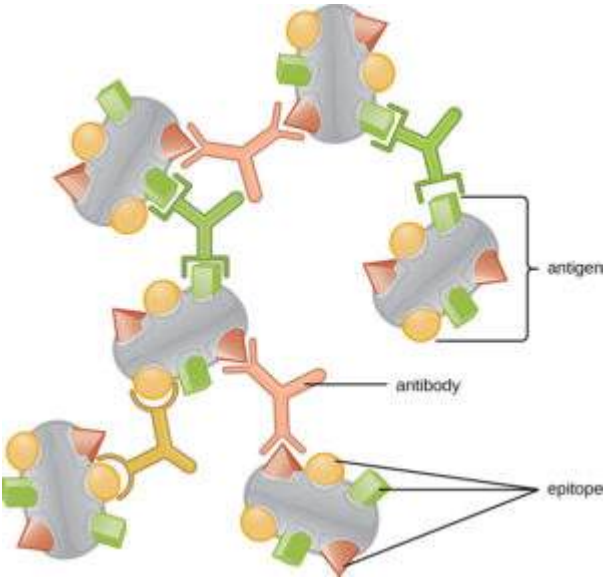

Figure 4-2 Polyclonal Antibodies. Antibodies are B-cell receptors that are secreted into blood and other tissues. Each B-cell clone has a receptor for a single epitope. Since antigens have multiple epitopes, they typically trigger the production of antibodies from multiple B-cell clones, making the antibody mixture *poly*clonal.

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4.5 Antigen Presenting Cells

In the previous section, we introduced *clones* of B-cells and T-cells each binding and responding to different epitopes. Once these cells respond to the bound antigen, they set in action a cascade of events that may produce a longterm immune response toward the antigen, commonly known as 'immunity'.

However, the adaptive immune response is not triggered by antigen freely floating in the interstitial space between cells. Instead, there are a special subset of **phagocytes** called **antigen presenting cells (APCs)** that display potential antigen molecules on their surface and assist in the initiation of the adaptive immune cascade. These antigen presenting cells include **macrophages**, **dendritic cells** and **B-cells**.

In a standard immune response, **macrophages and dendritic cells** phagocytose, degrade and process pathogen cells and debris, and then present the molecular fragments on their surface to elicit a response from an important T-cell subpopulation called **helper T-cells**. The helper T-cells then activate other T-cells and/or B-cells to produce a tailored and appropriate response to the infectious agent. This antigen presentation process is performed by a large molecular complex called the **major histocompatibility complex, type 2 (MHC-II).**

Like macrophages and dendritic cells, B-cells are also APCs. However. unlike macrophages and dendritic cells, B-cells *only* phagocytize cells or debris that have a matching antigen to the BCR. B-cells then present internalized antigen to already activated helper T-cells and the B-cell becomes activated in response to this interaction.

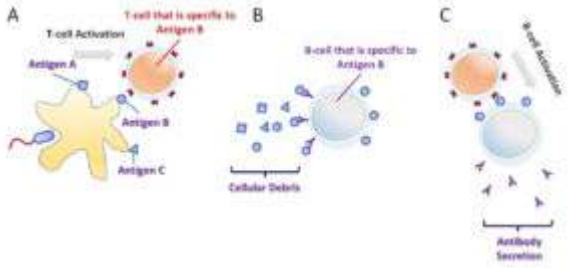

Figure 4-3 Antigen Presentation. (A) A macrophage or a dendritic cell phagocytize a pathogen and presents its antigens on the cell surface. A helper T-cell that is specific to one antigen binds the APC and becomes activated. (B)

Independently, cellular debris flows in the vicinity of a B-cell. The B-cell receptor is specific to one antigen and this antigen is phagocytized and presented by the B-cell. (C) When there is contact between an activated helper T-cell and a B-cell recognizing the same antigen, the B-cell becomes activated and release their antigen-specific B-cell receptor as antibodies.

A secondary form of antigen presentation involves the **major histocompatibility complex, type 1 (MHC-I)**. This process is performed by all nucleated cells, not just by phagocytes. Proteins that are produced within the cell are routinely shuttled to the cell surface and presented by MHC-I. Abnormal proteins, such as those produced as a result of viral infection or within a tumor cell may be detected by **cytotoxic T-cells.** While helper T-cells function by triggering a broader immune response to an antigen, cytotoxic T-cells simply kill the cell that presents the foreign antigen. The cytotoxic response is an effective way to prevent the spread of infection from one cell to another. However. in the context of a large-scale or chronic infection, the cytotoxic response can kill cells that perform an important function and thereby severely damage the human tissue.

A classic clinical example where cytotoxic T-cells severely damage human tissue is seen in organ transplant rejection. When an individual receives an organ transplant, such as a kidney or heart, from a donor, the immune system of the recipient recognizes the transplanted organ as foreign due to differences in the major histocompatibility complex (MHC) molecules themselves. The cytotoxic T-cells will destroy the grafted tissue because of these foreign molecular markers and *reject* the transplanted tissue.

The adaptive immune response begins with phagocytosis by macrophages and dendritic cells. APCs will process the phagocytized pathogen and present it on the cell surface using a molecular complex called the **major histocompatability complex** (MHC).

MHC-I – All nucleated cells present elements of the proteins they produce, using the MHC-I complex. If the cell is infected by a virus or develops into a tumor cell, the aberrant proteins produced from those events are presented on the cell surface to alert immune cells to this problem.

MHC-II – In addition to MHC-I presentation, some cells also present products of phagocytosis using the MHC-II complex. These *professional* **antigen presenting cells** include macrophages, dendritic cells, and B-cells. Other immune cells can present antigen to a lesser degree.

MHC and Tissue Transplantation

In transfusion medicine, the **MHC** is known by a different name: **Human leukocyte antigen, or HLA**. MHC-I and HLA-I are synonymous, as are MHC-II and HLA-II. All of the genes that make up the HLAs are clustered together on chromosome 6, and are often described as a "molecular barcode" that is virtually unique to every person.

- **Human MHC-I** consists of three genes, or **isotypes**, called HLA-A, HLA-B, HLA-C.
- **Human MHC-II** also consists of three **isotypes**, called HLA-DP, HLA-DQ and HLA-DR.

Recall that MHC-I antigens will be expressed on all nucleated cells, while MHC-II are mainly restricted to antigen presenting cells (monocytes, dendritic cells, macrophages, B-cells).

For each of these isotypes there are thousands of possible

variations from one person to another, creating an incredible diversity of possibilities. As such, the possibility of perfectly matching all six isotypes between two individuals at random is virtually impossible.

Making a perfect match even less likely because the set of HLA-A, -B, -C, DP, -DQ and -DR on one chromosome constitutes only half of the entire repertoire, called a **haplotype**. Because of **codominant genetics**, a person inherits two distinct chromosomes. These chromosomes originate from each of the two parents. The result is that perfect matching would require a match of 12 different isotypes, where each isotype has thousands of possible variations.

In the context of a tissue or organ transplant from a donor to a recipient, it might seem virtually impossible to establish a match and avoid rejection. It should be noted, not all mismatches are equally deleterious.

- Different isotypes occur at **different frequencies** in human populations. Since HLA isotypes are inherited, these frequencies depend on a person's ancestry.
- Not all HLAs equally immunogenic. The most immunogenic is **HLA-DR, followed by HLA-B and -A**. These 3 loci are therefore the most important for matching donor and recipient.
- Some **mismatches are tolerated** better than others because of a person's intrinsic immune tolerance.

Consequently, determining the *best* donor-recipient match is a complex task but can have a significant impact on the success of a tissue or organ graft.

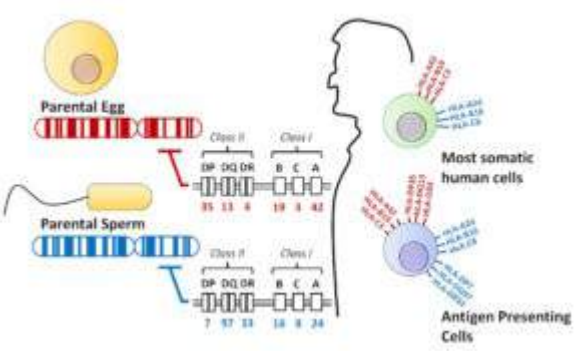

Figure 4-4 MHC Genetics. Class I and class II MHC/HLA are both encoded on chromosome 6. Each person receives two copies of this chromosome from the parental egg and sperm. Most somatic cells only express the class I MHC. Antigen presenting cells express both class I and class II MHC on their surface.

4.6 Coordination of the Components of the Adaptive Immune Response

Each component of the adaptive immune response acts in a coordinated an orchestrated manner with one another.

The adaptive immune response emerges from a persistent innate immune response to infection, as shown in the series of events below.

Persistent infection induces **inflammation** and recruitment of **antigen presenting cells** to the tissue, such as macrophages and dendritic cells.

Antigen presenting cells **phagocytose** pathogen and debris, presenting the foreign material on the cell surface using **MHC-II**.

Helper T-cells with T-cell receptors that match this particular foreign antigen will bind with the antigen presenting cells and signal a broader adaptive immune response.

The adaptive immune response may include activation of **cytotoxic T-cells**, which scan all proteins presented by **MHC-I** on the surface of almost all cells in the body. If foreign protein is produced inside the cell (due to infection or cancer) that antigen will be detected and the aberrant cell will be killed.

The adaptive response may include activation of **B-cells**, which release their B-cell receptors as antibodies to support in the defense against infection.

One outcome of the adaptive immune response is that future infections will normally elicit a faster and stronger response, which we often regard as "**immunity**"

Summary

- The adaptive immune system recognizes antigens found on the surface of pathogens or foreign substances.
- Antigen recognition is mediated by specialized lymphocytes, primarily B cells and T cells.
- Antigen-presenting cells (APCs), such as dendritic cells, engulf and process pathogens, breaking them down into smaller antigen fragments.
- These antigen fragments are then displayed on the surface of APCs along with major histocompatibility complex (MHC) molecules.
- T cells are activated when they recognize antigens presented by APCs. There are two main types of T cells: helper T cells (CD4+) and cytotoxic T cells (CD8+). Helper T cells stimulate other immune cells, while cytotoxic T cells directly target and kill infected cells.
- B cells are activated when they encounter antigens matching their specific antibody receptors. This activation leads to the production of antibodies, proteins that can bind to and neutralize the pathogen.
- Human leukocyte antigen (HLA) play a pivotal role in organ transplantation.
- HLA molecules are responsible for presenting antigens to immune cells, particularly T cells. They serve as a bridge between the innate and adaptive immune responses.
- HLAs are highly polymorphic, meaning they exist in numerous different forms or alleles within the human population. This diversity allows the immune system to recognize a wide range of antigens.

- Matching HLAs between donors and recipients is critical for the success of organ and tissue transplantation. A close HLA match reduces the risk of graft rejection, as the immune system is less likely to recognize the transplanted organ as foreign.

Chapter Review

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://pressbooks.bccampus.ca/appliedimmunology/?p=34#h5p-10

Case Study Review

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://pressbooks.bccampus.ca/appliedimmunology/?p=34#h5p-9

Chapter 5: Development and Stimulation of Adaptive Immune Response

Learning Objectives

By the end of this chapter you will be able to:

- Outline the series of events that occur during the adaptive immune response
- Identify the different T-cell populations and how they regulate the immune response
- Describe the origin and development of T and B cells
- Discriminate between the humoral and cell mediated immune responses
- Describe the cytotoxic mechanism of cytotoxic T-cells, and related lymphocytes
- Explain the role of memory cells in protecting

the body from reinfection

- Distinguish between the T-dependent and T-independent humoral immune response

Case Study

After finishing college Bernice decided to experience international travel. As a health conscious traveller, Bernice received the necessary travel vaccines. Her vaccines included the hepatitis A vaccine, since this viral infection spreads from contaminated food and water in places throughout the world and can produce acute liver damage, leading to jaundice, nausea, vomiting and lethargy.

The hepatitis A vaccine involves injecting inactivated virus into the human body and using two doses. While travelling, Bernice was not exposed to hepatitis A. She returned home healthy and tremendously enriched by the experience. Five years later, Bernice consumed a raw oyster that was contaminated with hepatitis A. Bernice was infected by the virus but did not have any noticeable illness. Bernice's immune system rapidly cleared the infection underscoring the significance of vaccination in bolstering the body's immune repertoire and enhancing its ability to combat infectious diseases.

1. *Why does Bernice need travellers' vaccines when she received multiple vaccines, including hepatitis A, as a*

child?

2. *When should Bernice get the vaccine, prior to travelling?*
3. *Why did Bernice experience no symptoms when infected by hepatitis A virus from oysters? Could a vaccinated person develop symptoms of infection?*
4. *What mechanisms allow the immune system to eliminate viruses, like hepatitis A?*

Answers to these questions are at the end of the chapter.

5.1 Lymphocytes Diversity

As mentioned in the previous chapter, lymphocytes include T-cells, B-cells and natural killer cells. Lymphocytes can be further divided into cell subsets with distinct and important roles in the immune response. The most diverse lymphocyte is the T-cell. T-cells can be broadly divided into **CD4** and **CD8** cells, based on "clusters of differentiation" (CD) proteins on their surface.

CD4 binds MHC-II facilitating the previously described interaction between **helper T-cells** ($CD4^+$) and **antigen presenting cells** (APCs, $MHC\text{-}II^+$). When APCs present antigen to CD4 helper T-cells, those T-cells can stimulate a potent adaptive immune response. However, there are multiple types of CD4 cells that can produce a tailored immune response to different antigens as shown below. CD8 binds MHC-I and allows cytotoxic T-cells to perform surveillance on all nucleated human cells. If these CD8 cytotoxic T-cells detect foreign antigen on the surface of a cell, they presume the cell is infected and destroy it.

B-cells can also be subdivided based on the context of antibody secretion. The cells that secrete antibody are

designated **plasma cells.** Alternatively, B-cells may form **memory cells** that contribute to longterm immunity.

T-cells have an antigen-specific receptor, called a "T-cell receptor" and participates in the **cell-mediated adaptive** immune response. Upon binding their antigen, T-cells may have different responses, depending on the type of T-cell:

- **Type 1 Helper T-cell ($CD4^+$ Th1)** – Effector cells that secrete cytokines to stimulate an immune response. If human cells are *infected* by intracellular pathogens (e.g. viruses or bacteria), or have developed into tumor cells, the Th1 will stimulate cytotoxic T-cells to kill the infected cell. Th1 also stimulate innate immune cells, such as macrophages, neutrophils and NK cells.
- **Type 2 Helper T-cell ($CD4^+$ Th2)** – Effector cells that secrete cytokines to stimulate an immune response. Coordinate the humoral response that involved B-cell differentiation and secretion of antibodies.
- **Type 17 Helper T-cell ($CD4^+$ Th17)** – Effector cells that secrete proinflammatory cytokines to stimulate an immune response. Important in defense against extracellular bacteria and fungal of the skin and **mucous membranes**.
- **Regulatory T-cell ($CD4^+$ Treg)** – Cells that regulate and often suppress the antigen-specific immune response. Secretes cytokines that promote tolerance to microbes that are normally resident to the human body and suppresses immune responses against the body's own tissues (autoimmune responses).

- **Cytotoxic T-cell ($CD8^+$ CTL or Tc)** – These cells can bind MHC-I on the surface of nucleated cells and monitor antigens produced within the cell. If the cell has recognized abnormal antigen, indicative of infection or tumor aberrant protein expression, the cell is killed. The CTL releases **granzymes** to trigger **apoptosis** controlled cell death in the target cell as well as **perforins** to form pores that disrupt the cell surface and lyse (*burst*) the cell.
- **Memory T-cell –** Similar to memory B-cells, memory T-cells persist after the infection and result in a faster and more exaggerated secondary immune response, for future infections with the pathogen.

B-cells have an antigen-specific receptor, called a "B-cell receptor" and participates in the **humoral adaptive** immune response. Like monocytes, B-cells phagocytose pathogens with these antigens on their surface. Also like monocytes, B-cells present antigen on their surface. In contrast to monocyte antigen presentation, B-cells do not activate T-cells but instead *become* activated through this interaction. B-cells differentiate into **plasma cells** that secrete their B-cell receptor as **antibodies**. Some B-cells also differentiate into **memory cells** that persist after the infection and can stimulate a more effective *secondary* response upon future exposure to the pathogen.

Natural Killer (NK) cells are cells of the **innate immune response** and recognize **PAMPs** using cellular receptors like those found in neutrophils. Unlike neutrophils, NK cells are not phagocytes. They kill the target cell using the same mechanisms employed by cytotoxic T-cells.

Maturation of Lymphocytes

As with other white blood cells (leukocytes), B-cells, T-cells and NK-cells (lymphocytes) are produced through the process of hematopoiesis (see Chapter 2.3). B-cells and T-cells undergo further maturation, which involves the development of lymphocyte receptors, called **B-cell receptors (BCR)** and **T-cell receptors (TCR)**. During maturation, these receptors undergo genetic **V(D)J recombination** to produce a unique receptor on each lymphocyte that is specific to a single target antigen (or few structurally related antigens).

> **V(D)J Recombination** allows individual T-cells and B-cells to bind different antigens. Since lymphocytes are constantly being produced in large number, the combination of all of the antigens that can be bound by B-cells and T-cells is known as the **immune repertoire**. The BCR and TCR receptors are different in structure but are composed of multiple interconnected chains that are held together by disulfide chemical bonds.
>
> - BCRs are made of four chains that include a pair of 'heavy chains' and a pair of 'light chains' that attach to form a characteristic 'Y' shape.
> - TCRs are typically formed from two attached chains, called the alpha (α) and beta chains (β).
> - In some T-cells, alternative gamma (γ) and delta (δ) chains make up the TCR.

