Introductory Immunology

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Basic Concepts for Interdisciplinary Applications

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Radarweg 29, PO Box 211, 1000 AE Amsterdam, The Netherlands
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First published 2014

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Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

ISBN: 978-0-12-420030-2

PREFACE

DEDICATION

To my father, Paul Actor, PhD, who instilled in me a sense of excitement about the wonders of science and the curiosity to seek questions about how biological systems function.

PREFACE

Our bodies have evolved a protective set of mechanisms, comprised of cells and organs, as a primary defense to maintain health. In essence, we have developed internal tools to preserve health and homeostasis. Indeed, a working definition for "health" embraces the effective elimination or control of life-threatening agents. This includes both infectious agents attacking from the outside and internal threats, such as tumors. Immune responses are therefore designed to interact with, and respond to, the environment to protect the host against pathogenic invaders and internal dangers. The goal of this book is to appreciate the components of the human immune system that work together to confer protection.

We will begin our discussion by establishing a foundation for subsequent chapters, through presentation of the systems and cells involved in immune responses. **Chapter 1** will give a general overview on mechanisms in place to fight against disease. Components and pathways will be defined to allow presentation of concepts of innate (always present) and adaptive (inducible and specific) responses, and how these responses interact with one another to form the basis for everyday protection. These concepts will form the foundation to examine the process of defense against different classes of pathogens. **Chapter 2** will examine the coordinated effort of cells and blood components in development of inflammation as related to protection against infection. **Chapter 3** will introduce the basis for function of adaptive components, exploring the generation of B lymphocytes and

the nature of antibodies. **Chapter 4** will extend this discussion to T-lymphocyte populations and examine how they serve as ringleaders for immune function. **Chapter 5** will discuss immune responses with an element of detail focused on infectious organisms commonly encountered. This overview will also contain how initial engagement of pathogens by innate components leads to triggering of pathways to cause inflammation. A special section will introduce opportunistic infections and diminished response when individuals are immunocompromised.

Effective immune surveillance is paramount to maintaining health. **Chapter 6** will examine basic disorders of immune function. Too little of a response results in an inability to control threats, thus is ineffective to eliminate infectious agents. This lack of reactivity (hyporeactivity) leads to holes in our immune repertoire. This may be the result of genetic deficiencies or due to acquired compromisation of immune function. In the same manner, responses representing excessive activity can also lead to damage to the host. This overaggressive response, a state of hyperreactivity, may reflect a productive response that increases in intensity and duration without effective control. The dysregulation leads to tissue-damaging events and eventual states of disease.

The chief function of the immune system is to distinguish between what is you and what constitutes external threats. When the ability to distinguish these elements is compromised, autoimmunity may arise. In **Chapter 7**, autoimmune dysfunction will be addressed, moving from basic concepts to specific mechanisms involved in major clinical disorders. This includes a detailed discussion of how "self" is recognized, as well as mechanisms involved in tolerance to limit reactivity to our own tissues. The goals are to present clinical manifestation of autoimmunity in a manner so that outward symptoms are understood through investigation of the molecular targets involved in the host immune self-recognition response. At other times, misdirected recognition of nonself elements, such as environmental allergens that typically are considered harmless, result in development of clinical presentations. **Chapter 8** will therefore examine the processes involved in manifestation of immune dysfunction, examining the concepts of immune hypersensitivities which lead to clinical disease.

The general topic of vaccines will be addressed in **Chapter 9**, including both how they work and a frank discussion of the relative truths and myths surrounding their use. This chapter will also contain

information on "newer" therapeutics that are grounded in methods that lead to immune modification and factors which promote a healthy immune response (for example, lifestyle activities and good common practices). Indeed, it is critical that we maintain a healthy balance throughout our lives to ensure functional immune response as we age. The challenges faced at each stage of our lives, from that found in the prenatal/newborn to midlife to "mature" status, are mentioned in a way to encourage a healthy condition to allow optimization of immune function.

A discussion of natural (effective) response to tumor development in **Chapter 10** will allow an investigation into components of immune function to naturally eliminate potentially dangerous precancerous events. This will be followed by a discussion of the challenges faced when protective responses fail and tumors develop. A section will also contain information on cancers of the immune system, and the problems that arise when the protective cells themselves become the cause of tumorigenic activity.

Chapter 11 will delve once more into details underlying concepts of "self" versus "nonself" and blood types, with the goal to present genetic relationships (similarities as well as differences) between individuals. The mechanisms of the immunobiology of transplantation will be discussed, with details on the contributing cells and factors involved in transplant acceptance versus rejection. The challenge is to appreciate the importance of innate and adaptive components in graft recognition, as well as to recognize clinical consequences of transplantation that affect aspects of daily activities. Rejection topics will be discussed, including graft versus host disease, as well as modern immune-based therapeutics designed to alter immune function to limit graft rejection.

Finally, additional information and resources will be provided in **Chapter 12** to allow the reader to develop an immune-based knowledge foundation to understand clinical tests associated with identifying immune parameters that arise during development of disease states. As such, this includes an introduction to mechanisms that form the basis of immune-related diagnostics and identification of immune properties of the blood during disorders.

All in all, the hope is to present a working understanding of the concept of the immune system so that the reader may better

understand immune-based diseases resulting from either immune system component deficiencies or excess activity. This book is aimed at those who want to know more and to encourage the reader to explore deeper. It is aimed at the curious who have never previously considered facets underlying effective immune function. To the student who wishes to expand upon basic knowledge of biological systems. To the physician seeking a refreshed understanding of immune concepts that cause clinical disease. To the nurse who desires to expand their view of symptom development in patients. To the patient who desires a simple explanation for the complex way their bodies respond in the context of the world they inhabit. To all who seek to ask how the body confers protection against infectious agents, maintains everyday homeostasis, and guards against dysregulation of normal response to confer health and control development of disease.

ACKNOWLEDGMENTS

I would like to give a special thanks to Keri Smith, PhD, for academic contribution to the chapters on antibodies and on immunoassays. In addition, thanks goes to Henry Blum for sharing his positive outlook on life and showing me the ability to embrace joy through study; to Ranon Teller for embracing and accepting those who learn from different perspectives; to my children, Jonas and Amanda, for sharing a thirst for knowledge and for continuing to ask questions; and to my wife Lori for her love and patience and for understanding my desire to complete this project. Finally, I extend gratitude to both Halima Williams and Anusha Sambamoorthy for their assistance in bringing this project to fruition.

CHAPTER 1

A Functional Overview of the Immune System and Immune Components

Chapter Focus: To establish a foundation to appreciate how components of the immune system work together to protect against development of clinical disease. The basic systems and cells involved in immune responses will be presented to give a general overview of functional immunity. Components and systems will be defined to allow an understanding of concepts of innate (always present) and adaptive (inducible and specific) responses, and how these responses interact with one another to form the basis for protection against disease.