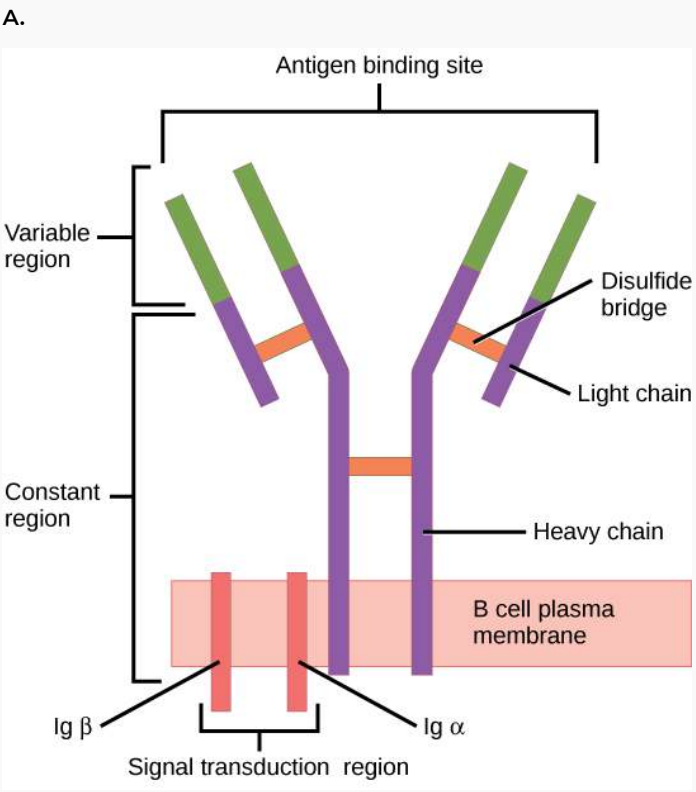

Figure 5-1 Lymphocyte Receptors. (A) B-cell receptor is a membrane bound immunoglobulin that consists of four polypeptide chains: two light chains and two heavy chains. This produces two sites within the 'variable regions' for antigen binding on each immunoglobulin molecule. (B) T-cell receptor consists of two membrane-bound polypeptide chains that come together to form a single antigen-binding 'variable region'.

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Commons; (B) By OpenStax CC BY 3.0, via Wikimedia Commons

V(D)J recombination is achieved using **RAG proteins** that remove DNA from the part of the lymphocyte genome that codes for the variable region of the lymphocyte receptor, which is the region that binds antigen. The diversity of these chains occur because there are many repeating copies of the relevant gene segments, which are named *Variable*, *Diversity* and *Joining* genes. The RAG proteins remove all copies except one of each gene segment.

- The BCR heavy chain as well as the TCR β-chain undergo VDJ recombination, where the peptide receives three randomly selected genes.
- The BCR light chain and TCR α-chain undergo VJ recombination.

Therefore, a BCR antigen binding site that consists of both heavy and light chain (**Figure 5-1**) combines five gene segments that were each randomly selected from a collection of segments (**Figure 5-2**). This produces a tremendous range of diverse antigen binding sites and gives each lymphocyte its unique binding properties.

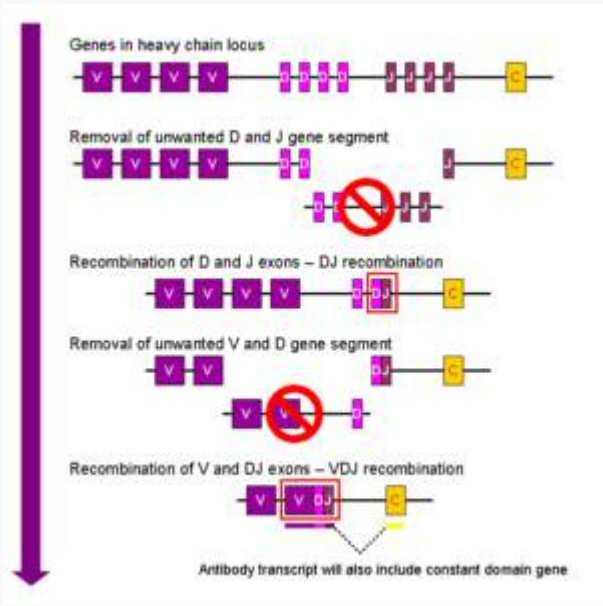

Figure 5-2 VDJ Recombination. A simplified diagram of how single variable (V), diversity (D) and Joining (J) gene segments are selected randomly from a collection and combined to achieve a tremendous diversity of antigen binding sites in B-cell and T-cell receptors.

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Once a B-cell or T-cell develops a unique receptor by VDJ recombination, all the progeny cells that are produced from this cell will also have that same lymphocyte receptor. This is called a ***clonal population*** and these lymphocytes that all

recognize the same antigen are called ***clones***.

5.2 Selection of Lymphocytes During Maturation

Lymphocytes selection is described by the ***clonal selection theory*** and involves two basic steps:

1. **Positive selection** promotes development of functional cells and eliminates any non-functional cells by apoptosis cell death.
2. **Negative selection** ensures that the lymphocytes do not harm human tissues by eliminating *auto-reactive* cells that bind human tissues with '*self antigen*'.

B-cell maturation and selection occurs in the **bone marrow**. First, during **positive selection** a *checkpoint* confirms that the **heavy chain** is functional and can bind a surrogate light chain. If this holds true the pre-B cells avoid death by apoptosis and instead proliferate rapidly. Next, **negative selection** involves testing the B-cell receptor, called IgM, against *human antigens* within the bone marrow. If B-cell clones are auto-reactive and bind human tissue, they may have an opportunity to edit their light chain. Immature B-cells that still have self-reactive receptors are retained in the bone marrow and are eventually removed in a process called **clonal deletion**. B-cells that survive both positive and negative clonal selection develop a second

IgD receptor and are released into the bloodstream. These functional B cells migrate to lymph nodes and other secondary lymphatic tissues.

T-cells are also produced in the bone marrow but mature and undergo selection in the **thymus**, in a process called *thymic education*. T-cells enter the thymus as immature **double negative (DN) thymocytes,** because they lack the expression of both CD4 and CD8 co-receptors. These thymocytes initially express both CD4 and CD8 within the thymus, to become **double positive (DP) thymocytes**. To survive, DP thymocytes must bind to *self-antigen* on the surface of thymus cortical epithelial cells. After binding these T-cells receive *survival* signals, leading to the differentiation of either CD4+ or CD8+ **single positive (SP) thymocytes**.

The need for T-cells to bind *self-antigen* may seem counter productive to the role of these cells in detecting *foreign* pathogens. However, deeper within the medullary region of the thymus, the self-reactive cells are eliminated by negative selection. **Negative selection** involves the interaction of SP thymocytes with medullary *"nurse"* epithelial cells presenting a diverse range of self-antigens. SP thymocytes with high-affinity interactions to self-antigens are eliminated through apoptosis, preventing the development of T-cells that would be overly reactive against self-tissues. This process is crucial for establishing immune tolerance and preventing autoimmune responses.

> The outcome of clonal selection during maturation are a population of lymphocytes that are capable of binding to target antigen but are poorly reactive to self-antigen.

Importance of leukocyte clusters of differentiation (CD) proteins

CD proteins are antigens on the surface of leukocytes that have been adopted as potent markers of cell identity and maturation. We have already alluded to the importance of these proteins when describing CD4 and CD8 as markers for T-cell subtypes. However, CD proteins can also be used more broadly to define specific immune cell populations, maturation state or even the emergence of tumor cells.

Important CD protein cell markers

- **Hematopoietic stem cells** give rise to all red blood cells, white blood cells and platelets. As a consequence, identification and extraction of these cells are a critical part of **bone marrow transplant** to recapitulate a failing immune system. The central marker for hematopoietic stem cells is **CD34**.
- **T-cells** have the common shared feature of a **T-cell receptor**. The **CD3** protein is a defining component of the T-cell receptor. For example, helper T-cells are $CD3^{+}CD4^{+}CD8^{-}$ and cytotoxic T-cells are $CD3^{+}CD4^{-}CD8^{+}$.
- **B-cells** in their naive (non-activated) form have the surface proteins **CD19** and **CD20**.

5.3 Primary and Secondary Immune Response

Another phase of **positive selection** occurs when lymphocytes are exposed to their TCR-matched antigen. Following antigen presentation, helper T-cells secrete cytokines to stimulate their reproduction. Similarly, helper T-cells stimulate proliferation of B-cells, upon activation. This process of ***clonal expansion*** is a hallmark of the adaptive immune response and results in up to a thousand-fold increase in antigen-specific lymphocytes. Furthermore, some lymphocytes differentiate into **memory** cells that survive as a population for extended periods of time. Memory lymphocytes are immediately activated by antigen and do not require antigen presentation, making the secondary response faster. These memory lymphocytes are responsible for long-term 'immunity' to specific pathogens.

Consequently, the first time a pathogen antigen is encountered, the *primary* immune response that consists of a gradual enhancement in pathogen-specific immune defense. Every subsequent exposure to pathogen produces a *secondary* immune response than produces a faster and more effective immune defense.

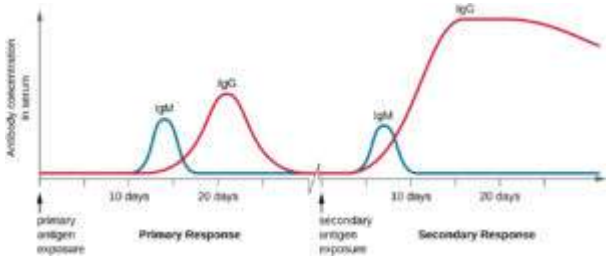

Figure 5-3 Primary and Secondary Immune Response. On first exposure to a pathogen, some antibodies (IgM and IgG) are produced. By the end of the first exposure, clonal expansion and memory lymphocytes are produced. This causes the

secondary immune response to respond more quickly and to produce a far greater abundance of antibodies.

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Table 5-1. Contrast of Plasma B-cells and Memory B-cells

PLASMA B-CELLS	MEMORY B-CELLS
Produced is **large number** during infection.	Produced is **small numbers** following infection.
No surface immunoglobulin because it was secreted as antibody.	Retains **surface immunoglobulin**, typically IgA or IgG.
Short lifespan (*days*).	**Long lifespan** as the cell population is capable of self-renewal.
Actively **secretes antibody**.	Provides **long term immunity** through rapid response to future infection.

When a pathogen is first encountered by the immune system, there is a massive expansion of lymphocyte clones specific to that pathogen's antigens. Some lymphocytes become long-lived memory cells that make any subsequent secondary response faster and more effective.

5.4 Humoral Response: Antibody Production

The word *humoral* refers to the body fluids, where protective **antibodies** may be suspended to protect us from pathogens. Antibodies are **B-cell receptors** that are secreted into the bloodstream. Like the lymphocyte receptor, they will bind specifically only to pathogens that have the matching surface antigen molecule. Antibodies do not directly harm the target microbe. This fact is important because it means that antibodies can be used as tools to label and detect cells without harming them. Within the body, cells that are bound by antibodies are more likely to be phagocytized or bound by complement proteins that cause cell lysis.

B-Cell Activation

Naive B-cells have undergone V(D)J recombination and therefore each have an antigen-specific B-cell receptor. These

naive B-cells **phagocytose** pathogen and debris but phagocytosis is not their main function since they only phagocytose the specific antigen, matching the B-cell receptor. Rather, the B-cell acts as an **antigen presenting cell (APC)** by displaying parts of the pathogen on its surface using the MHC-II complex.

Other APCs (e.g. **macrophage**, **dendritic cell**) use phagocytosis as their primary function, consuming a broad range of pathogens with surface **PAMPs**. These APCs present antigen similarly to a B-cell (**Figure 5-4**).

Both B-cells and other APCs interact with T-cells, but the outcome of these interactions are different. When a macrophage migrates from the site of infection with pathogen **PAMP** on its surface, it will be bound by an antigen-matched helper T-cell (Th). The **Th cell receives stimulatory signals from the macrophage** to become activated and can differentiate into more specialized cell subsets (*see section 3.4*). If the Th differentiates into the **Th2 subset**, these cells will specialize in B-cell activation. When a B-cell consumes and presents the same antigen to the Th2 cell, the **B-cell receives signals to become activated** and differentiate toward antibody secreting **plasma cells** as well as a less mature state representing the **memory cell**. In this way, cell activation is relayed from the macrophage to the Th cell and finally to the antibody-producing B-cell.

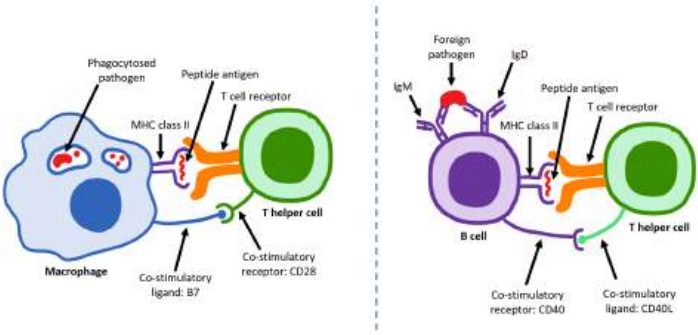

Figure 5-4 B-Cell Activation. (Left) At the site of infection,

macrophages broadly consume antigen and present them via MHC-II. These cells migrate toward a local lymph node, where they interact with and activate B-cells. (Right) Within the lymph node, the B-cell phagocytosed antigen that has filtered in from the infected tissue. Upon presenting this antigen to Th2 cells, the B-cell is activated.

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T-Dependent and T-Independent B-cell Activation

The section above describes activation of B-cells upon signalling from helper T-cells in a process called **T-dependent** B-cell activation. However some antigens have special characteristics that allow them to activate B-cells without T-cell signalling. **T-independent** B-cell activation can be classified into two types:

- **T-independent Type 1 (TI-1)** antigens have an epitope that is bound by the B-cell receptor, as is normal for B-cells. However, other parts of the antigen bind to toll-like receptors on the surface of the B-cell and provide the additional signalling needed to activate the B-cell.
- **T-independent Type 2 (TI-2)** antigens have a structure where the epitope is repeated multiple times. As the B-cell bind the antigen, B-cell receptors cluster together and crosslink so they can bind the repeating epitopes. The clustering B-cell receptors provide activation signals to one another to activate the B-cell.

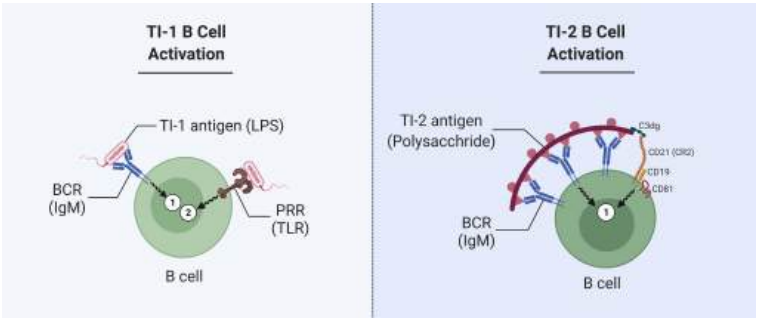

Figure 5-5 T-Independent B-cell Activation. TI-1 activation involves dual recognition of an antigen using a B-cell receptor (BCR) as well as a Toll-like receptor (TLR) in tandem to stimulate B-cell activation in a T-cell independent manner. TI-2 activation involves B-cell activation by an antigen with repeating units to provide a more intense activation signal and to induce T-cell independent B-cell activation.

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Superantigen

A superantigen causes B-cell activation in a T-cell dependent manner but bypasses the antigen-specificity of the T-cell. Superantigens elicit a massive and uncontrolled activation of T-cells by simultaneously binding to specific regions of the **TCR and MHC** molecules. *Staphylococcus aureus* is an example of a bacterial species that has adapted superantigens as toxins to support infection. The toxin TSST-1 produces a severe dysregulated cytokine response that results in potentially fatal toxic shock syndrome.

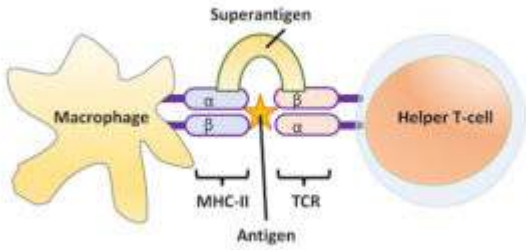

Figure 5-6 Superantigen. A macrophage presents antigen via MHC-II that is normally mismatched with the T-cell receptor (TCR). The superantigen binds the two receptors together, forcing T-cell activation despite the mis-match.

Antibody Secretion and Class Switching

Activated B-cells differentiate into **plasma cells**, which secrete their surface B-cell receptor to function as antibodies, and into **memory cells** that persist for long periods and contribute to the secondary immune response. Plasma cells initially secrete **IgM,** which is secreted as a immunoglobulin monomers but assemble into pentamers that consist of five immunoglobulins connected together by a **J-chain** peptide.

As the primary immune response progresses, activated B-cells undergo **class switching** that involves recombination of the DNA encoding the immunoglobulin Fc domain. Class switching produces new immunoglobulin isotypes with distinct characteristics.

> The functions of antibodies include:

Immunoprecipitation and Agglutination – Each immunoglobulin has two antigen-binding Fab regions. IgM pentamers have ten Fabs. Since each immunoglobulin can bind multiple antigen molecules, it can attach these molecules together and cause soluble molecules to become insoluble (*immunoprecipitation*). When the immunoglobulins connect multiple cells together, it causes the cells to clump together in the process of *agglutination*.

Opsonization – The Fc region of immunoglobulins are actively bound by phagocytes, making antigens and antibody-bound cells more likely to be phagocytosed.

Neutralization – Pathogens (bacteria, viruses) use proteins on their surface to adhere to and infect human tissues. By covering the surface of the pathogen, antibodies can prevent it from adhering to human tissues and infecting human cells.

Complement Fixation – The functions of antibodies are made significantly more potent by binding to complement proteins. Complement proteins form a MAC complex in the target cells and induce cell lysis. Complement proteins also enhance opsonization.

Antibody-Dependent Cell Mediated Cytotoxicity (ADCC) – The Fc region of immunoglobulins are bound by cells that may secrete toxins, such as eosinophils, macrophages and natural killer (NK) cells. This response is commonly used to eliminate pathogens that are too large to phagocytose.