IMMUNE HOMEOSTASIS

A functional immune system offers constant surveillance of ourselves in relationship to the world. It confers a balanced state of health through effective elimination of infectious agents (bacteria, viruses, fungi, and parasites) and through control of malignancies. Indeed, the immune system has evolved to allow cells and organs to interact with the environment to protect against harmful invaders. At the same time, mechanisms are in place for tolerance toward the naturally occurring microbiome (microbial and viral agents) that reside within us in symbiotic ways. Taken together, these responses represent a balance of components that ward off development of clinical disease.

SELF VS. NON-SELF

Discrimination between "self" and "non-self" is considered the chief function of the immune system. We are under constant assault by invaders. Our bodies represent prime substrates for organisms to grow and reside, with an abundance of nutrients, warmth, and protection from the outside elements. The immune system is basically a series of obstacles to limit and inhibit pathogen entry and then attack and

Introductory Immunology. DOI: http://dx.doi.org/10.1016/B978-0-12-420030-2.00001-9

destroy those organisms once they enter the body. The immune response is exquisitely designed to recognize these invaders as "foreign." In fact, the major feature that renders our immune system so effective is its ability to distinguish our body's own cells ("**self**") from that which it considers foreign (termed "**non-self**"). Each one of our cells carries specific tags, or molecular markers, that label it as "self." These markers are important, as they not only determine what is unique about us, but they also distinguish one person from another.

Almost anything and everything that registers as "non-self" will trigger an immune response. An intricate system of molecular communication and cellular interactions allows immune components to function in concert to combat disease-causing organisms. The foreign agent (microbe, virus, parasite, etc.), or any part of it that can be specifically recognized, is called an **antigen**. Simply put, an antigen is defined as any substance that can be recognized by the immune system. Major classes of antigens include proteins, carbohydrates, lipids, and nucleic acids. If an antigen is of high complexity and weight, it can trigger full immune activity and become **immunogenic**.

The ability to distinguish our own cells from the outside world is critical in maintaining functional protection. If this ability is lost, e.g., when "self" tissue is seen as foreign, then our immune system launches an aggressive response against our own tissues. This is what happens during **autoimmunity**, where destruction of "self" leads to clinical disease.

The immune system maintains a balance of responsiveness. Too little a response is ineffective, while too aggressive a response can lead to targeted destruction of bystander tissues. Both scenarios are equally as devastating and may result in clinical disease. The regulation of immune function and overall immuno-homeostasis is under control of multiple factors that include genetic components and environmental cues. The intensity and duration of response must be sufficient to protect against invading pathogens, with prompt and specific downregulation when the foreign material (the antigen) is no longer present. The clinical state that arises when immune responses are not properly regulated is termed **hypersensitivity**; a state of excessive or inappropriate responses leads to disease. As one might imagine, hypersensitivity can occur in many different forms, depending upon which arm of the immune system is dysregulated.

INNATE AND ADAPTIVE IMMUNITY

The immune system is loosely divided into two major functional categories termed **innate** and **adaptive immunity**. Innate immune mechanisms provide the first line of defense from infectious disease (Table 1.1). The innate immune components are present from birth and consist of components available prior to the onset of infection. These defensive components include both physical barriers and biochemical factors. Defensive innate mechanisms may be anatomic (skin, mucous membranes), physiologic (temperature, low pH, chemical mediators), phagocytic (digestion of microorganisms), or inflammatory (vascular fluid leakage).

Innate mechanisms are particularly powerful at limiting infections. However, once the infectious agent is established inside the body, a more focused set of reactive molecules and cellular components are required to specifically combat the organism. An intricate system of molecular communication and cellular contact allows components of the innate immune group to trigger cells involved in adaptive immunity. In essence, both innate and adaptive components must function in concert to combat and control disease.

Table 1.1 Innate Defensive Components

Component	Effectors	Function
Anatomic and physiologic barriers	Skin and mucous membranes	– Physical barriers to limit entry, spread and replication of pathogens
	Temperature, acidic pH, lactic acid	
	Chemical mediators	
Inflammatory mediators	Complement	– Direct lysis of pathogen or infected cells
	Cytokines and interferons	– Activation of other immune components
	Lysozymes, defensins	– Bacterial destruction
	Acute phase proteins and lactoferrin	– Mediation of response
	Leukotrienes and prostaglandins	– Vasodilation and increased vascular permeability
Cellular components	Polymorphonuclear cells • Neutrophils, eosinophils • Basophils, mast cells	– Phagocytosis and intracellular destruction of microorganisms
	Phagocytic–endocytic cells • Monocytes and macrophages • Dendritic cells	– Presentation of foreign antigen to lymphocytes

The adaptive (also called "**acquired**") immune response accounts for specificity in recognition of foreign antigenic substances. It is critical to understand that specificity of the adaptive immune response lies within two distinct subsets of white blood cells, called **lymphocytes**. Lymphocyte recognition of unique shapes associated with foreign antigens is accomplished by functional receptors residing on their cellular surface. Key elements of the acquired immune responses are compared to innate functional elements, as listed in Table 1.2.

The adaptive immune response is subdivided into functional groups representing **humoral and cellular immunity**, based on participation of the two major cell types. Humoral immunity involves **B lymphocytes** (also called **B cells**) which synthesize and secrete **antibodies**. Cellular immunity involves effector **T lymphocytes** (also called **T cells**) which secrete immune regulatory factors following interaction with specialized processing cells (called **antigen presenting cells**; **APC**s) that show the lymphocytes foreign material in the context of self-molecules.

ANATOMY OF THE IMMUNE SYSTEM

The immune system is just that: a "system." It is a network of protective barriers, organs, cells, and molecules. Specifically, there are subsets of primarily bone-marrow-derived cells which circulate throughout the body. Indeed, the power of the "system" is that contributing immune cells can be found within every major organ and in every tissue. These cells are available to be called into action at very short notice.

Table 1.2 Key Elements of Innate and Acquired Immune Responses

Innate	Adaptive
Rapid response (minutes to hours)	Slow response (days to weeks)
PMNs and phagocytes NK cells	B cells and T cells NKT cells
Preformed effectors with limited variability Pattern recognition molecules recognizing structural motifs	B-cell and T-cell receptors with highly selective specificities to foreign agents
Soluble activators Proinflammatory mediators	Antibodies (humoral) Cytokines (cellular)
Nonspecific	Specific
No memory, no increase in response upon secondary exposure	Memory, maturation of secondary response upon reexposure

The interactions are managed by a series of central **lymphoid organs** (e.g., bone marrow, thymus, spleen, and lymph nodes) containing high levels of lymphocytes. The immune-based lymphoid organs are where leukocytes of myeloid and lymphoid origin mature, differentiate, and multiply (Figure 1.1). Cells also accumulate outside these major organs, residing in less defined areas (e.g., throughout the gut or skin), to allow for protective responses at local sites when responses are rapidly needed.

Primary lymphoid organs are the sites where lymphoid cells are generated. This act of cellular development, or **lymphopoiesis**, occurs in the liver in the fetus, and then in the bone marrow after birth. Islands or progenitor stem cells give rise to immune system cells that are subsequently released into the blood. A specialized primary lymphoid organ is the **thymus**, a pyramid-shaped gland that is located beneath the

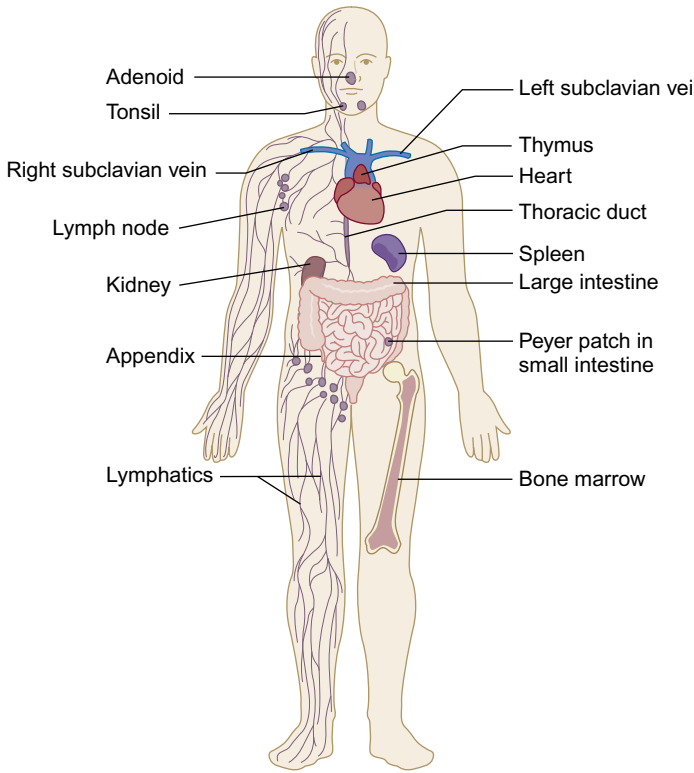


Figure 1.1 ***Distribution of lymphoid tissues.*** *Primary lymphoid organs, such as the bone marrow and thymus, are the major sites of lymphopoiesis and where lymphocytes differentiate. Secondary lymphoid organs, such as the spleen and lymph nodes, are sites where antigen-driven proliferation and maturation of lymphocytes occur.*

breastbone at the same level as the heart (Figure 1.2). Immature lymphocytes leave the bone marrow and circulate directly through the thymus. The principal function of the thymus gland is to educate T lymphocytes to distinguish between what is you (self) and what is not (non-self). When lymphocytes leave the thymus, they are committed along a certain pathway of activity and are ready to perform their effector functions. The other set of lymphoid organs, called **secondary lymphoid** organs, are structured compartments that provide a favorable environment for cell contact and activation of committed cells.

Lymphocytes continuously leave the blood vessels, migrating throughout the body where they perform surveillance activities. The return path to circulation begins when cells mix with fluids that naturally bathe tissues. The mixture eventually "drains" through small vessels to return materials into the blood supply. The fluid in the tissue is called **lymph**, and it carries cells and debris through small vessel **lymphatics**. The lymphatics direct the lymph and cells through secondary lymphoid organs before reaching the thoracic duct, where fluid and cells are returned to the venous circulation of the blood supply.

Lymph nodes are focal nodules connected by way of the draining lymphatic highway. They are placed throughout the body, with groupings found in the groin, armpits, and abdomen. They represent local nodes for antigen and cellular drainage. It is here where lymphocytes can interact and communicate with APCs, allowing a local presentation of antigenic particulates found in nearby regions of the body. Think of the regional lymph nodes as reststops along the highway, where cells can mingle and discuss local and system-wide information. If there is need for immediate response, cells can actively mobilize efforts to defend or repair tissues. In essence, this is where antigen-driven proliferation and differentiation occurs. Local lymph nodes become swollen and painful as cells respond to regional damage and drained materials lead to activation of the immune response team. Within the lymph nodes, the areas of response are called **germinal centers**. Indeed, the term "germinal center" is used to describe any local foci of responding lymphocytes in secondary immune reactive sites.

Just as the lymph nodes are connecting nodes for the lymphatics, the **spleen** is a filtering organ for circulating blood. The spleen, located in the upper portion of the abdomen, can be considered a holding facility where both innate and resting adaptive cells reside in specialized compartments.

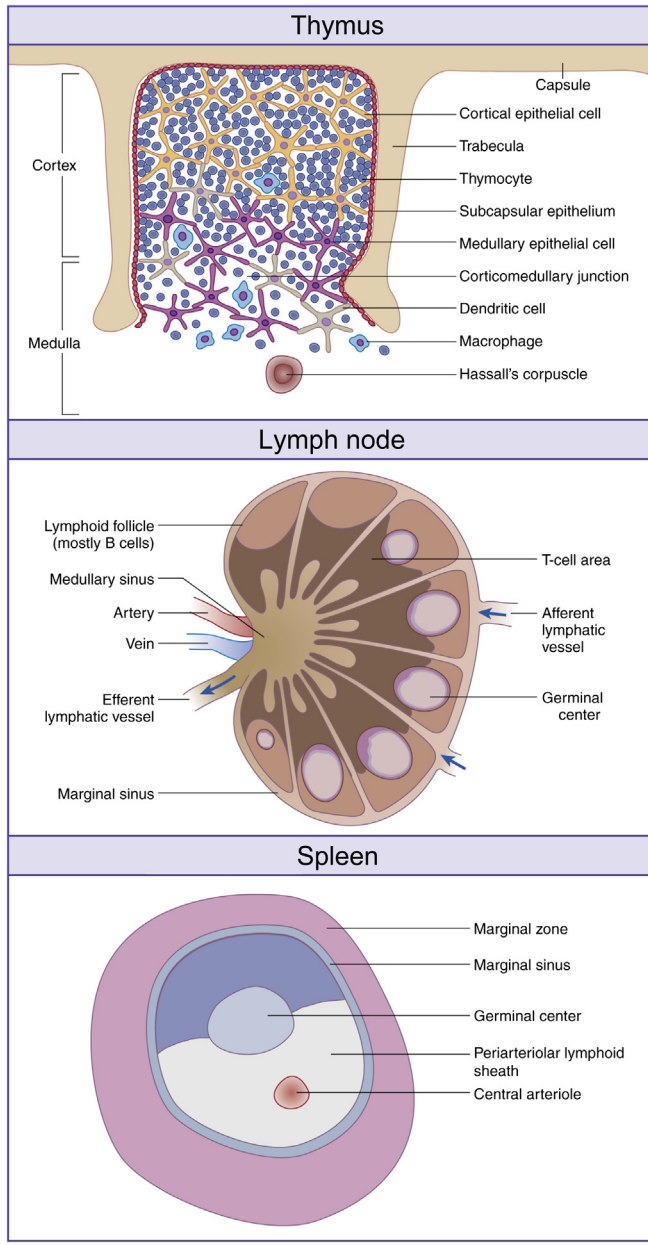


Figure 1.2 ***Major organs of the immune system.*** *The thymus is a primary organ responsible for education of lymphocytes to differentiate between self and non-self. The lymph nodes are secondary organs placed throughout the body, as focal nodules where lymphocytes interact and communicate with APCs. The spleen is a secondary organ, where resting lymphocytes reside to readily mobilize in response to detection of foreign materials.*

The main areas of the tissue are either comprised of lymphoid cells (called the "white pulp") where immune cells interact or comprised of red blood cells (RBCs) and associated areas where RBCs flow (called the "red pulp"). These different compartments for cellular activation allow cells to readily activate and mobilize in response to communication signals indicating foreign materials have been identified.