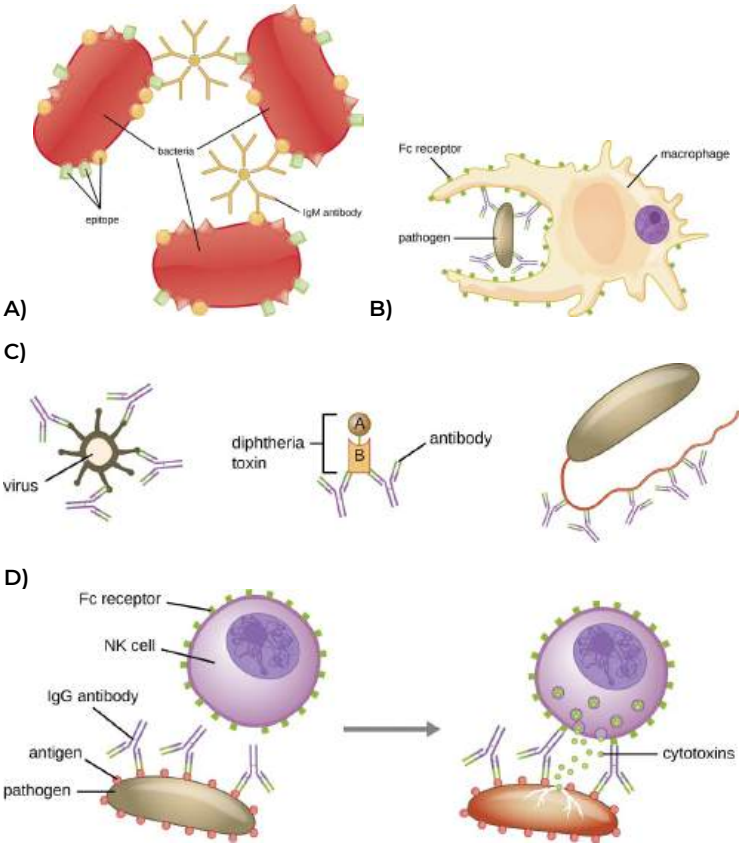

Figure 5-6 Effector functions of antibodies. (A) ***Agglutination*** involves attachment of antibodies to multiple cells in order to cause cells to clump together. (B) ***Opsonization*** occurs because phagocytes have receptors to the Fc region of some immunoglobulins. This causes the phagocyte to bind to and consume antibody-bound cells and material. (C) ***Neutralization*** occurs when antibodies bind the surface of cellular structures, viruses and toxins. The layer of antibodies prevent the agent from interacting with human cells, negating their harmful effects. (D) ***Antibody-dependent cell mediated cytotoxicity (ADCC)*** is similar to opsonization in that it involves

leukocytes binding the Fc region of immunoglobulins. However, the target cell is typically too large to phagocytose and is therefore eliminated by secretion of toxic substances.

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The Five Immunoglobulin (Ig) Classes					
Properties	**IgG monomer**	**IgM pentamer**	**Secretory IgA dimer**	**IgD monomer**	**IgE monomer**
Structure					
Heavy chains	γ	μ	α	δ	ε
Number of antigen-binding sites	2	10	4	2	2
Molecular weight (Daltons)	150,000	900,000	385,000	180,000	200,000
Percentage of total antibody in serum	80%	6%	13% (monomer)	<1%	<1%
Crosses placenta	yes	no	no	no	no
Fixes complement	yes	yes	no	no	no
Fc binds to	phagocytes				mast cells and basophils
Function	Neutralization, agglutination, complement activation, opsonization, and antibody-dependent cell-mediated cyotoxicity.	Neutralization, agglutination, and complement activation. The monomer form serves as the B-cell receptor.	Neutralization and trapping of pathogens in mucus.	B-cell receptor.	Activation of basophils and mast cells against parasites and allergens.

Figure 5-7 Characteristics of different immunoglobulin classes.

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5.5 Cell Mediated Response: Overcoming Intracellular Pathogens and Cancer

In *section 3.6*, we observed that antigen presentation may induce helper T-cells to differentiate into Th2 cells and promote the activation of B-cells. However, helper T-cells may also differentiate into **Th1 cells** that secrete a different collection of cytokines. These cytokines activate **macrophages** and promote development of **cytotoxic T-cells**.

The activated macrophages will be induced to phagocytose pathogen more efficiently and will secrete cytokines that promote inflammation at the site of infection. For this reason, the Th1 response is regarded as a pro-inflammatory immune response.

The cytotoxic T-cells have **CD8** on their surface, which causes them to attach to virtually all cells via binding MHC-I. Normal human cells routinely load peptides from inside the cell onto MHC-I and display these peptides on the cell surface. If a cell becomes infected or produces abnormal cancer proteins, those foreign proteins would also be displayed by MHC-I. The **T-cell receptor** then scans the MHC-I and peptides on the cell surface and, if an abnormal peptide is recognized, the cytotoxic T-cell will kill the target cell. Even though MHC-I displays normal human peptide as well as any viral or cancer peptide, cytotoxic T-cells will not respond to the normal peptides because of negative selection during maturation.

> ***The binding of a T-cell to its target depends on an immune synapse***
>
> When a T-cell encounters an antigen-presenting cell

(APC), one factor that decides whether the two cells will bind is compatibility with MHC type. Recall, CD8 binds MHC-I and CD4 binds MHC-II. However, a sustained **immune synapse** that results in T-cell activation requires an orchestrated interplay of molecular interactions between receptors on the T-cell and the APC. The central supramolecular activation cluster (cSMAC) forms, concentrating T-cell receptor (TCR)-MHC interactions, while the peripheral SMAC (pSMAC) and distal SMAC (dSMAC) arrange adhesion and signaling molecules. This dynamic structure facilitates the transmission of signals from the TCR complex to the T-cell's interior, initiating a cascade of events that lead to T-cell activation, proliferation, and subsequent immune responses.

The cytotoxic T-cell uses various methods to kill the target cell:

Perforins are related to complement proteins and are capable of producing pores in the target cell membrane.

Granzymes are enzymes that enter through the pores and induce apoptosis death in the target cell.

Fas ligand (FasL) is a protein on the surface of cytotoxic T-cells. FasL binds the Fas receptor on the target cell and induces an apoptosis suicide pathway in that cell.

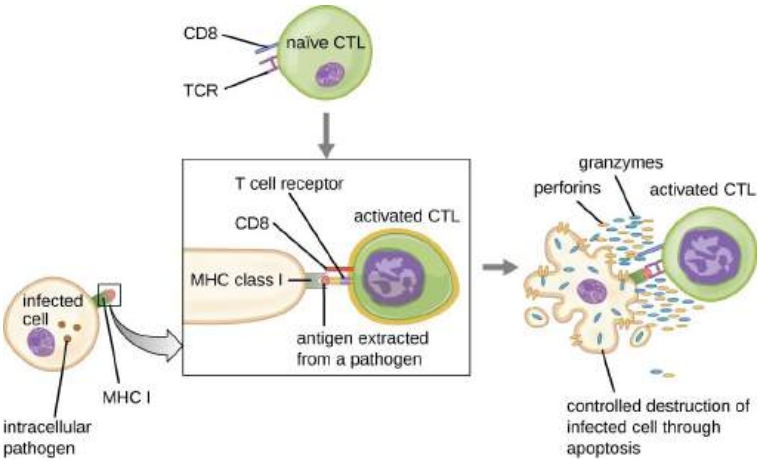

Figure 5-8 Effector function of cytotoxic T-cell. Human cells display their internal peptides using MHC-I. This means that an infected cell will also present the peptides from that intracellular pathogen. Cytotoxic T-cells are characterized by CD8 on their surface as well as an antigen-specific T-cell receptor. CD8 will attach the cytotoxic T-cell to MHC-I, while the T-cell receptor recognizes the pathogen peptide. Upon recognizing the infected cell, the cytotoxic T-cell will destroy the cell with granzymes and perforins.

5.6 Immune Tolerance

To function properly, the immune system must be able to recognize and respond to foreign invaders, such as pathogens, while avoiding inappropriate responses against the body's own tissues or microbiome that is a normal component of the body surface. **Immune tolerance** refers to the collective mechanisms by which the immune system prevents undesirable immune responses to self-antigens and commensal microbes.

Immune tolerance can be categorized into two types:

Central tolerance involves the negative selection process that occurs during the development of immune cells in the thymus (T cells) and bone marrow (B cells). Developing lymphocytes that recognize self-antigens with high affinity are eliminated or rendered non-functional. This ensures that only lymphocytes with the ability to recognize foreign antigens are allowed to mature and participate in immune responses.

Peripheral Tolerance involves suppression or elimination of self-reactive lymphocytes that are not eliminated by central tolerance. The mechanisms for peripheral tolerance include:

1. **Regulatory T cells (Tregs):** Like helper T-cells, Treg respond to specific antigen. However, they suppress the activity of other immune cells either through direct interaction with these cells or by secretion of immunosuppressive cytokines.
2. **Clonal Anergy and Clonal Deletion**: Anergy and deletion may occur in both B-cells and T-cells but their role in peripheral tolerance is characteristic of T-cells. Antigen presentation normally occurs in the context of inflammation, where the antigen presenting cell presents antigen via MHC-II but also expresses proteins called co-stimulatory proteins. If a T-cell interacts with antigen outside of the context of infection and inflammation, the T-cell clones may be deleted by apoptosis or may expand into a non-responsive cell population (*anergy*).
3. **Immune Privilege:** Certain tissues or organs in the body, such as the eyes, brain, and reproductive

> organs have specialized mechanisms that limit immune responses within their boundaries, reducing the likelihood of immune-mediated damage to these critical structures.

Summary

- B-cells and T-cells are lymphocytes that are involved in the immune response. B-cells secrete antibodies, while T-cells may induce an immune response (Helper T), suppress an immune response (Regulatory T) or kill virus-infected or cancer cells (Cytotoxic T).
- Each B-cell and T-cell has a unique receptor that is produced by randomly assorting genomic DNA segments, as V(D)J recombination. This process defines the antigen to which the lymphocyte will react.
- Lymphocytes undergo positive selection to ensure they are functional as well as negative selection that eliminates autoreactive cells.
- In addition to the 'effector' lymphocytes, B-cell and T-cell memory cells produce immunological memory that generates a faster and stronger immune response to future infections.
- B-cell activation requires that the B-cell binds its antigen via the B-cell receptor, phagocytoses and presents the antigen using MHC-II. Independently, an antigen presenting cell (APC) also phagocytizes and presents the same antigen. A helper T-cell must be activated by the APC and then interact with the antigen-bearing B-cell in order to secrete the cytokines needed to activate that B-

cell.

- B-cells can be activated independently of helper T-cells by additional toll-like receptor signalling (TI-1) or by repeating epitopes (TI-2).
- T-cells can be activated independently of a matched antigen via superantigen
- B-cells secrete antibodies that have five basic functions: (1) Agglutination/Immunoprecipitation, (2) Opsonization, (3) Neutralization or (4) Complement fixation, (5) Antibody-dependent cell-mediated cytotoxicity
- After B-cell activation, some cells undergo class switching. Each antibody class has different roles in the immune response.
- Cytotoxic T-cells kill virus-infected or cancer cells by lysing ("bursting") the cell using perforin proteins, or by inducing apoptotic death in that cell using granzymes or Fas ligand signalling
- Not all lymphocytes specifically induce immune response. Tolerance of self-cells or commonly present microbes may be achieved by elimination of some lymphocyte clones as well as regulatory T-cells to actively suppress the immune response.

Chapter Review

An interactive H5P element has been excluded from this version of the text. You can view it online

here:
https://pressbooks.bccampus.ca/appliedimmunology/?p=473#h5p-12

Case Study Review

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://pressbooks.bccampus.ca/appliedimmunology/?p=473#h5p-11

Chapter 6: Immunoprophylaxis

Learning Objectives

By the end of this chapter you will be able to:

- Explain the role of individual and herd immunity in public immunization programs.
- Distinguish between passive and active immunity as well as natural and artificial immunity.
- Describe the historical basis of vaccines and the different types of modern approaches for immunization.

Case Study

Alex was diagnosed with Common Variable Immunodeficiency (CVID) in their 30s. CVID is a primary immunodeficiency disorder characterized by a variable impairment of the immune system, specifically affecting antibody production. The first indications for Alex's condition were frequent

recurrent infections of the respiratory tract and ears. Blood tests showed hypogammaglobulinemia.

Alex has been hospitalized for infection several times and requires regular antibiotics treatments. Due to the impaired immune response, vaccinations are not effective for Alex. As a consequence, Alex relies on herd immunity, which is a strategy that uses vaccination or previous infections to reduce the overall spread of pathogens within the community.

Immunodiagnostic tests, such as ELISA, are used to monitor Alex' antibody levels and Alex receives intravenous immunoglobulin (IVIG) therapy in an effort to establish passive immunity and prevent severe infection.

1. *What do you think caused this immunodeficiency disorder in Alex?*
2. *Why might vaccines not work for Alex and how does herd immunity compensate for this situation?*
3. *How can components of the immune system be used to develop the immunotherapies (e.g. IVIG) that help keep Alex safe?*

Answers to these questions are at the end of the chapter.

6.1 Individual and Population Level Immunity

The term immunity is often used to describe the resistance to infection by a particular pathogen or to infectious disease achieved by engaging the adaptive immune response. The adaptive immune response produces memory lymphocytes that can be activated by antigen in a manner independent of antigen presentation. The resulting secondary immune

response occurs more quickly, is more intense and may eliminate a pathogen before a person experiences infectious disease. As such, there is a benefit when an otherwise healthy person recovers from infection because they develop immunity to protect themselves when their immune system is more vulnerable.

In addition to immunity in an individual person, the concept of immunity can be extended to entire populations. As more people recover from an infection, there are fewer potential hosts in the population for the pathogen to infect and spread. This creates a phenomenon called **herd immunity**, where the initial rapid spread of infection ultimately slows down and the pathogen may even be eliminated from the population if there are no suitable hosts to allow it to spread.

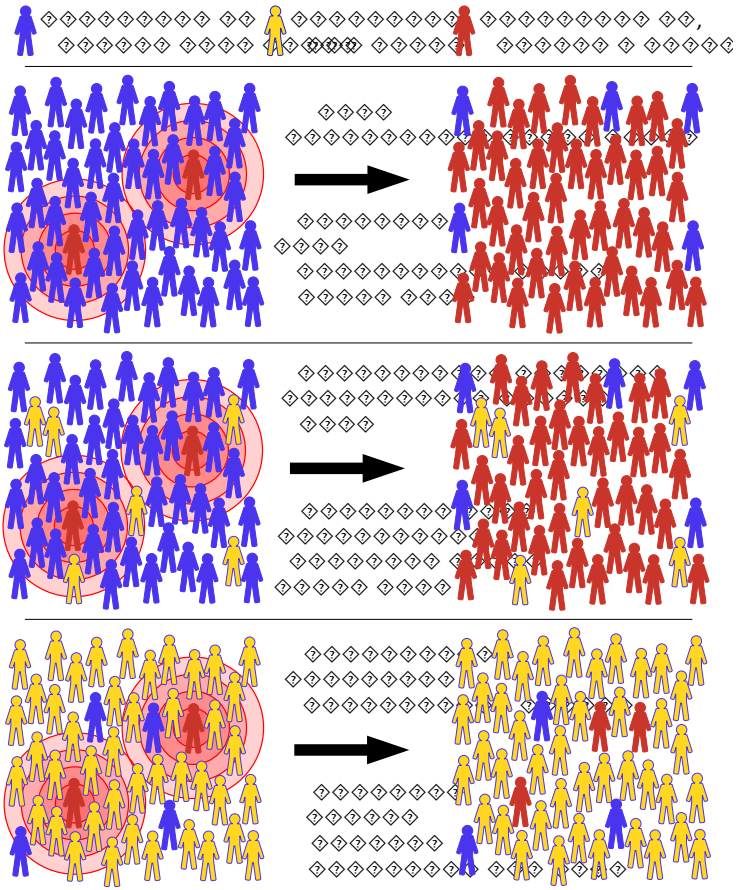

Figure 6-1 Herd Immunity. The **top** image illustrates a scenario where there is no pre-existing immunity. Almost everybody in the population becomes infected, except a few individuals who are perhaps no in contact with other people in the community or are genetically resistant to this particular pathogen. The **middle** image shows a small fraction of previously immune individuals. These people are individually immune but the pathogen spreads effectively to individuals who did not have prior immunity. The **bottom** image illustrates **herd** immunity, where a majority of people are immune from

previous exposure. Even those people who are not immune are protected from infection because the pathogen will not spread within this population.

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Individual Immunity

Immunity provides significant personal protection from specific pathogens. Since immunity operates through prior exposure to pathogens, inducing a person's immunity could serves as **prophylaxis**. *Prophylaxis* is a method of *preventative* medicine used before the onset of a disease whereas *treatment* options are used to manage an existing disease condition.

Immunity may be acquired through **active** or **passive** mechanisms. Active immunity involves *actively* triggering a person's own adaptive immune response. Passive immunity refers to the transfer of adaptive immune components from an individual or animal that is actively immune. These two strategies have distinct benefits and limitations. For example, passive immunization provides an immediate supply of antibodies, which contributes to immediate immune protection that is available to a person whose immune system may be otherwise compromised. However, the supplied antibodies are consumed over time since passive immunization do not generate the memory B-cells needed for longterm immunity. In contrast, active immunization uses a person's immune system to generate a response. However, the immune protection is delayed by 5-10 days as the adaptive immune system is engaged and may not occur at all if a person is immunocompromised. Active immunity provides longterm protection due to the formation of memory lymphocytes.

Natural and artificial mechanisms to induce active immunity:

Natural Active Immunity – Natural active immunity is developed following an infection. However, in this situation the benefits of exposure should be balanced against the risks of infection. For example, prior to vaccine availability, *pox parties* were promoted in certain communities, where parents intentionally exposed their children to v*aricella zoster virus* (chickenpox). The belief was that symptoms were more mild in children compared to adults and that infected children would develop longterm immunity. However, chickenpox infection can be fatal even in children and these intentional exposures contributed to the death of 100-150 U.S. children annually.

Artificial Active Immunity – Artificial active immunity involved vaccination. Rather than obtaining immunity by infection with the natural or wild form of a pathogen, individuals are given a "live" vaccine, which incorporates a weakened or **"attenuated**" version of the pathogen generated in the lab. Live vaccines are effective, but people who are immunocompromised may (albeit rarely) develop an infection from even an attenuated pathogen in a process called *reversion*. Therefore, live vaccines are not recommended for immunocompromised individuals.

Natural and artificial mechanisms to induce passive immunity:

Natural Passive Immunity – Nature has devised a powerful mechanism to extend a mother's immunity to

protect the newborn baby. Before birth, maternal IgG cross the placenta and accumulate within the circulatory system of the fetus. This **placental transfer** of antibodies can provide immune protection for six months after birth. After birth, a mother may express breastmilk infused with secretory IgA. These antibodies encase potential pathogens and prevent them from binding and infecting the feeding infant.

Artificial Passive Immunity – Antibodies can be purified from immunized animals (polyclonal antibodies) or may be produced in cell lines within a laboratory (monoclonal antibodies). These purified antibodies may be used as a prophylaxis, prior to pathogen exposure during **intravenous immunoglobulin (IVIG) therapies**. For example, palivizumab is an antibody that can be offered to children who are at high risk for infection by respiratory syncytial virus (RSV). IVIGs can also be used when a person is exposed to a pathogen if provided within a specific period of time through **post-exposure prophylaxis (PEP)**. When treated with PEP within the window of opportunity antibodies are able to block the infection process. Rabies virus or hepatitis B virus exposures are examples of infections that can be treated with PEP.