Another major secondary immune organ is not a truly defined organ, but rather represents a loosely associated cellular aggregation in areas where contact with foreign material is common. This type of immune aggregation is found in tissue layers that line the intestines, the lung, and the nasal cavities. These aggregates are called **mucosa-associated lymphoid tissue**, or **MALT**, and represent areas of rapid surveillance and detection for organisms entering through major openings in our bodies. The tonsils, adenoids, appendix, and Peyer's patches (organized tissue in the large intestines) represent a more formal association of parenchyma that shares the same functional parameters as the MALT. In an analogous manner, aggregates lining bronchial regions are called **BALT** (**bronchial/tracheal-associated lymphoid tissue**), and those lining the intestinal tract are referred to as the **GALT** (**gut-associated lymphoid tissue**). There are specialized cells in some of these aggregates; **M cells**, or microfold cells, can be found in the follicle-associated epithelium of the Peyer's patch. M cells sample antigen from the lumen of the small intestine and deliver it via transcytosis to immune cells located on their basolateral side.

CELLS OF THE IMMUNE SYSTEM

A **leukocyte** is the term given to any white blood cells that play a functional role in either innate or adaptive responses. This population of cells can be broken into two main groups, referred to as **myeloid** or **lymphoid** cells, depending on which developmental path was taken by the stem cells in the bone marrow during development (Figure 1.3). Myeloid cells are considered as the first line of defense and thus constitute the major cells involved in innate immunity (Table 1.3). Myeloid cells include highly phagocytic, motile **neutrophils**, **monocytes** and **macrophages**, and **dendritic cells** that provide relatively immediate protection against most pathogens. The other myeloid cells, including **eosinophils**, **basophils**, and their tissue counterparts, **mast cells**, are involved in defense against parasites and in the genesis of allergic reactions. In contrast, lymphoid cell types include

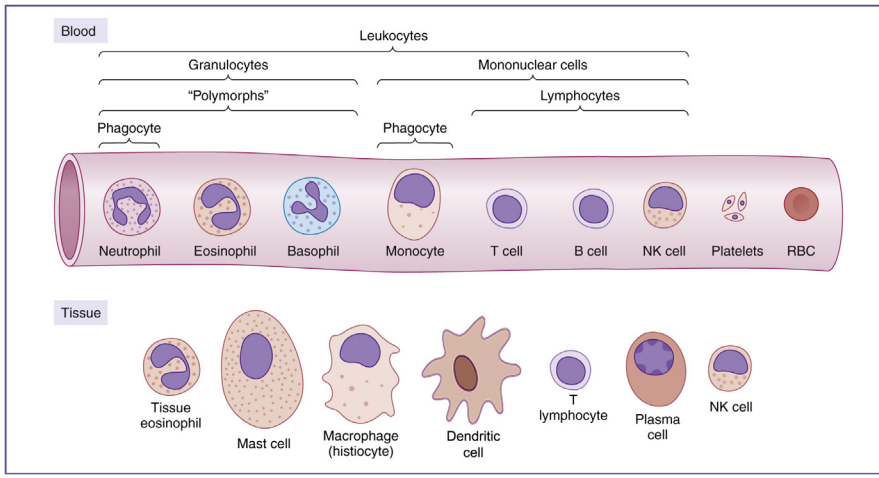


Figure 1.3 ***Nomenclature and location of immune system cells.***

Table 1.3 Myeloid Leukocytes and Their Properties

Phenotype	Morphology	Circulating Differential Cell Count[a]	Effector Function
Neutrophil	PMN granulocyte	$2–7.5 \times 10^9$/L	Phagocytosis and digestion of microbes
Eosinophil	PMN granulocyte	$0.04–0.44 \times 10^9$/L	Immediate hypersensitivity (allergic) reactions; defense against helminths
Basophil	PMN granulocyte	$0–0.1 \times 10^9$/L	Immediate hypersensitivity (allergic) reactions
Mast cell	PMN granulocyte	Tissue specific	Immediate hypersensitivity (allergic) reactions
Monocytes	Monocytic	$0.2–0.8 \times 10^9$/L	Circulating macrophage precursor
Macrophage	Monocytic	Tissue specific	Phagocytosis and digestion of microbes; antigen presentation to T cells
Dendritic cell	Monocytic	Tissue specific	Antigen presentation to naïve T cells; initiation of adaptive responses

PMN, polymorphonuclear.
[a]*Normal range for 95% of population, ± standard deviations.*

those that mediate specific immunity; simply put they are the cell types (**phenotypes**) that have defined receptors to physically interact with and recognize foreign materials (Table 1.4). These types of cells fall into the acquired category and include the B lymphocytes and the T lymphocytes that were mentioned above. In addition, a group of cells called **natural killer T cells** (**NKT cells**) exist which are a specialized subset of

Table 1.4 Lymphoid Leukocytes and Their Properties			
Total Lymphocytes 1.3–3.5 × 10^9/L			**Effector Function**
B Cell	Monocytic	Adaptive	Humoral immunity
Plasma Cell	Monocytic	Adaptive	Terminally differentiated, antibody secreting B cell
T Cell	Monocytic	Adaptive	Cell-mediated immunity, immune response regulation
NKT Cell	Monocytic	Adaptive	Cell-mediated immunity (glycolipids)
NK Cell	Monocytic	Innate	Innate response to microbial or viral infection

lymphocytes. A functionally related set of lymphoid cells that are also considered lymphoid in origin are the **natural killer cells** (**NK cells**). Although similar in name, and slightly confusing, NK cells are distinct from NKT cells.

FIRST LINE DEFENDERS: THE MYELOID CELLS

Neutrophils are highly adherent, motile, phagocytic leukocytes which are typically the first cells recruited to acute inflammatory sites. Neutrophils are the most abundant of the myeloid populations. They are stored in the bone marrow and are readily released during infection. Neutrophils engulf (**phagocytose**) and devour pathogens, after which they use specialized destructive enzymes and toxic molecules to kill the ingested organism. Many of these enzymes regulate reactive oxygen species, such as superoxide and nitric oxide, to mediate killing. This **respiratory burst**, the phase of elevated oxygen consumption shortly after cellular ingestion of organisms, allows the neutrophil to limit expansion and pathogen growth. In essence, the neutrophil acts as a first responder, giving immediate help and slowing spread of infection while the next phases of the immune response are mobilized.

Like the neutrophil, **eosinophils** also have specialized molecules, stored as granules, which they release in defensive response to infection. While the neutrophil is adept at engulfing smaller organisms, the eosinophil is successful against large multicellular parasites that are too big to fit inside any one cell.

Basophils, and their tissue counterparts called **mast cells**, produce cytokines that help defend against parasites. However, these cells are best known clinically for their role in allergic inflammation. Basophils and mast cells display surface membrane receptors for a specific class

of antibodies; they release a host of molecules, such as histamine and vascular mediators that affect blood flow, when cell-bound antibodies recognize allergens.

Macrophages are also involved in phagocytosis and intracellular killing of microorganisms. Macrophages can reside for long periods in tissues (outside of the bloodstream). These cells are highly adherent, motile, and phagocytic; they marshal and regulate other cells of the immune system, such as T lymphocytes. In a similar manner, **dendritic cells** also provide a critical link between innate and adaptive immunity by interacting with T cells. The unique function of dendritic cells lies in the manner of how they deliver strong signals for development of memory responses. Dendritic cells recognize foreign agents through a series of unique receptors that recognize general shapes (motifs) on foreign organisms. Different cellular subsets exist, enabling this group of cells to both prime and dictate how subsequent responses will develop.

Macrophages and dendritic cells are aptly called **APCs**. After they destroy pathogens, they show (present) chopped pieces to T lymphocytes and thereby mediate a connection with the adaptive immune response. Basically, the pathogen is digested inside the presenting cell, and small fragments are shown on their cell surface for recognition by adaptive lymphocytes. Indeed, the macrophages and dendritic cells, along with other specialized APCs, contain a myriad of surface molecules that directionally drive responsiveness and focus adaptive responses to allow productive elimination of microorganisms.

Finally, **platelets and erythrocytes** (RBCs) also arise from bone-marrow-derived myeloid megakaryocyte precursors. Platelets are involved in blood clotting and wound repair. During the process of wound repair, they release inflammatory mediators involved in innate immune activation.

ADAPTIVE AVENGERS: THE LYMPHOID CELLS

Lymphoid cells provide efficient, specific, and long-lasting immunity against microbes and pathogens, and are responsible for acquired immunity. As a group, they respond to infectious invasion only after the myeloid cells have begun their work. Indeed, the innate responding cells discussed above send signals to the lymphoid population to "stop and smell the inflammation" and engage them to specifically respond

in a directional manner. **Lymphocytes** differentiate into separate lineages. The **B lymphocytes** secrete antibodies. The **T lymphocytes** operate in a supervising role to mediate cellular and humoral immunity. A third group, the NK cells mentioned previously, is critical in defense against viral agents. B and T lymphocytes produce and express specific receptors for antigens, while NK cells do not.

LYMPHOCYTES

The adaptive ("acquired") immune response accounts for specificity in recognition of foreign substances, or antigens, by functional receptors

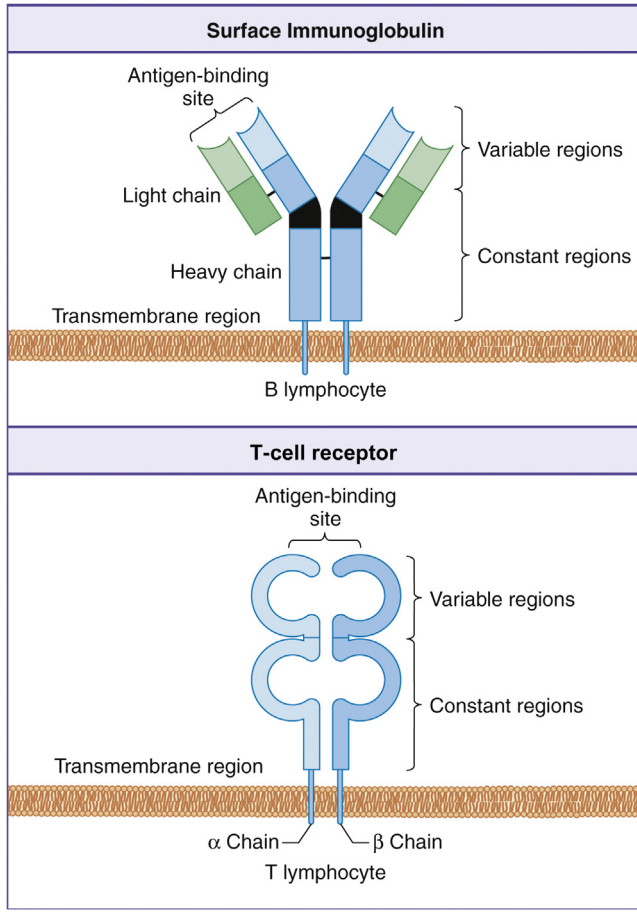


Figure 1.4 ***Basic structure of the antigen receptors.*** *Depicted are the antigen receptor on the surface of a B cell (the immunoglobulin B-cell receptor, BCR) and on the T cell (the TCR).*

residing on their cell surfaces (Figure 1.4). The **B-cell antigen receptor** is the surface **immunoglobulin**, an integral membrane protein with unique regions to bind specific antigenic shapes. There can be thousands of identical copies of the receptor present on the surface of a single B lymphocyte (simply called a **B cell**). B-cell activation occurs when the receptor encounters the antigen. This leads to a morphological change in B cells, which now multiply to become secretory factories to make and release soluble immunoglobulins, or **antibodies**. B cells that actively secrete antibodies are called **plasma cells**. We will see in subsequent chapters how antibodies are critical to target and neutralize invading organisms.

The T lymphocyte matures as it passes through the thymus. Like its B cell counterpart, it also has a surface receptor, called the **TCR**. The TCR is structurally similar to the antibody; it too recognizes specific pieces of the antigen, although we will see in later chapters that there are restrictive constraints on this interactive process. Unlike the antibody, the TCR is only present on its surface and is not secreted when T cells are activated. The process of T-cell activation is quite complex and requires a group of cells to physically show pieces of the antigen to the T cell. These assisting cells are the APCs described above. The surface molecules on the presenting cell which show antigen to the T cells are also involved as tags for "self" identity. These molecules are called **major histocompatibility molecules** (**MHC molecules**). Together, these cellular events form a regulated pathway to present foreign antigens for subsequent recognition and triggering of specific responses to protect against disease.

The T lymphocytes are the true ringleaders of the adaptive response. Different subsets control different functions. Some help B cells produce antibodies. Others help the myeloid cells become more efficient to destroy pathogens. Some preferentially function to kill viral infected cells or tumor cell targets. Finally, some function as regulators, conferring immune tolerance or establishing limits on responsiveness.