Table 6-1. Comparing Passive and Active Immunization

PASSIVE IMMUNITY	ACTIVE IMMUNITY
Pros: Immediate effect, does not depend on the recipient's immune status	**Pros**: Provides longterm immunological memory
Cons: No immunological memory	**Cons**: Response delayed 5-10 days. Immunocompromised persons may have a poor response.
Natural: Placental transfer, Breastmilk	**Natural**: Exposure to pathogen
Artificial: Antibodies purified from animals or engineered in laboratory, e.g. "rabies shot"	**Artificial**: Vaccines consisting of attenuated pathogen, pathogen subunit, pathogen proteins or genetic material coding for those proteins.

Vaccination

The history behind smallpox variolation and vaccination stands as a testament to humanity's quest for protection against a deadly disease. Smallpox, caused by the *variola virus*, is a highly contagious and often fatal disease that afflicted countless individuals throughout history. However, individuals who survive smallpox infections develop robust immunity to future infection.

Variolation is a process believed to have originated from 10th century China where healthy individuals are deliberately inoculated with pustules or scabs from an infected person. While the procedure risked both infection and spread of smallpox, most people treated this way experienced only mild disease.

The advent of modern **vaccination** is attributed to the groundbreaking work of Dr Edward Jenner, an English physician, in the late 18th century. In 1796, Jenner noticed that milkmaids who contracted cowpox, a related but milder disease, were immune to smallpox. Jenner hypothesized that exposure to cowpox could protect against smallpox. Jenner conducted an experiment in which he deliberately inoculated a young boy with material from cowpox lesions and later exposed him to smallpox. The boy remained unaffected by smallpox, confirming Jenner's hypothesis. This marked the inception of vaccination, derived from the Latin word "vacca," meaning cow where antigens from the pathogen are utilized to develop immunological memory.

> ***The vaccine antigen may include a full and competent pathogen or other pathogen components that are immunogenic.***

Live Attenuated Vaccines: These vaccines use a weakened form of the live pathogen, which can still reproduce within the host but causes only a mild or asymptomatic infection. Because they closely mimic natural infections, live attenuated vaccines tend to provide strong and long-lasting immunity. Examples include the measles, mumps, and rubella (MMR) vaccine and the oral polio vaccine (OPV). However, live vaccines are generally not suitable for individuals with weakened immune systems.

Inactivated or Killed Vaccines: In these vaccines, the pathogen is rendered non-infectious through physical or chemical processes, such as heat or formaldehyde treatment. Examples include the inactivated polio vaccine (IPV) and the hepatitis A vaccine. While they are safe, inactivated vaccines often require booster shots because they may not induce as long-lasting immunity as live vaccines.

Subunit or Recombinant Vaccines: These vaccines contain only specific proteins or protein subunits from the pathogen, rather than the entire microorganism. **Subunit vaccines** can be biochemically extracted from the pathogen surface and purified. **Recombinant vaccines** are produced by engineering pathogen genetic material into other organisms like baker's yeast or certain plants. The engineered pathogen proteins are purified to generate the vaccine antigen. Subunit and recombinant vaccines are safer than live vaccines but may require adjuvants (substances that enhance the immune response) to be effective. Examples include the hepatitis B vaccine and the human papilloma virus (HPV) vaccine.

Nucleic Acid Vaccines: These are a newer class of vaccines that use only the genetic material from the

pathogen, such as DNA or RNA, to induce an immune response. They do not contain live or inactivated pathogens and are considered safe. COVID-19 mRNA vaccines, like the Pfizer-BioNTech and Moderna vaccines, are examples of nucleic acid vaccines.

Virus-Like Particle (VLP) Vaccines: VLP vaccines are designed to mimic the structure of a virus without containing its genetic material. They are made from self-assembled proteins and can stimulate a strong immune response. The human papilloma virus (HPV) vaccine and some experimental vaccines for other viruses utilize VLP technology.

Toxoid Vaccines: These vaccines use toxins produced by certain bacteria rather than the microbe themselves. Toxins are chemically inactivated to create toxoids, which are used as antigens. The tetanus and diphtheria vaccines are examples of toxoid vaccines.

Conjugate Vaccines: Conjugate vaccines combine a weak antigen from the pathogen with a strong antigen from another source (often a protein carrier). This approach is used to enhance the immune response, especially in young children whose immune systems may not respond well to certain antigens. The Haemophilus influenzae type b (Hib) vaccine and certain pneumococcal vaccines are conjugate vaccines.

Table 6-2. A comparison of vaccine types

Vaccine Type	PROS	CONS
Live Attenuated Pathogen	Similar to natural infection and produces a strong immune response. Booster vaccines *may* not be necessary.	Possibility of reversion to a harmful form of pathogen in people who are immunocompromised. Serological blood tests cannot determine if the immune response is to the vaccine or the 'wild' pathogen.
Inactivated Pathogen	No live pathogen used in the vaccine product, making it safer.	Since vaccine pathogen does not replicate, additional vaccine immunizations may be needed to serve as boosters. Serological blood tests cannot determine if the immune response is to the vaccine or the 'wild' pathogen.
Purified pathogen subunit or recombinant protein	Only a segment of the pathogen is used, making these vaccines safer than live pathogen. Recombinant vaccines are inexpensive to mass produce. Since only part of the pathogen is used, serological blood tests can scan the antibodies produced and determine if the immune response is against the vaccine or 'wild' pathogen.	Since the number of antigen is low, immune stimulator chemicals ("adjuvants") as well as booster shots may be needed. There are fewer epitopes being engaged in the immune response, compared to inactivated pathogen. The pathogen may evolve and adapt resistance against the antibodies produced by the vaccine.

Nucleic Acid Vaccines	All of the advantages of a recombinant vaccine but even less expensive to produce.	All of the disadvantages of recombinant vaccine.

Summary

- Herd immunity involves immunizing most individuals in a population in order to protect those individuals who cannot be immunized.
- For an individual, immunization by vaccine can provide a prophylaxis that is given before exposure to pathogen to minimize the impact of an infectious disease.
    - Immunization can be natural (infection, breastfeeding) or artificial (immunoglobulin therapy, vaccine).
    - Immunization can be passive (provide antibodies, no immunity; e.g. breastfeeding, immunoglobulin therapy) or active (generate immune response to antigen; e.g. infection, vaccine).
- Different types of vaccines are available with different benefits and limitations

Chapter Review

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://pressbooks.bccampus.ca/appliedimmunology/?p=233#h5p-13

Case Study Review

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://pressbooks.bccampus.ca/appliedimmunology/?p=233#h5p-14

Chapter 7: Immunology in Health and Medicine

Learning Objectives

By the end of this chapter you will be able to:

- Explain the deficiencies in immune function that can contribute to disease pathology.
- Classify hypersensitivities into four categories.
- Discuss testing, manifestations and treatments for hypersensitivity disease.
- Describe how components of the immune system are used in diagnostic techniques.

Case Study

Sarah, age 38, was diagnosed with rheumatoid arthritis (RA) at the age of 30. Rheumatoid arthritis involves the accumulation of antibodies within the joints as antibody-antigen complexes. Infiltration of immune cells and their interaction with immune

complexes produces inflammation within the joint. Sarah initially presented with joint pain, stiffness, and swelling, predominantly in hands and wrists. Sarah underwent testing for rheumatoid factor, which is an antibody produced by some RA patients that binds IgG. This test involved observing agglutination of IgG-coated latex beads. An ELISA was performed to measure the amount of antibodies that bind Cyclic citrullinated peptide (anti-CCP). These antibodies may be produced because RA patients overproduce CCP within the joints. In addition to non-steroidal anti-inflammatory drugs (NSAIDs) and disease-modifying antirheumatic drugs (DMARDs) to reduce disease-associated inflammation, Sarah has received Adalimumab (Humira) therapy that involves infusion of a monoclonal antibody targeting tumor necrosis factor alpha (anti-TNF).

Sarah disease management is an ongoing process that involves periodic MRI scans to assess inflammation and tissue damage. However, with new advances in immunodiagnostics and immunotherapy, Sarah feels confident in being able to manage this complex immunological disorder.

1. *What type of hypersensitivity might rheumatoid arthritis be?*
2. What is the characteristic of immunoglobulins that causes IgG-conjugated latex beads to agglutinate in the presence of rheumatoid factor?
3. *What is the rationale for using monoclonal antibodies in immunotherapy for RA?*

Answers to these questions are at the end of the chapter.

The immune system plays a critical role in preventing infection and repairing damaged tissues. However, the immune system is also a complex biological system that can

adversely affect a person's health when this system is perturbed. This chapter will discuss immune dysfunction and its effect on a person's health as well as technologies that leverage the mechanisms of the immune system to diagnose and treat infection, cancer and immune disorders.

7.1 Immune Dysfunction

The immune system must maintain a balance between mounting an immune response to pathogens and immune tolerance to human cells and the microbiome. **Immunopathology** involves the study of disorders that emerge from disrupting this balance.

> ***Immunopathologies fall into two categories:***
>
> **Immunodeficiency** occurs when the immune system fails to respond appropriately to pathogens or other threats. Deficiencies may emerge when the immune system is not completely developed or when it is suppressed or destroyed. For example, an individual may become immunodeficient as a consequence of cancer, since the immune response to cancer consumes resources that would be otherwise available to address infection.
>
> **Hypersensitivity** occurs when the immune system responds too intensely or inappropriately to stimuli. **Allergy** involves an inappropriate immune response to environmental stimuli that do not usually pose risks to a person's health. **Autoimmune disease** is an inappropriate immune response against a person's own tissues. In the context of **tissue graft and organ transplant**, the immune

> response to the grafted tissue is considered to be a hypersensitivity as it is not a desirable outcome following a transplant.

Immunodeficiency

Immunodeficiencies are disorders associated with absent or functionally defective immune responses and may be either inherited before birth ("**primary**" or "**congenital**") or acquired after birth (**"secondary**" or "**acquired**").

Primary immunodeficiencies are typically inherited, however, the exact nature of the disorder is not always known.

Chronic granulomatous disease involves genetic defects that prevent phagocytes from producing antimicrobial superoxide. This disorder affects 1 in 200,000 people and is typically diagnosed in children (1-4 years old). These individuals are at higher risk for infection, particularly relating to catalase-positive bacteria (e.g. *S. aureus, E. coli, Listeria*) as well as A*sprigillus* and *Candida* fungus.

A person may produce low amounts of protective IgG antibody (**hypogammaglobulinemia**) or none at all **(agammaglobulinemia).** These individuals are at risk for frequent infection and are treated with immune serum globulin as well as continuous antibiotic therapy.

DiGeorge syndrome involves a defect in the development of the thymus, which results in a failure in T-cell development. The effect of this disorder is most apparent in the cell-mediated response because T-cells are involved as both inducers and effectors of this response. As a consequence, individuals with DiGeorge syndrome experience persistent fungal, protozoan and viral infections. In addition, since CD4 helper T-cells mature in the thymus and are necessary to activate B-cells, a person

with DiGeorge syndrome may also lack antibodies. A thymus transplant can restore immune function for these individuals.

Severe combined immunodeficiency (SCID) describes the most severe form of immunodeficiency because affected individuals are unable to produce T-cells or B-cells. In one form of SCID known as **adenosine deaminase (ADA) deficiency**, developing lymphocytes lack adenosine deaminase, an enzyme needed to detoxify cells. **X-SCID**, where the affected gene is found on the X chromosome most frequently affects males. X-SCID involves a deficiency in interleukin receptors such that lymphocytes do not respond to the signals necessary to grow and become responsive. Since SCID places individuals at such high risk for recurrent infection patients will undergo a bone marrow transplant to replace their hematopoietic cells from a donor. While a bone marrow transplant has a success rate of 50% in curing SCID, there is a risk for **graft-versus-host disease (GvHD)** where T-cells within the bone marrow may induce an immune response against the new host. Donor bone marrow must be carefully processed to remove reactive T-cells prior to transplantation to reduce the risk of GvHD.

Secondary immunodeficiency typically arises as a consequence of **infection** (*e.g. viral hepatitis or HIV infection*), **immunosuppressive therapy** (*e.g. cancer chemotherapy, radiation, graft rejection drugs*) or **prolonged disorders** that impair normal body function (*e.g. malnutrition, diabetes*). Unlike primary immunodeficiencies that are typically a consequence of genetic defects, secondary immunodeficiency results from environmental and other exposures and may be reversible. However if untreated, individuals with secondary immunodeficiencies may have recurrent infections but also may develop **rare cancers** and **opportunistic infections**, which involves infection by microbes that are normally harmless (e.g. *Candida* yeast infection or *Cryptosporidium* diarrheal disease).

Acquired immunodeficiency syndrome (AIDS) is the best-known secondary immunodeficiency and is a consequence of

infection by the human immunodeficiency virus (HIV). The HIV virus uses the CD4 protein as a viral receptor to attach to and enter helper T-cells. Infected T cells subsequently die of infection and through a cellular apoptosis response called *pyroptosis*. The profound decrease in CD4 T cells results in impairment of the adaptive immune response.

Hypersensitivity

Hypersensitivity involves an exaggerated or inappropriate immune response.

Hypersensitivities can be classified into four types:

Type I: Involve exposure to specific environmental antigens called **allergens**. The adaptive immune response to these allergens produces **IgE** antibodies as well as memory lymphocytes that perpetuate the disorder. IgE binds the surface of inflammatory cells, such as mast cells, which have internal granules containing **histamine.** Upon future exposure to the antigen, mast cells *degranulate* and the histamine is released to trigger inflammation.

Type II: Specific IgG and IgM **antibodies** are produced, which **target cellular antigens**. The target cells and tissues are damaged in the subsequent immune response, resulting in a loss of function for that particular tissue.

Type III: Antibodies and antigens agglutinate to form a web of **immune complexes**. These complexes leak into the bloodstream and deposit at various sites throughout the body. Where immune complexes accumulate, the local tissue becomes damaged and inflamed.

> **Type IV: T-cell–mediated** reactions damage tissues through activation of macrophages and cytotoxic T cells. These reactions normally occur more than 12 hours after exposure to allergen, leading it to be called a *delayed-type* hypersensitivity.

Type I Hypersensitivity

Type I hypersensitivity has significant parallels to the standard adaptive immune response. The antigens that trigger type I hypersensitivity are called **allergens** and are typically harmless environmental substances, such as skin flakes (*dander*) from animals, pollen, molds or certain foods, such as peanut or shellfish.

Stage 1: Sensitization

As with the standard humoral immune response, allergen-specific Th2 cells activate B-cells that are also specific to the same allergen. Following clonal expansion, differentiation into plasma cells and IgM secretion, the B-cells undergo class switching. The divergence of an immune response and an allergy response arises because class-switching produces **IgE**-secreting B-cells. IgE is distributed in the body by the bloodstream and deposits on the surface of **mast cells**. This first exposure to allergen does not necessarily produce allergy symptoms but instead *sensitizes* a person to future potential allergy response.

Stage 2: Provocation

After IgE class switching and sensitization, exposure to allergen will cause the allergen to bind the IgE that have deposited on the mast cell surface. Granules inside the mast cell hold **histamine** and this histamine is released by a process called *degranulation*. Other factors like serotonin and bradykinin are also released by degranulation. Together, these

inflammatory cytokines induce inflammation and allergy symptoms within 10-15 minutes of exposure. Activation of mast cells also induces metabolism of arachidonic acid, leading to the production of prostaglandins and leukotrienes. These cytokines can produce a more sustained allergy response.

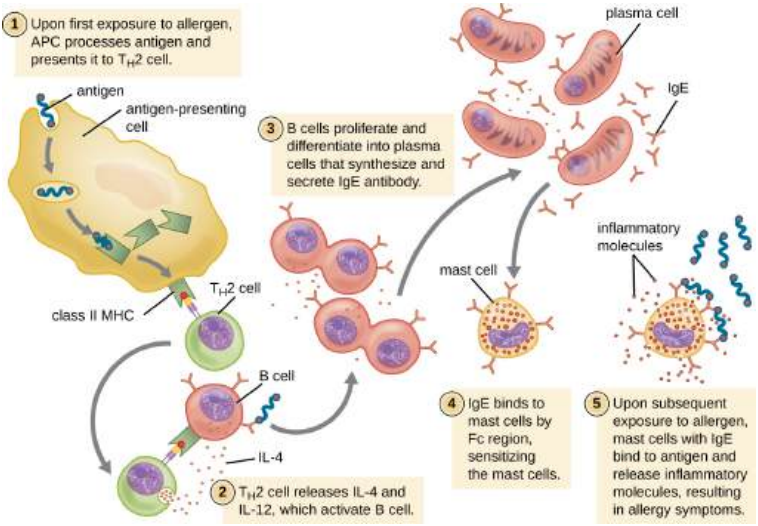


Figure 7-1 Allergy Sensitization and Provocation. The ***sensitization*** phase of allergy involves allergen detection by both Th2 and B-cells (1-2). The B-cells undergo class switching to secrete IgE and the IgE deposits on the surface of mast cells (3-4). The ***provocation*** phase of allergy occurs on subsequent exposure to allergen, where allergen binds to IgE on the mast cell surface and induces degranulation to release histamine and other inflammatory molecules (5).

Image Source: By OpenStax CC BY 4.0, via Wikimedia Commons

Atopic Hypersensitivity Responses

Atopic diseases traditionally included asthma, eczema, and hay fever because they all run in families. However, the classification has since been expanded to describe the

personal or familial tendency to produce IgE antibodies in response to allergens.

Atopic allergies may present as either **local** or **systemic** reactions. Local reactions include inflammation in a confined organ, such as airway inflammation of atopic asthma or skin irritation in allergic eczema (*atopic dermatitis*). A systemic response is called **anaphylaxis** and is characterized by inflammation in distinct organs and tissues throughout the body, resulting in swelling of the tongue and trachea, blockage of airways, and a dramatic drop in blood pressure leading to potentially fatal *anaphylactic shock*.

Allergy Therapy

Therapies for allergy responses are based on the mechanisms associated with this immunological process. Since allergies depend on plasma B-cell secretion of IgE, effective therapies can target the activity of the plasma cells (corticosteroids) or can reduce the systemic accumulation of IgE (anti-IgE antibodies). The IgE bind the surface of mast cells, *priming* the mast cell by acting as a *de facto* allergen-specific receptor. When primed mast cells interact with allergen, they degranulate to release histamine. Cromolyn acts to inhibit degranulation while anti-histamines block the histamine receptor. Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin or ibuprofen, can mitigate the inflammation that results from an allergic response.

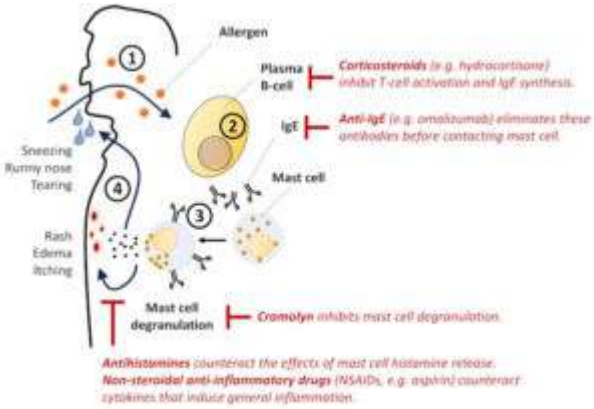


Figure 7-2 Allergy Therapy. Allergens are environmental agents that induce plasma B-cells to secrete IgE (1). Corticosteroids and anti-IgE reduce the production and accumulation of IgE within the body (2). IgE binds to and primes mast cells, which are then sensitized to degranulate on exposure to allergen (3). Cromolyn inhibits mast cell degranulation while antihistamines block the action of histamine that is released through this process. NSAIDs also inhibit the inflammation that occurs during allergic response.

Type II Hypersensitivity

Type II hypersensitivities involves IgM and IgG binding to human cells, followed by damage to those cells by lysis, phagocytosis and/or antibody-dependent cell-mediated cytotoxicity (ADCC). The surrounding tissue may become inflamed and type II hypersensitivities underlie most autoimmune diseases.

Blood Type Incompatibility

Blood type incompatibility may occur in response to blood transfusion or pregnancy and involves antibodies that eliminate or "reject" the non-self red blood cells. Blood types

are determined by the presence or absence of specific antigens on the surface of red blood cells.

The two most widely known blood type systems are the **ABO system** and the **Rh system**.

ABO blood type is determine by the presence or absence of carbohydrates 'A' and 'B' on the red blood cell surface, where 'O' represents the absence of either carbohydrate. ABO blood type is inherited as a gene copy (*allele*) of the blood antigen from each parent, where *genotype* describes each gene copy. For example, AO indicates a person has one allele to produce surface carbohydrate A and an allele that does not produce carbohydrate antigen. Since, A-antigen is produced the red blood cell is A-type.

> ***The specific genotypes and corresponding blood types are listed below:***
>
> - **A-Type:** *AO, AA* – red blood cells have only A carbohydrate on cell surface
> - **B-Type:** *BO, BB* – red blood cells have only B carbohydrate on cell surface
> - **AB-Type:** *AB* – red blood cells have both A and B carbohydrates on cell surface
> - **O-Type:** *OO* – red blood cells have neither A nor B carbohydrates on cell surface

The immune system generates IgM antibodies, called **isohemagglutinins**, against red blood cells with blood group antigens not found on an individual's own red blood cells. These antibodies are produced early in life and are maintained, such that red blood cell rejection could occur with every transfusion.

A person with A-type blood (*AO* or *AA*) would make antibodies against the foreign B-antigen (*antibodies are called anti-B*). Conversely, a person with AB-type blood would not make antibodies against either A-antigen or B-antigen. The AB-type individual is called the *universal recipient* because they can receive blood from any donor without rejection of blood on the basis of ABO blood group antigens. The O-type individual will reject RBCs from donors with every blood type except O-type. Importantly, individuals with O-type blood are regarded as *universal donors* because the absence of surface antigen allows the transfusion of their RBCs into persons of other ABO blood types, without rejection on the basis of these antigens.

ABO Blood Typing

See the Appendix A1-1 for more information on ABO Blood typing

Rhesus (Rh) factor blood type is described as the presence (Rh+) or absence (Rh-) of Rh factor (Rho/D antigen) on the surface of red blood cells. A person who is Rh+ can receive Rh- transfusion without consequence, because Rh- blood has no antigen.

A person who is Rh- and receives a transfusion with Rh+ blood will initiate a primary immune response. However, unlike the ABO blood group, anti-Rh IgMs do not pre-exist as such the rejection of transfusion is delayed by two weeks. Following the initial sensitizing event, subsequent transfusions will trigger a rapid and more potent secondary response involving IgG.

Rh-incompatibility must be considered during pregnancy if a Rh- mother is pregnant with a Rh+ fetus. During childbirth the mother's immune system is likely to become sensitized

toward the Rh antigen. In subsequent pregnancies, anti-Rh IgG produced by the mother and will cross the placenta resulting in red blood cell hemolysis in the fetus and the potentially life-threatening **hemolytic disease of the newborn**.

Through routine screening before and during pregnancy, affected mothers can be identified and treated with human Rho/D immune globulin (e.g., RhoGAM). RhoGAM is administered to the mother during the 28th week of their pregnancy and within 72 hours after delivery. These immunoglobulins function by binding and eliminating fetal Rh+ red blood cells before they come into contact with and sensitize the lymphocytes of the mother.

Drug-Induced Immune Hemolytic Anemia (DIIHA)

DIIHA is commonly observed when patients have been treated with penicillin and some cephalosporin class antibiotics. It is suspected that the antibiotic becomes attached to the surface of red blood cells. Antibodies (IgM or IgG) bind the antibiotics and mark the red blood cell for destruction by complement fixation and hemolysis. DIIHA depends on the presence of the drug and will disappear when the drug is discontinued.

Type II Hypersensitivity Autoimmune Disorders

Antibodies (IgM/IgG) may contribute to autoimmune disorders in multiple different ways. Antibodies may bind cell receptor proteins to turn on or off signals to the cell. For example, in Grave's disease, autoantibodies bind and activate the receptors for thyroid stimulating hormone (TSH), which causes the thyroid to become hyper-activated and release excess thyroid hormones. Affected persons experience weight loss, rapid heartbeat, nervousness and related effects.

Alternatively, antibodies may bind human cells and cause

damage to the tissues of the affected person. Hashimoto's disease involves antibody-mediated damage to the thyroid that results in an underproduction of thyroid hormones leading to slowed heartbeat, fatigue, weight gain and other effects that contrast directly with Grave's disease. Similar tissue dysfunction may occur from antibody-mediated damage to lungs and kidney basement membrane (*Goodpasture's syndrome*), heart muscle (*rheumatic fever*), peripheral nerves (*Guillain–Barré syndrome*) as well as a range of other tissues.

Type III Hypersensitivity

Type III hypersensitivity results when antigen exists in a soluble form, not bound to the surface of a cell. This may occur as a consequence of some infections (e.g. *post-streptococcal glomerulonephritis*), reactions to serum or vaccine components, or diseases with genetic predisposition (*systemic lupus erythematosus*, *rheumatoid arthritis*).

Antibodies produced against these soluble antigens form antigen-antibody **immune complexes** that disseminate through the bloodstream and deposit throughout the body. Common sites of immune complex deposition include the glomerulus of the kidney, where blood is filtered, joints and in tissues with high oxygen demand and consequently develop networks of many small blood vessels (heart, lungs, brain, etc.). The affected tissues become inflamed and attract immune cells resulting in tissue destruction.

Type IV Hypersensitivity

Type IV hypersensitivities are regulated by T-cells, however, unlike the other hypersensitivity types, the **effector cells** are

activated in an **antibody-independent manner**. Type IV hypersensitivities may be delayed-type hypersensitivities, where the immune response may occur up to 12 hours post-exposure to antigen. These immune hypersensitivities may occur when antigen is presented by antigen presenting cells (APCs) to induce a Th1-specific immune response. Subsequent stimulation of cytotoxic T-cells and monocytes/macrophages contribute to local inflammation and tissue damage.

- **Contact dermatitis** is an example where chemical agents on the skin bind to cells and proteins to produce a larger antigen *("hapten")* that sensitizes a person to that chemical. Upon future exposure, the chemical agent becomes the target of an Th1 immune response. Influx and stimulation of immune cells that secrete inflammatory cytokines induces **swelling,** itching and pain at the site of contact. Contact dermatitis resulting from contact with poison ivy is a specific example of this type of hypersensitivity.
- **Tuberculin-type** hypersensitivity is used as the basis of the tuberculosis skin test. Bacterial tuberculin protein is purified and administered by intradermal injection. Influx of inflammatory cells produces a local induration and swelling that is typically measured at 48-72 hours after the injection. The diameter of induration indicates the type four hypersensitivity that occurs if a person was previously exposed to *Mycobacterium tuberculosis*.
- Some **organ transplant rejection** is a consequence of type IV hypersensitivity. APCs present foreign antigen of the grafted tissue, resulting in a Th1 immune response. CD8 cytotoxic T-cells will be induced and will actively eliminate the transplanted tissue.

Autoimmunity and Autoimmune Disease

Autoimmunity is a complex and intriguing aspect of the immune system, involving the body's defense mechanisms turning against its own tissues and cells. Low levels of autoimmunity is normally observed and may be involved in coordinating tissue development or elimination of old cells. However, if specific autoimmune responses become enhanced or dysregulated, a person may experience an autoimmune disease because of damage incurred on a specific tissue.

Autoimmune diseases are believed to be affected by a multitude of environmental, physiological and genetic factors as such many of the detailed mechanisms behind autoimmunity is still unknown. For example, autoimmune diseases are generally more frequently experienced by individuals of female biological sex. Environmental factors include infection and chemical exposure while major physiological drivers of autoimmunity include age, pregnancy and hormonal changes. Some autoimmune diseases are observed in individuals with a family history, suggesting a genetic component as well.

Normally, the immune system distinguishes between self and non-self, targeting foreign invaders while sparing the body's own constituents. However, in autoimmune diseases, this self-recognition breaks down and immune cells mistakenly attack healthy tissues, which can result in chronic inflammation, tissue damage. There is a wide spectrum of autoimmune diseases, each characterized by specific targets. Conditions such as rheumatoid arthritis, systemic lupus erythematosus (SLE), and multiple sclerosis exemplify the diverse array of autoimmune disorders, each with its unique clinical manifestations. Understanding autoimmunity and autoimmune diseases is crucial for developing therapies that modulate or suppress the aberrant immune responses and alleviate the suffering of affected individuals.

7.2 Immunodiagnostics and Immunotherapy

Antibodies as Tools

Antibodies play a critical role in the immune response, by marking pathogens and foreign agents for elimination from the body. A key characteristic of antibodies is their tremendous binding specificity, which emerges due to VDJ recombination. Their high specificity makes antibodies valuable tools for both detecting illnesses (**immunodiagnostics**) and treating inflammatory or infectious diseases (**immunotherapy**).

Antibody specificity is determined by the antigen binding site, which forms primarily from VDJ recombination. Each B-cell receptor destined to become an antibody has a unique amino acid conformation resulting in a three dimensional shape and chemical characteristics (non-covalent bonds) that allows them to interact specifically with complementary shapes and chemical characteristics on an epitope of the target antigen.

The strength of binding between an antibody binding site and the epitope of its target antigen is known as **affinity**. Affinity can be measured in a laboratory assay. However, in nature interactions between antibodies and antigens depend on more than just the antigen binding site and the epitope. In this case **avidity**, which accounts for the overall structural arrangement of the whole antibody and the antigen is a more relevant measure. Avidity takes into account the interactions between all antibody binding sites and multivalent antigens. The distinction between these terms is exemplified by IgG and IgM. The J-chain of IgM connects five immunoglobulin molecules together. Consequently, if IgG and IgM have the same affinity (binding of antigen binding site to epitope), the IgM will still exhibit higher avidity because the antibody has five-fold more antigen binding sites.

In some cases, two antigens may be structurally similar and antibodies that specifically target one antigen may inadvertently bind another antigen as well. This unintentional binding of an antigen is called **cross-reactivity**. One example of cross-reactivity is the herpes simplex virus 2 (HSV-2) IgM test for evidence of early infection by this sexually transmitted disease. A false positive detection of HSV-2 may occur if the person has been infected by HSV-1 ("cold sore" oral herpes), which easily transmitted without sexual contact. The reason that this test fails is that HSV-1 and HSV-2 viruses share structurally similar epitopes that would bind the antibodies.

Another example of cross-reactivity results from **molecular-mimicry**, which is a phenomenon where pathogens acquire epitopes that are structurally similar to those found in human tissues, either by chance or possible evolution to evade immune detection. For example, *Streptococcus pyogenes* M-protein has structural similarities to myosin protein of the human heart. Antibodies produced during *S. pyogenes* infection can produce an autoimmune response against human heart tissue, leading to development of rheumatic fever that persists long after the bacterial infection has been resolved.

Another characteristic of antibodies that is relevant to immunodiagnostics and immunotherapy relates to how the antibodies are obtained. The classical approach to produce antibody involves immunization of an animal, such as a rabbit or goat, with a specific antigen. The animal will produce antibodies against this foreign antigen. Serum is harvested from the animal via bleeding and contains the antigen-specific antibodies. In this context, the serum is called antiserum to reflect the antibodies contained within. The **antiserum** preparation may also be called a **polyclonal antibody** because the animal produced a mixture of antibodies against the variety of epitopes on the antigen surface.

Immunodiagnostics

The high specificity of antibodies makes them an excellent tool for detecting and quantifying a broad array of targets, from drugs to serum proteins to microorganisms. With *in vitro* assays, antibodies can be used to precipitate soluble antigens, agglutinate (clump) cells, opsonize and kill bacteria with the assistance of complement, and neutralize drugs, toxins, and viruses.

The functional utility of antibodies can be extended via **conjugation.** The chemical fusion of dyes, enzymes, toxins, etc. to an antibody produces a conjugate that has the antigen-specificity of an antibody but the acquired function of the attached molecule.

Immunodiagnostics can be employed in a range of applications:

Serotyping: Microbial species differ in their cell surface composition. Subtle variation in antigens can even occur in subpopulations of microbes in the same species, called *serotypes*. One example of a serotyping method uses bead-conjugated antibodies that will specifically bind to some microbes. Binding of the bead to the microbe also causes the beads themselves to clump together (agglutination).

One or more interactive elements has been excluded from this version of the text. You can view them online here: https://pressbooks.bccampus.ca/appliedimmunology/?p=768#oembed-1

Serological Testing: The frequently used "blood test" for infection is also known as a serological test. Like serotyping, these methods may use antibodies specific to pathogen in order to detect infection in the bloodstream. However,

antibodies to identify human immune cells and cytokines can also play an important role in in determining the type as well as the stage of infection.

Serological testing can be used to test for a range of blood abnormalities:

Agglutination of red blood cells to monitor blood transfusion and infection

Hemagglutination assays take advantage of the agglutination (clumping) of red blood cells that is observed when they are bound by compatible antibodies. This agglutination can be used as a read-out for standard blood incompatibility testing (Appendix A1-1 and Appendix A1-2), testing for antibodies that emerge as a consequence of infection (Appendix A1-3) and determining the precise pathogen responsible for infection (Appendix A1-4).

Using red blood cell lysis to assess complement protein function

Since complement proteins are responsible for the lysis of red blood cells, this method can assess the function of classical complement pathway (Appendix A3-1) and alternative complement pathway (Appendix A3-2). Complement lysis can also be used to determine if a person has developed antibodies to a particular antigen (Appendix A3-3)

ELISA for quantification of pathogen or immune components

See the Appendix A5 for information on the ELISA methods to measure the amount of pathogen protein, antibodies or other molecules that may emerge during infection.

ELISPOT for quantification cytokine or antibody secretion

See the Appendix A6 for information on the ELISpot method that can test for a specific response from immune cells, such as the secretion of cytokines or antibodies in response to allergens, transplant tissues or other foreign materials. Immune cell secretions can confirm that these cells are responding to the foreign material and indicate the nature of that response.

Immunoblot/Western blot to capture protein size and identity.

See the Appendix A7 for information on Western blot method. This method is ideal when antibodies may cross-react with different proteins in the specimen. Confirming the size of the protein can ensure you are measuring the correct one.

Flow cytometry to count cells.
See the Appendix A8-1 for information on flow cytometry cell counting. Antibodies are used to fluorescence-label specific cells and then cytometry is used to count the number of cells that are labelled. This method is frequently used to assess the number of specific immune cells in blood in order to identify specific immune responses or possible immune deficiencies, as part of a complete blood cell count with differential (CBC-DIFF; Appendix A13-1).

Microscopy methods.
Antibodies may be used to visualize specific cells or cell structures by their conjugation with fluorescent dyes in immunofluorescence microscopy (Appendix A9) or to visualize cells with antibodies conjugated with enzymes that catalyze a localized colour change (Appendix A10).

Immunoprecipitation to visualize antigen.
Immunoprecipitation involves binding antigen and antibody molecules together into dense clumps that form a precipitate that is visible at the bottom of a tube or well (Appendix A11). This method can be

modified to quantify antibodies or antigen in precipitin ring assays (Appendix A12-1) or immunodiffusion assays (Appendix A12-2). Immunoprecipitation may be observed as simply cloudiness or turbidity in a tube, as seen in the CRP assay (Appendix A13-2)

Immunotherapy

Antibodies produced in the laboratory can be used to harnesses the body's immune system to combat diseases, including cancer and autoimmune disorders. Monoclonal antibody (mAb) therapies, like Rituximab, bind the surface of cancer cells and mark these cells for elimination by complement and phagocytes. Other mAb therapies, like Pembrolizumab, bind proteins on the surface of cells to modulate receptor function. In the context of Pembrolizumab, the PD-1 receptor on T-cells normally suppresses their function. Binding of this receptor by mAbs can preserve T-cell function. Moreover, antibodies are integral to the emerging field of theranostics, which combines therapy and diagnostics. In theranostics, antibodies can be radiolabelled for diagnostic monitoring of cancer, while conjugation of drug using a cleavable linker allows the drug to be simultaneously delivered to the target cell. For example, Brentuximab vedotin couples radiolabels or imaging agents with a drug payload that reduces the growth of lymphoma cancer cells. The versatility and specificity of antibodies make them potent tools in the development of novel immunotherapies and the advancement of precision medicine.