CLUSTER OF DIFFERENTIATION

The **cluster of differentiation** (**CD**) designation refers to cell surface proteins. Each unique molecule is assigned a different number designation,

which allows identification of cell phenotypes. Surface expression of a particular CD molecule may not be specific for just one cell or even a cell lineage; however, many are useful for characterization of cell phenotypes. The CD designation will be used throughout this text. The official listing of determinants has identified over 350 individual and unique markers. A simplified list of CD molecules and their associated cell function is given in Table 1.5. CD-specific diagnostic agents have been useful for determining the functions of proteins and for identifying their distribution in different cell populations. They have also been used for measuring changes in the proportion of cells carrying those markers in patients with disease.

Table 1.5 Selected CD Markers and Associated Functions

CD Marker	Biological Function
CD1	Presentation of glycolipids to NKT cells
CD2	T-cell adhesion molecule
CD3	Signaling chains associated with the TCR
CD4	Coreceptor for Class II MHC on T cells
CD8	Coreceptor for Class I MHC on T cells
CD11	Leukocyte adhesion
CD18	β2 integrin
CD19	B-cell signal transduction
CD20	B-cell calcium channel activation
CD21	B-cell activation
CD25	IL-2 receptor α chain
CD28	T-cell costimulatory molecule
CD32	IgG receptor
CD34	Hematopoietic stem cell marker
CD40	Class switching on B cells
CD44	Lymphocyte adhesion
CD54	Adhesion molecule
CD58	Adhesion molecule
CD59	Regulator of complement MAC assembly
CD62L	T-cell adhesion to high endothelial venules
CD80	Costimulatory receptor on APCs
CD86	Costimulatory receptor on APCs
CD95	Induction of apoptosis
CD152	Negative regulator for T cells
CD154	Involved in B-cell proliferation and class switching

SUMMARY

- Our immune responses are designed to interact with the environment to protect against pathogenic (disease causing) invaders.
- Immunity is based on functional discernment between self and non-self, a process that begins *in utero* and continues through adult life.
- A network of organs allows generation of functional cells and regional centers for cellular interaction and recognition of foreign (non-self) material.
- Physical barriers comprise the first line of defense against pathogens. Once inside the body, innate systems react on short notice to confer protection. Cells of the myeloid lineage (including neutrophils and macrophages) are readily available to allow containment of organisms until the specific adaptive immune response is engaged.
- Innate cells form a primary network to set the stage for arrival of adaptive cells. The macrophages and dendritic cells serve as a trigger to present foreign materials and give directionality to the next stage of adaptive cellular responses.
- Adaptive immunity allows discrimination of antigens using defined receptors. B lymphocytes make antibodies as a major defensive strategy. T lymphocytes perform as the functional ringleaders to direct the defensive response.

CHAPTER 2

The Inflammatory Response

Chapter Focus: To examine the coordinated effort of cells and blood components to elicit the inflammatory response. Information will be presented to allow an understanding of how initial organism detection launches an inflammatory cascade. Factors creating redness and swelling, heat, and pain will be addressed with an overall goal to identify and appreciate the processes involved in the development of inflammation as related to protection against infectious disease.

INFLAMMATION

Innate immune mechanisms provide the first line of defense against infectious disease. At the very basis of this response is a set of components that are continuously available for immediate response. These components are present prior to the onset of infection or tissue damage. These constitutive factors initiate a series of coordinated events that allow rapid detection of microorganisms and then triggering of a signaling cascade to call in cell types that are adept at controlling infection. While these events productively begin both healing and protective processes, they also culminate in inflammation.

Greek literature suggests that ancient healers held a strong knowledge of the inflammatory response. Yet it was the Romans that established the first written clinical definition of inflammation. According to Cornelius Celsus (circa the first century), **inflammation** was defined by four cardinal signs: RUBOR ET TUMOR CUM CALORE ET DOLORE. Redness and swelling with heat and pain. We will approach this topic by examining the response to infection, although realize that inflammation may arise in the absence of an infectious agent (think trauma or response to a bee sting). Manifestation of the four signs is related to physiological changes. Redness occurs due to changes in localized blood flow (vasodilation); swelling occurs from influx of fluid and cells leaving blood vessels to enter tissue; heat is the result of increased blood arriving to areas of damage; and pain results from edema (fluid accumulation) which increases pressure on local

Introductory Immunology. DOI: http://dx.doi.org/10.1016/B978-0-12-420030-2.00002-0

Table 2.1 Effects of Fluid Exudate

	Effectors	Function
Beneficial	Entry of plasma fluid	Delivery of nutrients and oxygen
		Dilution of toxins
		Delivery of immune response mediators
		Dilution of toxins
	Entry of antibodies	Lysis of microorganisms (complement)
		Assisted phagocytosis (opsonization)
		Neutralization of toxins
	Fibrin formation	Impede movement and trap microorganisms; facilitate phagocytosis of foreign agents
	Entry of cells	Initiation of innate and adaptive immunity
Detrimental	Excess innate cell activation	Release of lysosomal enzymes
		Digestion or destruction of normal tissues
	Excess plasma fluid	Obstruction of ducts, lymphatics
		Vascular constriction and ischemic damage
		Pressure on nerves (pain)
Outcomes	Drainage to lymphatics	Delivery of antigens to lymph nodes
		Antigen presentation to adaptive cells

nerves surrounding the damaged site (Table 2.1). If these four cardinal signs persist over time, a fifth component can be added to the definition, FUNCTIO LAESA, which encompasses loss of function to the inflamed area due to destruction of living tissue.

INITIATION OF THE INFLAMMATORY RESPONSE

The skin provides an effective mechanical barrier against microorganisms. Breach of that inflammatory barrier triggers an immediate cascade (Figure 2.1). A simple tear or rip in the skin and underlying tissue causes disruption of cells and release of lipids from cellular membranes. A series of bloodborne enzymes are designed to transform released membrane components into signaling molecules, which can subsequently exert effects on blood vessels. For example, production of **prostaglandins** and **leukotrienes** leads to vasodilation, in essence causing the endothelium lined blood barrier to become more or less "leaky."

Dilated local blood vessels, capillaries, and small venules/arterioles subsequently allow fluid (edema) to accumulate into the damaged area.

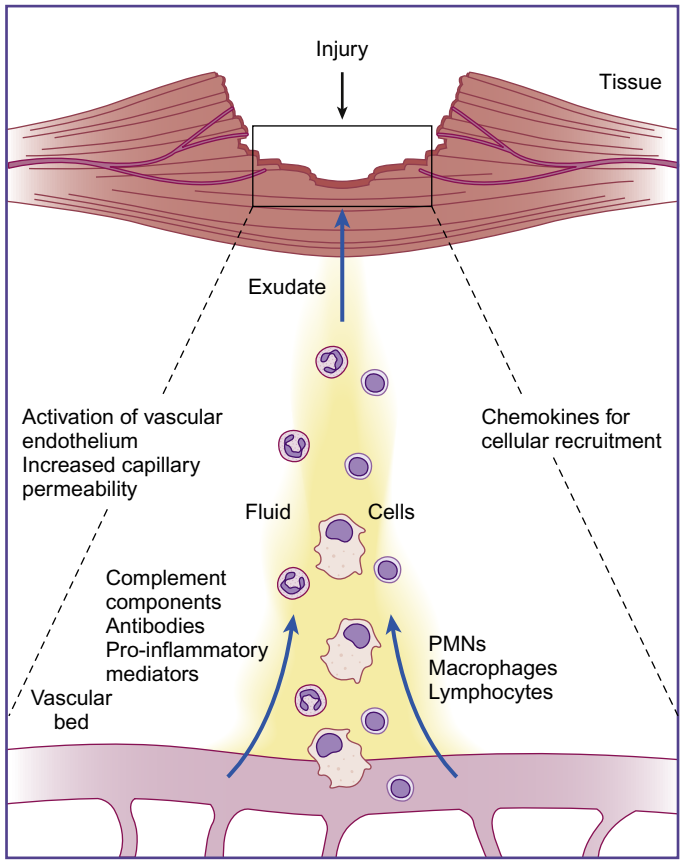


Figure 2.1 ***Inflammation as a result of tissue injury.*** *Inflammatory responses lead to vasodilation causing erythema (redness) and increased temperature, increased capillary permeability which allows exudate (fluid) to accumulate leading to tissue swelling (edema), and influx of cells to site of tissue damage. Cells entering the area of injury release chemotactic factors to recruit additional cells, leading to local activation at the damaged site.*

If the breach in the mechanical barrier is deep enough, damaged blood vessels also directly flood the local area with leukocytes and red blood cells. **Plasma** (blood minus the cells) contains critical components to mediate development of inflammation. On a positive side, the immediate consequence of tissue damage allows direct delivery of nutrients and oxygen. Fluid entering the site also contains liver-derived factors which are always present in circulating blood. Many of these molecules are effective at coagulation and repair, working to direct the **clotting cascade** and the production of **fibrin**. Plasma serine proteases, normally present as inactive molecules, are activated; some of these function to produce **kinins** to mediate vascular permeability and pain.

Other proteins and enzymes are released by platelets, which help further drive and direct the initial response. Indeed, these factors initiate molecular changes that, from an immunological standpoint, are extremely beneficial. Clotting and formation of a fibrin matrix impedes movement of microorganisms in the local area; generation of breakdown products in the cascading events also attracts and activates incoming leukocytes.

ROLE OF ANTIBODIES IN INFLAMMATION

A major benefit of the influx of fluid is the direct delivery of relatively high levels of antibodies to the area. The circulating plasma is filled with antibodies that, as a group, have the ability to recognize just about any shape or form of antigen. Depending on prior exposure or positive vaccination status, there may also be an abundance of specific antibodies reactive to antigenic features present on destructive pathogens.

Antibody entry to the area confers multiple functions, the most critical of which resides within the end of the antibody molecule that can recognize unique shapes and forms, with a specific and unique binding site for foreign substances. This allows for binding directly to the pathogen or to pathogen-derived proteins. In effect, antibodies recognize toxins and deleterious factors; recognition results in inhibition and neutralization of their toxic properties. Direct binding inhibits organism movement and can block attachment and adhesion to target host cells. Antibody recognition of multiple regions on the microorganism can result in a latticework structure, a precipitate, which promotes organism clearance.

The opposite end of the antibody molecule has a constant structure which confers biological function. Innate phagocytes contain surface receptors for the constant end of the antibody molecule. These receptors assist pathogen engulfment, specifically targeting foreign agent destruction using intracellular enzymes. The term for coating the organism with antibodies is **opsonization**. Targeted engulfment is referred to as **phagocytosis**.

BIOLOGICAL FUNCTIONS OF COMPLEMENT

Opsonization of organisms has another role. Simply coating the agent with antibody targets it for attack by serum enzymes that comprise the

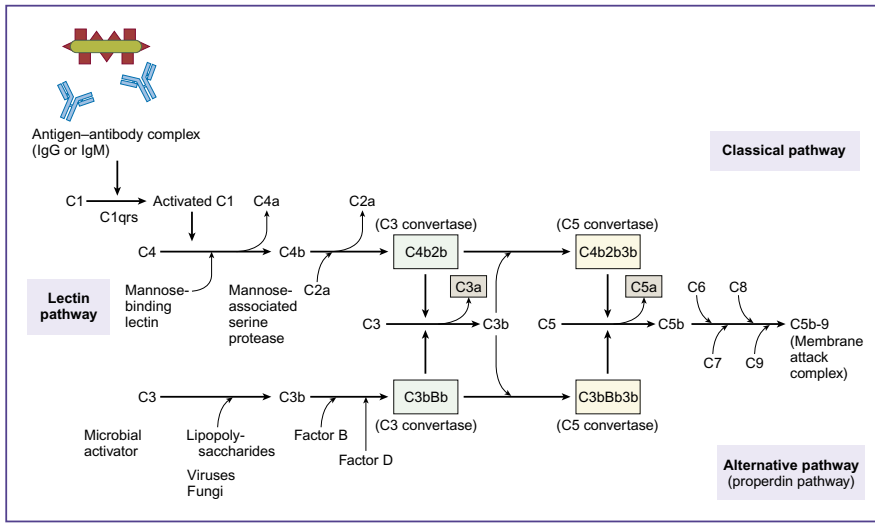


Figure 2.2 ***The complement cascade.*** *Activation of complement through the classical pathway (antigen–antibody complexes), the alternate pathway (recognition of foreign cell surfaces), or the lectin pathway (or mannose binding pathway) promotes activation of C3 and C5, leading to construction of the membrane attack complex.*

complement cascade (Figure 2.2). Complement is a term that refers to heat labile factors in the serum that causes immune cytolysis. As a group, complement comprises at least 30 distinct serum proteins that effect multiple biological functions. These components are normally inert in serum, however, a "cascade" of events occur when these molecules interact with signaling agents. Activation of complement enzymes results in subsequent cleavage of proteins to yield specific polypeptide fragments with short-lived enzymatic functions related to inflammation. In essence, proteolytic degradation of the complement components drives many aspects of immunity.

Different pathways exist to initiate complement activation. One pathway, referred to as the **classical pathway**, utilizes antibodies as the initiating signal. The sheer action of antibody bound to pathogenic determinants on organisms forms the basis of a physical structure. Complement components interact with the pathogen-bound antibodies. The "first" complement component (called C1) interacts with the constant portion of specific classes of antibody bound to the surface of the bacteria. This initiates a cascading series of reactions whereby a complex structure is built upon the bacterial cell surface. Synthesis of this structure culminates in a pore channel,

called a **membrane attack complex**, which causes osmotic lysis of the pathogen or infected cell.