Summary

- Immunodeficiency may occur in two ways:
    - Primary – defect in the development of immune cells or the production of immune components. Primary immunodeficiencies are inherited before birth.
    - Secondary – defect in immune function due to the influence of environment, infection, certain medications, or other occurrences after birth.
- Hypersensitivities are excessive or inappropriate immune responses and are divided into four types:
    - Type I – allergen-specific IgE-mediated response where mast cells or similar inflammatory cells degranulate to secrete histamine.
    - Type II – IgM or IgG antibodies that target a person's own tissues.
    - Type III – IgG-antigen complexes that deposit within the body, leading to inflammation and tissue damage.
    - Type IV – Damage to a person's own tissues due to auto-reactive cytotoxic T-cells and dysregulation of macrophages.
- Autoimmunity is part of normal immune homeostasis, where immune cells identify a person's own cells and eliminate ones that become old or non-functional. However, autoimmunity can be dysregulated to produce an autoimmune disease.
- Conjugation involves addition of dyes, enzyme or other functional components to antibodies to enable immunotherapy and immunodiagnostics.

Chapter Review

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://pressbooks.bccampus.ca/appliedimmunology/?p=768#h5p-15

Case Study Review

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://pressbooks.bccampus.ca/appliedimmunology/?p=768#h5p-16

Appendix: Summary of Lab Diagnostics using Immunological Techniques

APPENDIX
Applications of Immunology Techniques in Researc

An array of tools and techniques have played a pivotal role in advancing our understanding of the immune system and have become indispensable applications in diagnostic testing. This appendix is a summary of some of these tools and techniques employed in immunological research and diagnostic testing.

- A1: Agglutination tests.

    - A1-1 Hemagglutination and blood typing
    - A1-2 Antiglobulin tests
    - A1-3 *Treponema pallidum* hemagglutination (TPHA) test
    - A1-4 Tube agglutination test
- A2: Antibody generation
    - A2-1: Hybridoma technology
    - A2-2: Recombinant antibody technology
- A3: Complement pathway testing
    - A3-1: Total hemolytic complement (CH50) assay
    - A3-2: Alternative complement hemolytic (AH50) assay
- A4: Complement fixation test
- A5: Enzyme linked immunosorbent assays (ELISA)
- A6: Enzyme linked Immunosorbent spot assay (ELISpot)
- A7: Immunoblotting (Western blot)
- A8: Immune cell isolation
    - A8-1: Flow cytometry and fluorescence activated cell sorting (FACS)
    - A8-2: Immunomagnetic cell separation

- A9: Immunofluorescence microscopy
- A10: Immunohistochemistry (IHC)
- A11: Immunoprecipitation
- A12: Precipitin reaction
    - A12-1: Precipitin ring test
    - A12-2: Radial immunodiffusion assay
- Testing for general immune function
    - A13-1: Complete Blood Count with Differential (CBC diff)
    - A13-2: C-reactive Protein Test (CRP)
    - A13-3: Erythrocyte sedimentation rate (ESR)

A1: Agglutination tests

Agglutination tests detect and/or measure the presence of specific antigen or antibodies in a patient sample. They utilize one of the functions of immunoglobulins, agglutination or the clumping of particles when antibodies detect the corresponding antigens. In agglutination tests, antibodies or antibody-coated particles are mixed with a patient sample containing the corresponding antigen. Agglutination indicates the presence of the molecule being detected in the tested sample. The agglutination reactions can be observed

macroscopically as clumping or as the formation of an insoluble precipitate.

A1-1: Hemagglutination and blood phenotyping

The ABO blood phenotyping test is a hemagglutination assay, where agglutination of red blood cells (RBCs) is used to determine an individual's blood type. Recall that the ABO blood group system is based on the presence of two antigens on RBCs, antigen A and antigen B. Based on the presence of these antigen individuals are classified into four groups, individuals with antigen A (type A), individuals with antigen B (type B), individuals with antigen A and B (type AB) and individuals who do not bear either antigen (type O).

During a blood typing test, patient samples are mixed with antibodies against antigen A and antigen B. If there is an antigen-antibody interaction, RBCs will agglutinate forming visible clumps. A positive reaction indicates the presence of the corresponding antigen on the surface. Thus, the blood type of a patient can be determined by observing the agglutination pattern.

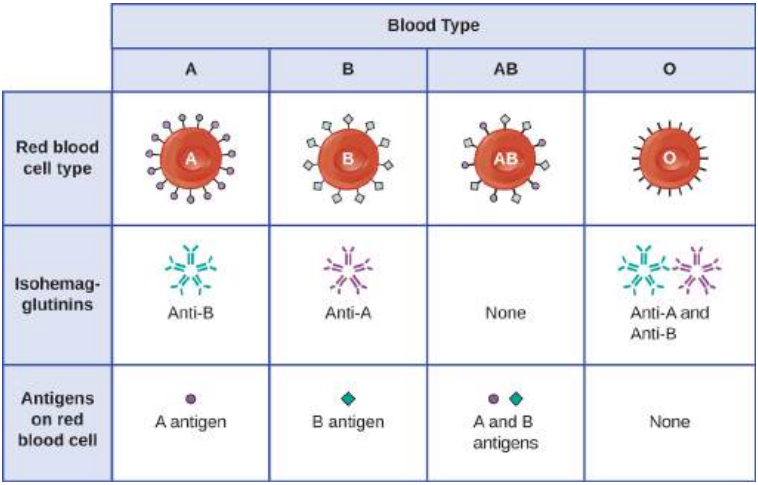
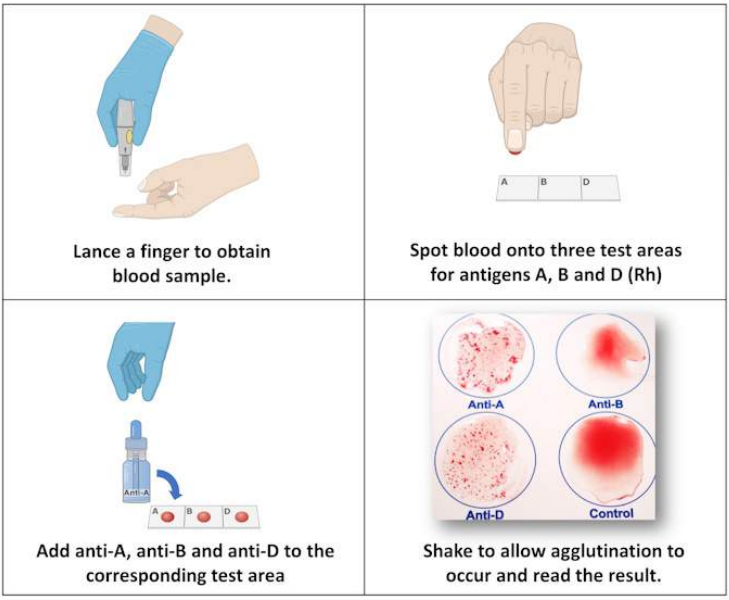


Figure A1-1-1 Blood Groups. A and B antigens constitute glycoproteins on the red blood cell (RBC) surface. A person with A-type blood has A-antigen on the RBC surface but not B-antigen. Consequently, this person produces antibodies anti-B that would reject any RBCs with surface B-antigen (B-type or AB-type). A person with O-type blood has no RBC surface antigen and can be transfused into any other person, since neither anti-A nor anti-B would bind these cells.

Image Source: By OpenStax CC BY 4.0, via Wikimedia Commons



Lance a finger to obtain blood sample.

Spot blood onto three test areas for antigens A, B and D (Rh)

Add anti-A, anti-B and anti-D to the corresponding test area

Shake to allow agglutination to occur and read the result.

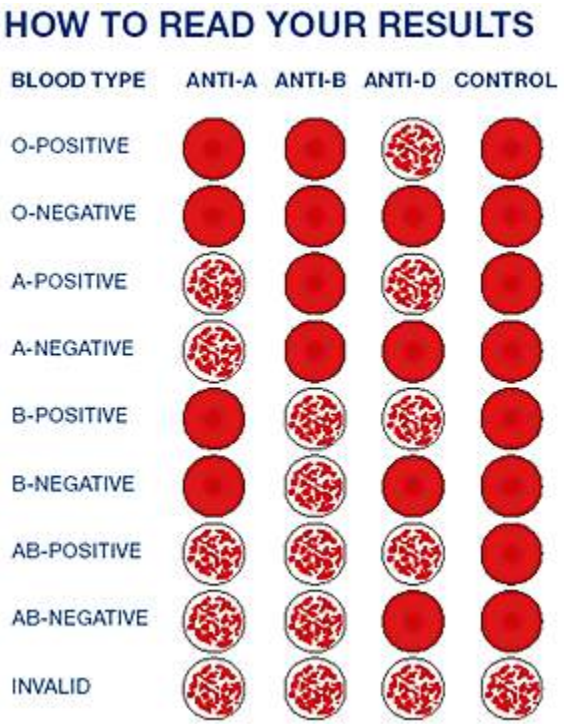


Figure A1-1-2 ABO Blood Typing. (Top) The procedure for blood typing involves spotting blood into three test areas and adding antibodies to A, B and D (Rh) antigens onto each labelled test area. (Bottom) Cell clumping indicates that the red cell has the test antigen on the cell surface.

Image Source: (Top) By Human Bio Media CC BY 4.0, via Blood Typing Lab Test Simulation; (Bottom) By Biology Corner CC BY 4.0, via BiologyCorner.com;

A1-2 Antiglobulin tests and detecting Rh incompatibility

Rh (Rhesus factor) incompatibility occurs in pregnancy, when Rh-negative mothers are exposed to Rh-positive fetal RBCs during delivery. Upon exposure, the mother will produce anti-Rh antibodies. In subsequent pregnancies these anti-Rh antibodies will be transported across the placenta to the fetus, which leads to the destruction fetal RBCs resulting in hemolytic disease of the newborn (HDN). The **antiglobulin** test, also known as the **Coombs test**, is an immuno-hematology assay that detects the presence of antibodies that lead to HDN. The tests make use of the Coombs reagent or anti-human-IgG antibodies to detect the presence of IgG antibodies.

In the **direct antiglobulin** (DAT) test, newborn blood samples are tested to detect antibody bound RBCs. The blood sample is mixed with the Coombs reagent. If the newborn RBCs are coated with antibodies this will result in agglutination. A positive DAT test indicates the mother has produced anti-Rh antibodies, which are responsible for HDN.

The **indirect antiglobulin** (IAT) test is performed on maternal blood to detect the presence of anti-Rh antibodies. The maternal blood sample is first mixed with Rh-positive RBCs. After incubation, the sample is washed to remove unbound antibodies, and the Coombs reagent is added to detect antibody-bound RBCs. Agglutination indicates a positive IAT test, which shows the presence of anti-Rh antibodies in maternal blood. The IAT test is often performed in the first trimester of pregnancy because RhD-immunoglobulins (RhoGAM) can be given to Rh-negative mothers to reduce the probability of sensitization and subsequent anti-Rh antibody production.

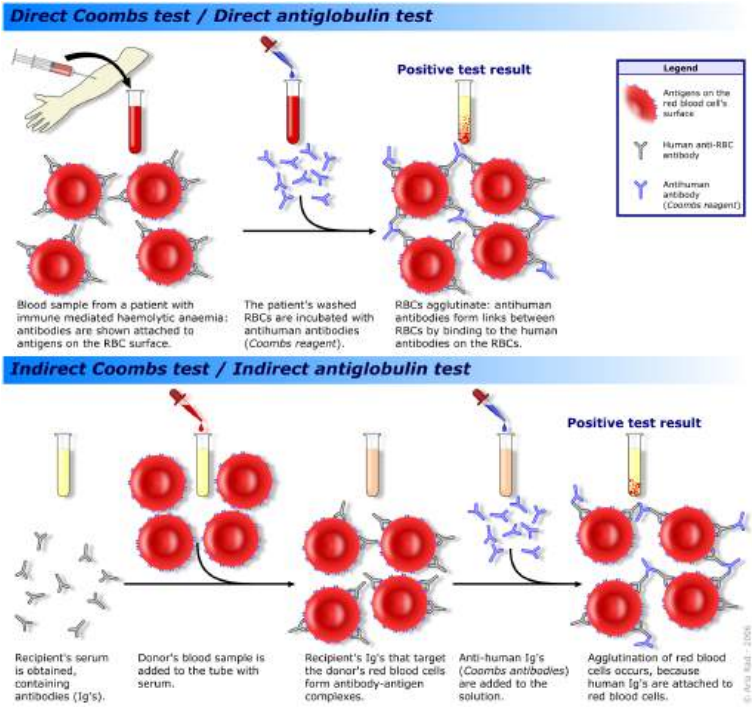


Figure A1-2-1 The Coombs test. (Top) The DAT assay is performed to detect the presence of maternal anti-Rh antibody bound RBCs in newborn with suspected HDN. A newborn blood sample is mixed with Coombs reagent containing human anti-IgG antibodies. Agglutination indicates a positive test for antibody-bound newborn RBCs. (Bottom) The IAT test is performed during the first trimester to detect presence of anti-Rh antibodies in maternal blood. In the first step Rh-positive RBCs are mixed with maternal serum containing antibodies. Following a wash step the Coombs reagent is added to the reaction. Agglutination indicates a positive test for anti-Rh-positive antibodies.

Image Source: By Rad~commonswiki CC BY 3.0, via Wikimedia Commons

A1-3 *Treponema pallidum* hemagglutination (TPHA) test

The **TPHA assay** is an indirect hemagglutination test used to diagnose syphilis, a sexually transmitted disease caused by the bacterium *Treponema pallidum*. It is a **treponemal test** that detects the presence of anti-treponemal antibodies in patient samples. The TPHA test is used as a confirmatory test to diagnose syphilis. It can also be used as a primary screening test for syphilis at all stages beyond the early primary stage of the disease.

TPHA assay uses RBCs sensitized with *T. pallidum* antigen. If anti-treponemal antibodies are found in the tested patient serum, the RBCs will agglutinate to form a smooth mat of cells at the top of the test plate. If antibodies are not present, the RBCs will settle to the bottom of the plate forming a compact button of non-agglutinated cells. Reactive results in a TPHA assay may indicate an active, past, or successfully treated infection. As such the TPHA test alone is not sufficient for a diagnosis.

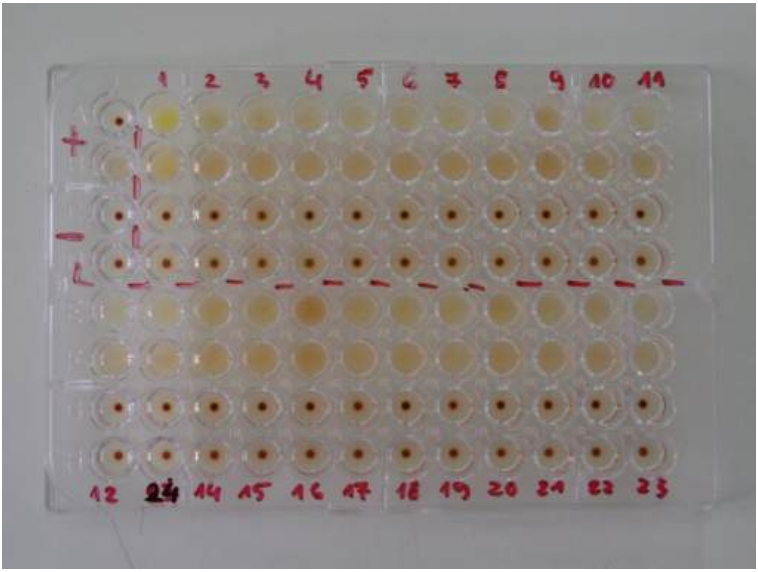


Figure A1-3-1: TPHA assay performed in a microwell plate. The left upper corner contains the positive controls, one with RBC aggregate seen as a dense button and one with agglutination observed as a smooth mat of cells. The negative controls are shown below containing two RBC aggregates. Lanes 1-4 show patient samples with the top two rows containing positive samples and the two bottom rows containing negative samples.

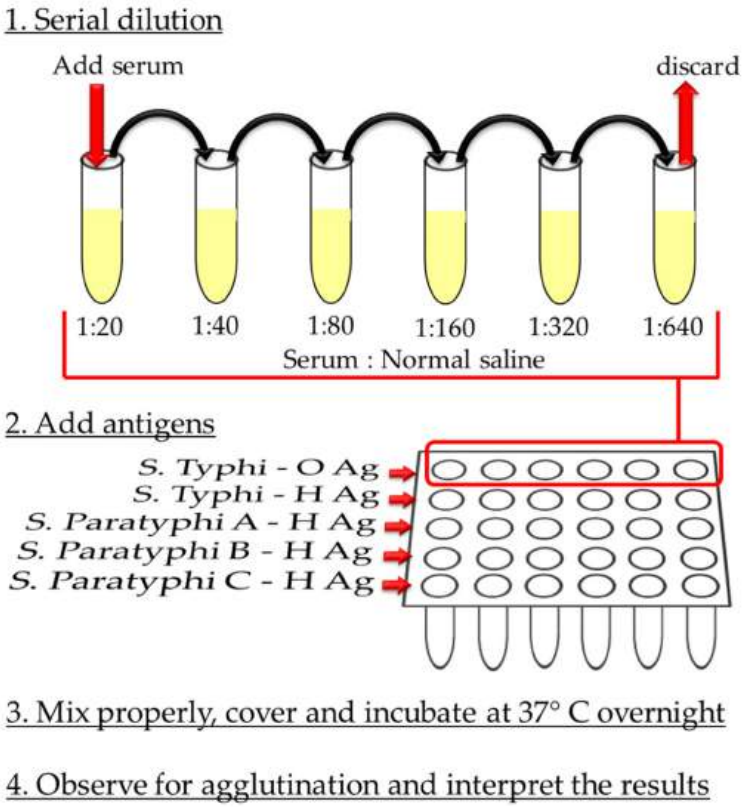
Image Source: By OpenStax CC BY 3.0 via Wikimedia Commons

A1-4 Tube agglutination test

The tube agglutination test is a technique used to detect the presence of antibodies or antigen in a sample. In this test, patient serum is incubated with a specific antigen or an

antibody. If the target antigen is recognized by the antibody, a visible reaction occurs in the form of agglutination or clumping. Agglutination is observed macroscopically by gently tipping or swirling the test tubes and observing visible clumps. Alternatively, the degree of agglutination can be quantified using a spectrophotometer or by performing agglutination titers.

The Widal test tube method used for diagnosing typhoid fever is an example of a tube agglutination test. This test involves mixing patient serum with specific antigen derived from *Salmonella typhi* the microbe causing typhoid fever. Agglutination is indicative of the presence of antibody in patient serum. The Widal test has been replaced with ELISA and nucleic acid detection methods in North America. However, it is still widely used in developing countries due to low cost and availability.



A1-4-1 Tube agglutination test for diagnosing typhoid fever. (Step 1) The patient samples is serially diluted (typically 8 dilutions 1:20, 1:40 etc). (Step 2) Antigens for relevant *Salmonella* serotypes are added to the wells. (Step 3) Mixture is incubated overnight and (Step 4) agglutination is observed. Agglutination is indicative of the presence of typhoid antibodies. This is a semiquantitative test, where the titre of the patient serum is equivalent to the highest dilution of the serum that gives visible agglutination.

Image Source: By Alhaj-Qasem*et al.* (2020) *Diagnostics*, *10*(7), 438. CC BY 3.0

A2: Antibody generation

Antibody generation is a fundamental tool in immunology that allows researchers to produce specific antibodies for various applications. Today, antibodies are developed in the lab to recognize and bind a variety of target molecules such as proteins, peptides, and cells. Lab-made antibodies can be used in a wide range of applications including basic research, diagnostic assays, therapeutics, and imaging techniques.

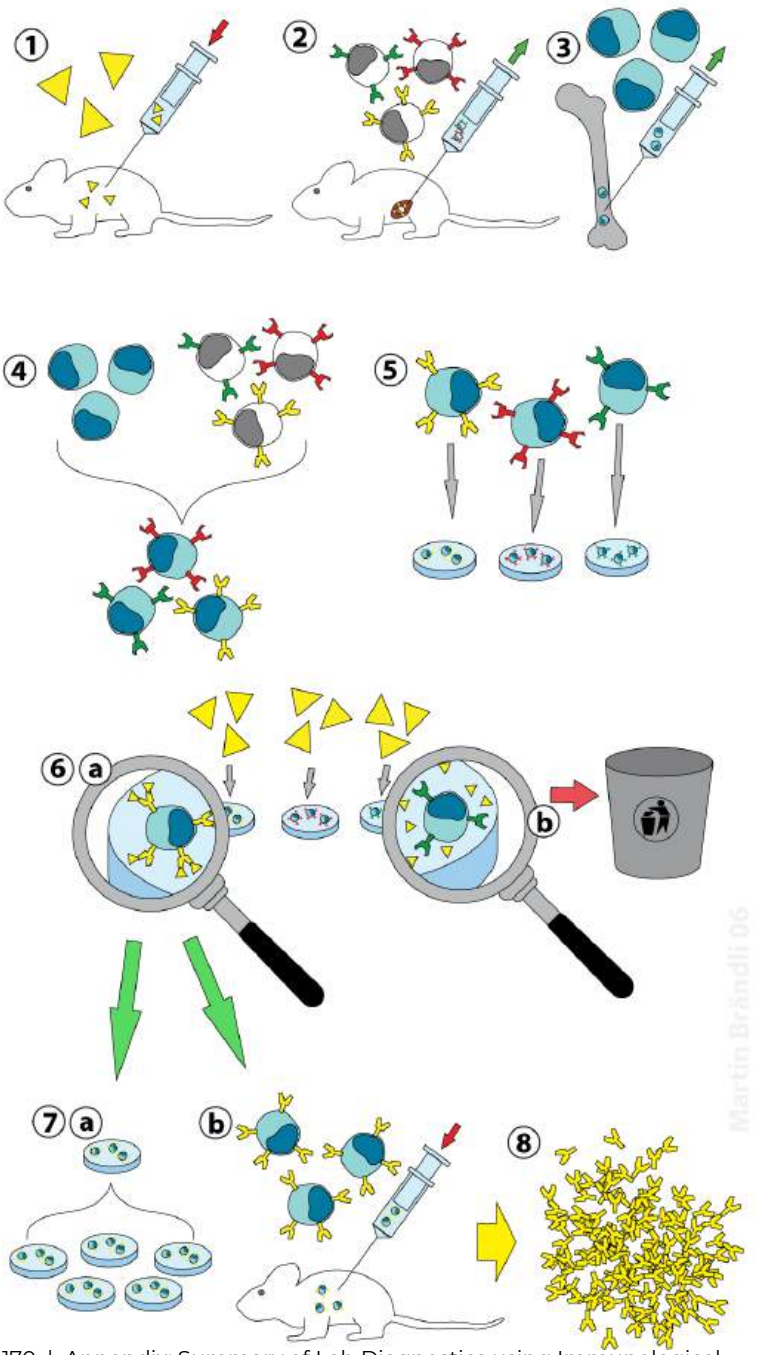
There are two types of lab-made antibodies: **monoclonal antibodies (mAbs)** and **polyclonal antibodies**. B cells are key to generating both monoclonal and polyclonal antibodies. In monoclonal antibody production, a single B cell is selected to ensure the generation of an antibody specific to a single epitope. In polyclonal antibody production, a diverse population of B cells are selected to generate a mixture of antibodies that respond to multiple epitopes or regions of the antigen. Monoclonal antibodies offer highly specific and uniform responses while polyclonal antibodies provide broader coverage because they originate from a collective response of multiple B cells.

A2-1 Hybridoma technology

Hybridoma technology was the most commonly used method for generating monoclonal antibodies in the past. In this process, antibody-producing B cells are isolated from immunized mice and are fused with myeloma cells to form hybrid cells, called **hybridoma** cells. Hybridoma cells are cultured in the lab and once stable lines are established these cells can be used to produce limitless amounts of antibodies. Antibodies generated using hybridoma cells have high sensitivity and specificity.

The general steps of generating monoclonal antibody using hybridomas include:

1. **Immunization**: The process begins by immunizing an animal (typically mice) with the antigen of interest using a series of injections over a period of several weeks. The foreign antigen will stimulate the immune system, leading to the production of antibody secretion B cells specific to the introduced antigen.
2. **B cell isolation**: Antibody producing B cells are isolated from the spleen, a rich source of B cells
3. **Fusion**: Isolated B cells are fused with a myeloma cell line, which is an immortal tumor cell line. The fusion is usually achieved using fusion agent such as polyethylene glycol (PEG)
4. **Selection**: Hybridoma cells are cultured using specific medium that will support only the growth of hybridomas.
5. **Screening**: Once established hybridoma cell lines are screened to identify those producing the most desirable antibody.
6. **Antibody production**: Selected hybridoma cells are grown in large-scale bioreactors or cell culture to make large quantities of antibodies. Antibodies are secreted by the hybridoma cells lines into the culture media, which can be easily harvested, purified, and used.


1
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Martin Brändli 06

Figure A2-1-1 Hybridoma technology. To produce monoclonal antibodies (1) a mouse is first immunized with a purified antigen and (2) antigen-reactive B-cells are extracted from the mouse. (3) Myeloma cancer cells are also obtained and are (4) chemically fused with the B-cell to produce a *hybridoma* cell that has the B-cell's capacity to produce antibody as well as the cancer cell's ability to grow indefinitely in culture. (5) Individual hybridoma cells are grown and expanded into clonal populations. (6a) Each hybridoma clone is tested for its ability to bind antigen and (6b) non-responders are discarded. Hybridoma cells are then grown in culture (7a) or in animals (7b) to (8)produce large amounts of epitope-specific (*monoclonal*) antibodies that can be harvested.

Image Source: By Martin Brändli CC BY 2.5, via Wikimedia Commons

A2-2 Recombinant antibody technology

While hybridoma technology was instrumental in generating monoclonal antibodies for years, recombinant antibody technology has gained significant popularity in recent years for antibody production. Recombinant antibodies are synthetic antibodies generated using recombinant DNA technology. Initial steps involved in making recombinant antibodies are similar to hybridoma technology. Animals are immunized with the desired antigen and B cells are extracted from these animals. However, once B cells are isolated, instead of generating hybridomas, B cells are sequenced to map their VDJ regions. The mapped genes are then introduced into a host system such as bacteria or yeast using genetic engineering.

Recombinant antibodies are more desirable than the traditional hybridoma based antibodies because they can be fully humanized, making them more compatible when using

as a human therapeutic. What is more, recombinant antibodies can be further customized based on the need of each individual application. Production of recombinant antibodies can be easily scaled up for large-scale production, providing cost-effective and efficient manufacturing. In contrast, maintaining individual hybridoma cells lines can be resource-intensive and time-consuming. The ability to screen large antibody libraries and rapidly generate recombinant antibodies accelerated the discovery of novel antibodies against various targets. Finally, the use of standardized systems for antibody production ensures greater batch-to-batch consistency and reproducibility, which is crucial for maintaining the safety and efficacy of therapeutic antibodies.

A3: Complement pathway testing

The complement system consists of more than 30 proteins that participates in the innate immune response. Complement testing is used to diagnose and monitor certain immune-related conditions. These tests evaluate the activity and levels of various components within the complement system.

A3-1 Total hemolytic complement (CH50) assay

CH50 Assay

The **CH50 assay** aka the total hemolytic complement assay is assesses the overall activity and function of the complement system, particularly the classical complement pathway. As discussed in Unit 2, the classical complement pathway is

initiated primarily by antigen-immunoglobulin complexes.

The CH50 assay measures the lysis of sheep red blood cells (SRBCs) coated with anti-sheep antibodies in the presence of antibodies. If classical complement pathways components such as C1, C2, C3 and C4 are all available in a patient sample, the membrane attack complex (MAC) will be assembled leading to SRBC lysis. However, if there are deficiencies or dysregulation within the complement system (C1-C9) or if there is consumption of complement due to immune (or autoimmune) complexes the CH50 assay results will be abnormal. The complement deficiencies can be hereditary or acquired.

For more information on how the CH50 assay is conducted, see the video and protocol below:

Costabile M. J Vis Exp. 2010 29;(37):1923.

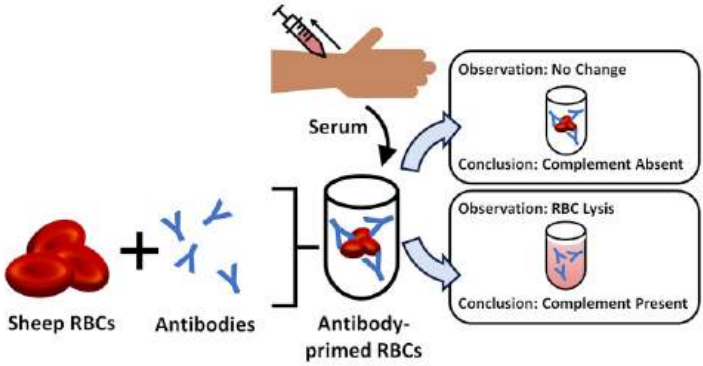


Figure A3-1-1 CH50 assay. Sheep red blood cells are sensitized with antibodies to serve as targets for complement mediated lysis. Patient samples are collected using standard venipuncture and centrifuged to separate plasma. Sensitized SRBCs and patient plasma is incubated under specified conditions to induce hemolysis. Samples are centrifuged, and the extent of RBC lysis is assessed via measuring the release of hemoglobin into the supernatant. The CH50 value is calculated based on the dilution of patient's plasma at which 50% of sensitized RBCs are lysed.

A3-2 Alternative complement hemolytic (AH50) assay

The AH50 assay is used to evaluate the function and activity of the alternative complement pathway. As discussed in Unit 2, the alternative pathway is spontaneously activated.

The **AH50** assay measures the lysis of rabbit red blood cells in the presence of complement proteins in a patient serum sample. Rabbit RBCs contain low levels of sialic acid, which makes them highly susceptible to alternative pathway mediated lysis. When alternative complement pathways

components are available in a patient sample, MAC will be assembled resulting in RBC lysis. Abnormal AH50 results indicate a potential alternative pathway component defect.

Interpretation of results

The alternative pathway shares C3 and C5-C9 components with the classical complement pathway. However, it also has unique complement components including Factors D, B and properidin. As such CH50 and AH50 assays are often run in conjunction to identify specific complement pathway abnormalities.

- Absent alternative pathway activity (AH50=0) in the presence of a normal CH50 (CH50=100) suggests an alternative pathway component deficiency.
- Normal AH50 (AH50=100) with absent CH50 (CH50=0) suggest deficiencies in early classic pathway involving C1, C2 or C4.
- Absent AH50 and CH50 (CH50=0 and AH50=0) suggest a late component deficiency involving C3, C5, C6, C7, C8 and C9.
- Normal CH50 and AH50 CH50=100 and AH50=100) in the presence of recurrent infections and continue suspicion of complement deficiency may require testing of the MBL pathway function.

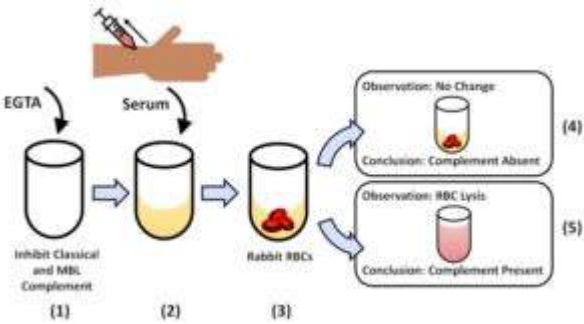


Figure A3-2-1 AH50 assay. (1) Collection tubes are prepared with EGTA, which removes calcium required for the activation of the classical and MBL pathways. (2) Patient samples are collected using standard venipuncture and centrifuged to separate plasma. (3) Rabbit red blood cells are used in this assay due to their low sialic acid content and susceptibility to lysis. Rabbit RBCs and patient plasma is incubated together to induce hemolysis. (4-5) Samples are centrifuged, and the extent of RBC lysis is assessed. The AH50 value is calculated based on the dilution of patient's plasma at which 50% of sensitized RBCs are lysed.

A4: Complement fixation (CF) test

The complement fixation test uses components of the complement system as reagents to detect the presence of specific antibodies in patient samples. While this test was widely used in the past to diagnose infections, today, ELISA assays and DNA-based methods have replaced complement fixation tests.

CF tests uses sheep RBCs, anti-sheep RBC antibody, and guinea pig serum as a source of complement proteins. When these three reagents are mixed under normal circumstances,

complement proteins bind to antibody coated RBCs resulting in MAC formation and hemolysis.

When performing a CF test, patient samples are first treated with heat to inactivate human complement proteins, as their presence will interfere with the results. Next, the known antigen is added to the serum sample. If the patient sample contains antibodies specific to the antigen antigen-antibody complexes will be made. Following this incubation, guinea pig complement proteins are added to the mixture, which will bind the antigen-antibody complexes. These complement proteins are now fixed on the antigen-antibody complexes. When sensitized sheep RBCs are added to this mixture (containing antigen-antibody-complement proteins) they will not be lysed since the complement proteins are already bound. In this assay, a lack hemolysis indicated a positive CF test. The patient sample contains the specific antibodies examined in the CF test.

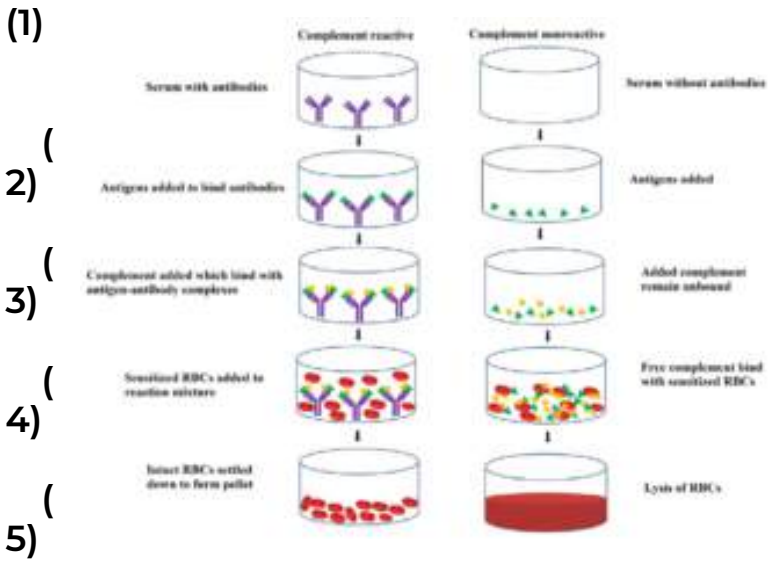


Figure A4-1 The complement fixation assay. (1) Patient samples are collected using standard venipuncture and heat treated to remove complement proteins. (2) The samples are then incubated with the test antigen. If the patient sample contains the antibody (*left*) they will form antigen-antibody complexes. (3) Next, complement proteins from guinea pig serum are added to these samples. If antigen-antibody complexes are present the complement proteins will be bound to these complexes becoming fixed (unavailable for further interactions). (4) Final step of the assay involves adding sensitized or antibody bound sheep red blood cells (SRBC) to the reaction. (5) If complement proteins are already fixed, SRBCs will not be lysed. This results in a positive CF test (*left*). If complement proteins are available, it will lead to MAC formation on SRBCs, leading to lysis. In this case, the patient sample did not contain the antibody being examined.

Image Source: Ranjan, et al. (2016). *J. adv. parasitol.*. 2. 80-99. 10.14737/journal.jap/2015/2.4.80.99. [*Shared under creative commons licence*]

A5: Enzyme linked immunosorbent assays (ELISA)

ELISAs use catalytic properties of enzymes to detect and quantify immune reactions. They can be used to detect and measure levels of antibodies in blood, to estimate the level of tumor markers, to track disease outbreaks, to screen donor blood and plasma and to detect past exposures to infectious agents.

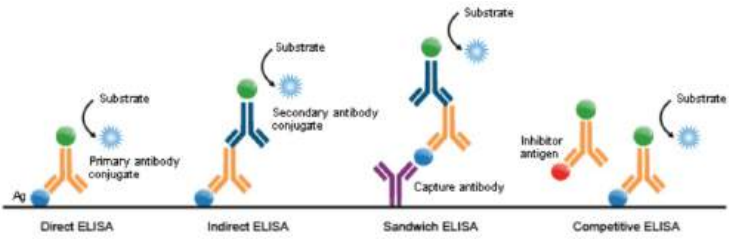
ELISA assays are performed in 96 or 384 well plates and depend on two basic elements: (1) capture or coating of antigen of interest and (2) detection of antibodies or antigen

in patient samples. **Antigen capture** on the microwell plate can be either direct or indirect. In **direct capture**, the antigen is adsorbed directly on the microwell plate. **Indirect capture** involves a capture antibody, which is first immobilized on the well plate. The target antigen is captured through its interaction with the capture antibody. Similarly, analyte detection can also be performed directly or indirectly. In **direct detection**, the analyte is detected with a labelled primary antibody. **Indirect detection** makes use of two antibodies, where the primary antibody binds to the analyte and a labelled secondary antibody interacts with the primary antibody.

> ***Three main types of ELISAs are relevant to diagnostic testing: direct ELISAs, indirect ELISAs and sandwich ELISAs.***
>
> **Direct ELISA** assays use **direct capture** and **direct detection.** Patient samples contains the target antigen, which is detected using an enzyme-labelled primary antibody. Next detection in carried out by adding a substrate that generates colour, fluorescence or chemiluminescence upon cleavage by the enzyme.
>
> **Indirect ELISA** assays use **direct capture** and **indirect detection**. Initial steps are identical to a direct ELISA assay with the exception of requiring two antibodies. Antigen of interest are adsorbed to the well plate. A patient sample containing the primary antibody is incubated with the antigen. Following the incubation and washing, an enzyme-labelled secondary antibody that recognizes the primary antibody is added to the sample. Due to the signal amplification that happens at the final step, where one primary antibody can be bound by several labelled

secondary antibodies indirect ELISAs offer higher sensitivity than direct ELISAs.

Sandwich ELISAs (generally known as antigen capture assays) are the most widely used ELISA format. They involve **indirect capture** and **indirect detection**. The assay is known as a "sandwich" ELISA because the analyte is bound between two primary antibodies, each detecting a different epitope of the antigen. Steps involved in a sandwich ELISA is identical to that of an indirect ELISA with the exception of indirect antigen capture with a capture antibody. Sandwich ELISAs offer high sensitivity and specificity as it uses three specific antibodies for analyte detection.



A5-1 Common ELISA assays. (1) In direct ELISA antigens of interest are detected using a labelled primary antibody. (2) Indirect ELISA involves two antibodies. The *primary* antibody that binds the antigen of interest, and the *secondary* antibody that binds the primary antibody. Since multiple secondary antibodies can bind a single primary antibody, the detection signal is amplified at this stage making indirect ELISAs more sensitive. (3) Sandwich ELISAS use indirect capture and indirect detection, making use of three antibodies. The capture antibody binds the antigen of interest. The primary antibody is found in patient samples. If they are present, the labelled-secondary antibody will bind to the antigen-antibody

complexes resulting in a positive test. (4) Competitive ELISA requires the test antigen to displace a competing antibody-bound antigen. This method provides higher consistency in quantification.

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A6: Enzyme linked Immunosorbent spot assay (ELISpot)

The **ELISpot assay** is a widely used immunological technique that allows the detection and quantification of individual **cytokine-secreting T cells or antibody secreting B cells**. ELISpot is based on the same principles as ELISA assays but combines it with the analysis of individual cells. ELISpot measures the secretion of cytokines, such as interferon-gamma, interleukins (ILs) and, and other immune-related molecules, by capturing and visualizing the proteins produced by individual cells.

The **interferon-gamma release assay (IGRA)** is an example of an ELISpot assay used to test for latent tuberculosis infections. Interferon-gamma (IFN-G) is the primary cytokine release in response to infections by the microbe Mycobacterium tuberculosis. Since people with latent tuberculosis do not display symptoms of the disease, the only way to identify past exposure is through a tuberculin skin test or a blood test. IGRA is more specific for identifying tuberculosis infections particularly in people who were vaccinated as children as it uses antigens that are not found in the tuberculosis strains used in the vaccine.

In the ELISpot assay, T cells from the patient sample are first stimulated with the tuberculosis antigen. The cells are then allowed to settle on a well plate coated with antibodies that

recognize IFN-G. If the activated T cells release IFN-G, the cytokine will be captured by the capture antibody. Antibody bound IFN-G is detected using a labelled-secondary antibody, which reveals a circle (spot) surrounding the position of each activated T cell. These circles give ELISpot assay its name.

In addition, ELISpot assays are also useful for assessing functionality of T and B cells, evaluating vaccine efficacy, and monitoring immune reactivity in diseases such as cancer and autoimmune disorders.

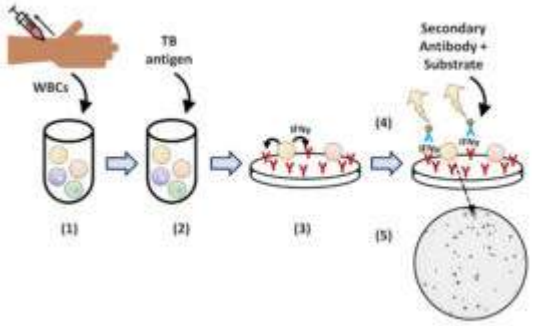

Figure A6-1 The T.SPOT TB assay. (1) White blood cells are isolated from a whole blood sample. (2) Cells are stimulated with TB antigen and transferred to cells pre-coated with capture antibodies for IFN-G. (3) IFN-G is bound to capture antibodies. (4) IFN-G is detected using a labelled-secondary antibody and detection reagent that reacts with enzyme labelled secondary antibody is added. (5) Reaction generates spots in the areas where IFN-G was released. Spots are enumerated for a T cell count.

A7: Immunoblotting (Western blotting)

Immunoblotting or Western blotting is a technique employed

to detect and analyze specific proteins within a biological sample using antibodies. Western blots can also be used to evaluate the size of a protein and to quantify the amount of protein.

The first step of **Western blotting** involves mixing the sample with a detergent called sodium dodecyl sulfate (SDS) to denature (unfold) the proteins. Proteins are then separated by size using gel electrophoresis. Following separation, proteins are transferred to a blotting membrane ensuring the proteins maintain their relative position from the gel onto the membrane. The membrane is the 'blocked' to prevent non-specific binding and subsequently incubated with a primary antibody that specifically recognizes the protein/antigen of interest. Unbound antibodies are washed off and the membrane is incubated with a labelled secondary antibody, which allows the detection and/or visualization of the protein. Addition of the detection substrate generates a signal that may be quantified.

Western blots are still used in Lyme disease testing, HIV confirmation tests and hepatitis C confirmation tests by diagnostics labs.

A8: Immune cell isolation

This section highlights techniques that selectively separate specific subsets of immune cells by using antibodies. They play a critical role in research, diagnostics, and therapeutics.

A8-1 Flow cytometry and FACS analysis

Flow cytometry allows individuals cells to be counted and analyzed based on various physical properties such as size,

complexity, and specific labelling. The basic principle of flow cytometry involves the passage of cells through a flow cell, where cells suspended in fluid are passed through a laser beam. As each cell pass through the laser, they scatter the light. The scattered light is collected by a series of detectors, which converts these signals into electrical impulses to provide information about the characteristics of a cell.

Two physical properties, the size and internal complexity (granularity and topology), determines the extent of light scattering. Two measures of scattered light are assessed, **forward-scattered light (FSC)** and **side-scattered light (SSC)**. FSC is proportional to cell size. FSC is a measure of diffracted light (light bent as the cell passes through the laser) and therefore is used to differentiate cells based on size. SSC is proportional to internal complexity or granularity. SSC measurements are collected by a lens placed 90 degrees to the laser beam, which measures refracted and reflected light.

A common method used to display light scatter data is a **dot plot**. FSC values are plotted on the x-axis and SSC is plotted on the y-axis, where each dot represents a cell. Based on FSC and SSC values different immune cell populations can be identified. For example, granulocytes (eg: neutrophils and basophils) will have larger FSC and SSC values than lymphocytes (T cells and B cells), which are smaller and less complex.

Fluorescence-activated cell sorting or **FACS** is a specialized application that combines flow cytometry with the ability to physically separate or sort cells based on fluorescent properties. In FACS, the flow cytometer is equipped with a **cell sorter**. The cell sorter can identify cells based on fluorescence and sort them into different collection tubes or plates.

Cells to be sorted by FACS are fluorescently labelled, using either fluorescent antibodies or fluorescent dyes. Fluorescence induces the device to put a charge on the fluid droplet containing the fluorescent cell. Since the charge is matched to a specific wavelength of fluorescent light, differentially labelled

cells can be given different charges. Sorting is accomplished using an electrostatic deflector that moves the charged droplet containing the cell into the assigned collection vessel.

A8-2 Immunomagnetic cell separation

Immunomagnetic cell separation is a technique for isolating cells using magnetic beads. The beads are coated with antibodies or ligands that recognize and bind specific surface markers on target cells allowing selective capture and separation of cells. Immunomagnetic cell separation is particularly useful for isolating specific immune cells for therapeutic applications.

To select for cells of interest (aka positive selection), the heterogeneous cell suspension is first incubated with coated magnetic beads, allowing the beads to bind the desired antigen expressing target cells. Next, a magnetic field is applied causing the magnetically labelled cells to be immobilized near the magnetic source. Non-target cells are then be removed by washing, while target cells remain bound to the beads. Final step involves the elution or release of the cells from the beads so they can used in downstream applications.

Alternatively, immunomagnetic cell separation is also useful for removing an unwanted cell type from a population (negative selection). Here the magnetic beads are coated with an antibody that will bind and contain the cells to be removed. Following incubation, these cells will remain bound to the magnetic source, resulting in their removal from the population.

Magnetic cell isolation is a faster and a much simple process compared to FACS. It also does not require specialized equipment. However, FACS offers many more capabilities as it is possible to sort multiple cell types simultaneously, isolate

cells based on internal markers, isolate cells based on protein levels and sort complex cell types with multiple markers to obtain samples with higher purity with FACS.

A9: Immunofluorescence

Antibodies are specific to their corresponding antigen. This specificity makes them an invaluable resource for probing and identifying specific molecules in cells, tissues, and biological fluids. In addition, antibodies can be conjugated or attached to other molecules such as fluorescent dyes. Antibodies labelled with fluorescent molecules are used in immunofluorescence assays (IFA) to detect and visualize specific antigens such as viral proteins.

Two main IFAs are used in diagnostic testing, direct immunofluorescence, and indirect immunofluorescence. In **direct immunofluorescence** assays, a fluorescently labelled primary antibody is used to identify analytes in patient samples. **Indirect immunofluorescence** assays involve two steps for detecting analytes. The unlabeled primary antibody binds specifically to the target molecule and the secondary antibody with the fluorescent molecule binds to the primary antibody.

Diagnostic tests that use both IFA techniques are available for syphilis testing caused by the spirochete Treponema pallidum. The **direct fluorescent antibody (DFA) assay** is used as a screening test for syphilis. The test is performed on smears of exudate (fluid) from lesions and chancres. Slides are incubated with a fluorescently labelled primary antibody specific to pathogenic treponemes. A positive result constitutes a visible fluorescence signal when slides are examined using a microscope.

The **fluorescent treponemal antibody absorption (FTA-ABS)**

test is an indirect fluorescent assay used to confirm a syphilis diagnosis. In this test, the patient serum is absorbed (ABS component of the test) by mixing with a sorbent that will remove non-specific antibodies. The absorbed serum is then tested on a microscope slide containing antigens from the Treponema strain specific to syphilis. If the patient serum contains syphilis specific antibodies, they will bind to the antigen. Washed slides are then incubated with fluorescently labelled anti-human antibodies. As with the DFA test, a positive result constitutes a visible fluorescence signal when slides are examined using a microscope.

The **ANA (anti-nuclear antibody)** test and the **CLIFT (Crithidia luciliae immunofluorescent)** test used to detect autoantibodies associated with autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus are two additional examples of diagnostic tests employing indirect immunofluorescence.

A10: Immunohistochemistry (IHC)

Immunohistochemistry is a technique that exploits the specific binding between an antigen and an antibody. It combines anatomical, immunological, and biological techniques to visualize the distribution and location of specific proteins within the context of cells and tissue in their histological context (in situ). IHC is used to identify and classify cancer cells, for characterizing tumours that guide treatment decisions, to detect infectious agents in tissue, and for diagnosing autoimmune diseases.

Antibodies used in IHC are chemically coupled to enzymes that convert a colourless substance to a coloured reaction product in situ. Tissue samples are incubated with the antibody followed by the addition of the substrate. In the presence of an

antigen-antibody complex, the coloured product is deposited at the sites of interaction and can be observed using a microscope.

A11: Immunoprecipitation

Immunoprecipitation (IP) is a technique used to isolate and purify proteins of interests from a mixture based on their interactions with antibodies. Antigen-specific antibodies employed in IPs to bind and precipitate the target protein of interest.

In immunoprecipitation, samples such as serum, cell extract, or tissue extract are incubated with specific antibodies. The antibody-antigen complex is then precipitated from the rest of the complex with the aid of agarose beads bound to protein A or protein G. Protein A and G are bacterial proteins that bind to antibodies with high affinity. After the precipitation, the samples are washed to remove any non-specifically bound proteins. The protein of interest is then eluted or released from the immunocomplex so it can be further analyzed using techniques such as Western blotting.

A12: Precipitin Assays

The precipitin test was the first assay developed for quantifying antigen and antibody titres. It relies on the interaction between antigens and antibodies and the resulting antigen-antibody complex formation. The immunocomplexes form a lattice structure that becomes visible as either a ring or a line. “Precipitin” in this assay refers to the precipitate that forms when the antibody reacts with the corresponding antigen.

The outcome of the precipitin reaction depends on the amount of antigen added:

1. **Prozone effect**
   The prozone effect occurs when there is an excess of antibodies relative to antigen. Each antigen molecule is bound by antibodies. However, the presence of excess antibodies prevents crosslinking of antigen molecules, the resulting complexes are generally small in size and do not form precipitates.

2. **Zone of equivalence**
   This zone represents the optimal conditions for forming precipitin complexes. In the zone of equivalence, the maximum number of antigen-antibody complex are formed resulting in a robust, detectable assay signal.

3. **Postzone effect**
   Postzone refers to the region in which the concentration of antigen exceeds the optimal range for immunocomplex formation. In this zone, the excess antigen can interfere with the formation of visible precipitates.

When performing precipitin assays, the prozone effect can be overcome by diluting samples to reduce the concentration of antibodies. This enables the formation of properly sized complexes for accurate detection. Various antigen concentrations may be tested to overcome the limitation of postzone effects.

A12-1 Precipitin ring test

The precipitin ring test is a liquid based assay used to detect the presence of antigen-antibody complexes. To perform the test, a fixed amount of patient serum is placed in the bottom of a clear test tube. Next varying amount of soluble antigen is gently layered above the antibody solution. If the patient sample contains the antibody, immunocomplexes will form, and a white ring will become visible at the interface of the two liquid layers. Absence of a visible ring is indicative of the absence of antigen-antibody complexes.

The precipitin ring test is quick and easy to perform, cost-effective and can be adapted to detect both antibodies and antigen in biological samples. However, due to limited sensitivity and the semi-quantitative nature of the assay, other immunological assays such as ELISAs have replaced the precipitin ring test for the most part.

A12-2 Radial immunodiffusion assay

The radial immunodiffusion assay (RID) is a semi-solid based method for quantifying antigen-antibody interactions. In this assay, a predetermined dilution of patient serum is mixed with agar or agarose to generate semi-solid media on a plate. The solidified gel is cut to create small wells, which are then filled with various concentrations of antigen. As the antigen diffuses into the gel, it interacts with the antibody present in the agar to form a distinct precipitin ring around the well. The diameter of the ring is directly proportional to the concentration of antigen in the solution.

To estimate the concentration of an unknown sample, the diameter of the ring produced by the sample is measured and plotted on a standard curve generated using known

concentrations of antigen. While RIA is a well-established method for quantifying immune products, more sensitive and automated immunoassays such as ELISAs have largely replaced RIA.

A13: Tests for General Immune Status

A13-1 Complete Blood Count with Differential (CBC diff)

The complete blood count (CBC) test is a common blood test that provides information about the overall health status of a patient. It measures various components in a blood sample including red blood cells (RBC), white blood cells (WBC) and platelets. The CBC test with differential provides additional information regarding WBC as it counts the total numbers of five different WBC types: neutrophils, basophils, eosinophils, monocytes. and lymphocytes. In this test, a blood sample from the patient is analyzed using an automated hematology analyzer, which can identify and bin cells according to size, shape, and specific markers.

The CBC with differential provides additional insight into immune function of a patient as abnormal WBC differential counts may be indicative of infections, autoimmune diseases, allergic reactions, or leukemia. For example, an increase in the neutrophil count may be due to an active infection, inflammation, or acute stress, while an increase in the lymphocyte count may be indicative of a chronic infection or lymphocytic leukemia.

A13-2 C-reactive Protein Test (CRP)

Various conditions such as infection, tissue injury, autoimmune diseases and certain cancers can increase inflammation in the body. The C-reactive protein (CRP) is an acute phase protein released by the liver in response to inflammation. Acute phase proteins (APPs) are a group of approximately 30 unrelated proteins that are regulated in response to inflammation or injury. They are produced and released by the liver in response to tissue damage or insult, and are mediators of the inflammatory response. Prothrombin, plasminogen, fibrinogen, alpha-1 antitrypsin, haptoglobin and complement proteins are some other examples of APPs.

CRP is one of the most well-known and commonly measured APPs. Its levels rise rapidly in response to inflammation and can be used as a marker of inflammation and infection. Elevated CRP levels are also linked to an increased risk of heart disease. Therefore, monitoring CRP levels can help identify individuals who are at higher risk of heart disease.

Immunoturbidimetry is the most common method used to measure CRP. This test relies on CRP specific antibodies and the change in turbidity (cloudiness) associated with antigen-antibody complex formation. Serum samples are mixed anti-CRP antibodies. CRP will bind the antibody leading to the formation of a cloudy product. The turbidity of the sample will be higher if greater than normal ranges of CRP is being present in the sample. ELISA assays for measuring CRP are also available.

CRP is a non-specific marker of inflammation. While elevated CRP levels are indicative of the presence of inflammation, the cause or location of the inflammation cannot be identified with this test.

A13-3 Erythrocyte Sedimentation Rate (ESR)

The ESR test also known as sedimentation rate (sed rate) test measures the rate at which RBCs settle in a vertical tube over a specific period of time. The ESR test is based on the principle that when blood is drawn into a narrow tube and allowed to stand undisturbed, the RBCs will gradually settle at the bottom due to gravity. The rate at which they sediment is influenced by various factors, including the presence of APPs. APPs neutralize the negative charge present on the surface of RBCs, increasing their "stickiness." When APP levels are elevated, the presence of sticky RBCs result in a corresponding increase in ESR.

To perform an ESR test, whole blood samples are placed in a tall, thin tube (Westergren tube) and allowed to stand upright for a specific duration, typically one hour. During this time the RBCs will sediment due to gravity. After the designated time, the height of the serum present at the top of the tube is measured. Higher ESR rates suggest more significant inflammation in the body.

Similar to the CRP test, the ESR test is a non-specific indicator of inflammation in the body. It is not specific to any disease or condition and can only provide an indication of the presence and severity of inflammation. Due to its low cost and reproducibility, the ESR is often used as a "sickness indicator" in conjunction with other diagnostic tests for detecting infections, autoimmune disorders, inflammatory diseases and certain cancers.