Two other pathways of complement activation, the **alternate pathway** and the **lectin pathway**, function to allow direct lysis of microorganisms in the absence of antibodies. In these enzymatic cascades, complement components bind directly to pathogens via recognition of bacterial sugars and lipids. Similar to events described above, the deposition of complement on the invading pathogen leads to a cascade of enzymatic reactions culminating in pore channel assembly on the organism surface. Furthermore, breakdown products of the cascading pathway results in the production of smaller molecules, **opsonins**, which remain deposited on the pathogens. The opsonins act as molecular beacons, allowing interaction with receptors on macrophages, monocytes, and neutrophils to enhance phagocytosis and elevate mechanisms of targeted organism destruction. Overall, complement and its related components exert multiple biological functions that are critical components of inflammation, including activation and regulation of both innate and adaptive immune functions (Figure 2.3). Complement will be discussed again when examining defense mechanisms against infectious agents.

ACTIVATION AND DIRECTED MIGRATION OF LEUKOCYTES

One of the most prevalent by-products of the complement enzymatic cascade is the production of molecules with the ability to call in and activate leukocytes. This chemical attraction is termed **chemotaxis**. Proteolytic degradation of complement components releases leukocyte chemotactic factors referred to as **anaphylatoxins**. For example, breakdown products of complement component C3 are chemotactic for eosinophils. Proteolytic digestion of component C5 produces a much more potent chemokine, attracting neutrophils, monocytes and macrophages, and eosinophils. Another major property of the enzymatic cascade is the production of factors that activate those incoming cells. For example, interaction of breakdown components C3a, C4a, or C5a with mast cells and basophils leads to release of histamine, serotonin, and other vasoactive amines which further drive vascular permeability. One can readily see that by-products of the complement cascade can directly influence local inflammatory responses.

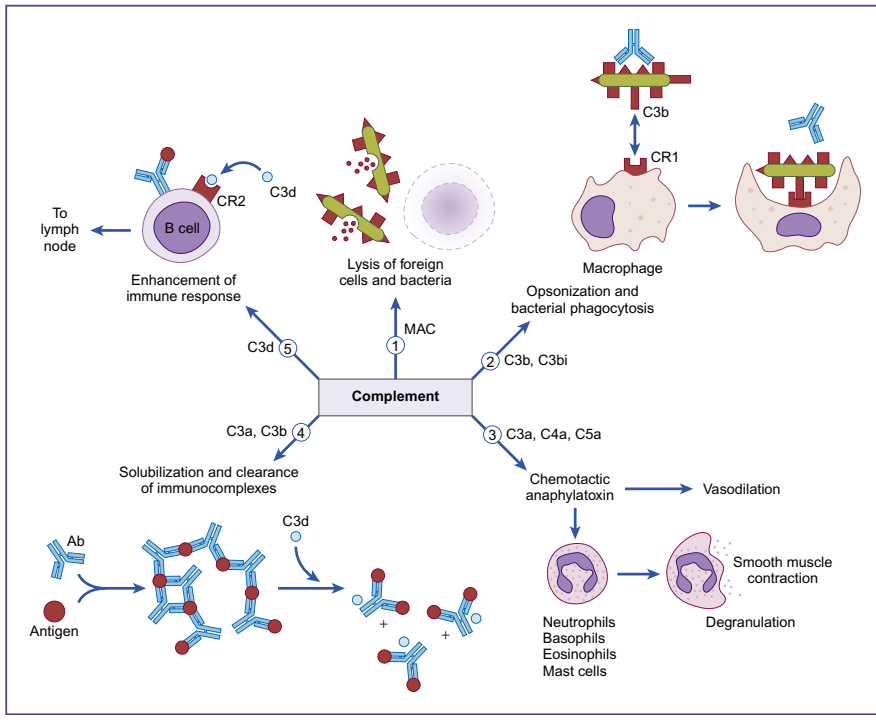


Figure 2.3 ***Biologic functions of complement.*** *Complement activation results in the formation of biologically active fragments that act as anaphylatoxins, opsonins, or chemotactic factors. Reactivity of complement with bacterial factors or with antibodies initiates the cascade. Breakdown products of the enzymatic cascade are recognized by receptors, leading to cellular activation and enhanced function. Multiple products also drive lymphocytic maturation (not shown).*

PATHOGEN RECOGNITION AND CYTOKINE SIGNALING

Phagocytes bear several unique receptors that recognize microbial components, bind bacterial carbohydrates, and induce phagocytosis. Recognition can occur directly through **mannose receptors**, **scavenger receptors**, or **Toll-like receptors** (**TLRs**), receptors which detect common pathogenic motifs (**pathogen-associated molecular patterns**; **PAMPs**). Recognition through any of these receptors represents a "danger" signal that initiates a proinflammatory response (Figure 2.4), accomplished through release of small chemical mediators called **cytokines**. Cytokines represent cell-derived secreted mediators that allow cells to communicate with each other. They regulate development and behavior of immune effector cells and facilitate cross-talk at low concentrations (10^{-10}–10^{-15} M). They are short-lived and bind to cell surface

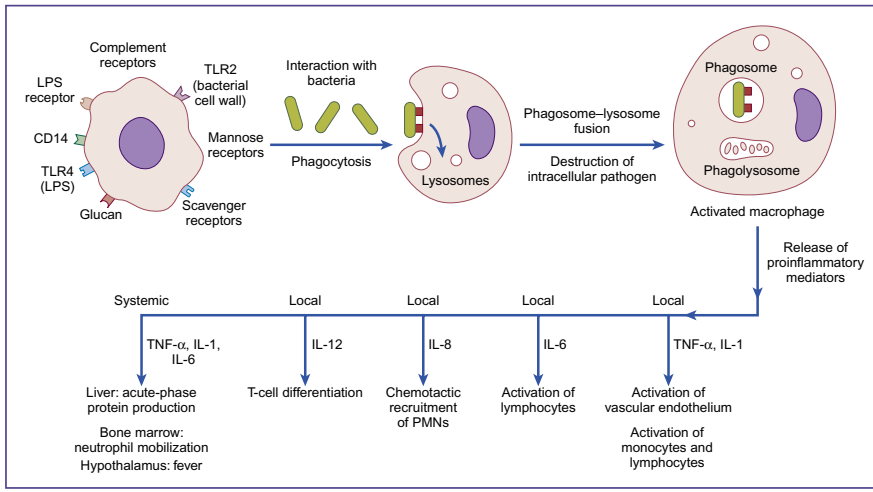


Figure 2.4 ***Important cytokines secreted by macrophages.*** *Recognition of bacteria and bacterial products induced cytokine that regulate both local and systemic events involved in the inflammatory response to contain infections. IL, interleukin; TNF-α, tumor necrosis factor-alpha; LPS, lipopolysaccharide; CD14, LPS and/or lipoteichoic acid receptor; PMN, polymorphonuclear cell; TLR, Toll-like receptor.*

receptors. They may act alone or in concert with one another synergistically. They are considered pleiotropic because they exert multiple actions on multiple different cell types, with overlapping and redundant functions. Another common name for these molecules is **lymphokines**, which are basically cytokines produced by lymphocytes. Finally, many cytokines also function as growth factors for specific cell subsets (Table 2.2).

FEEDBACK AND ADAPTATION FROM A DISTANCE

There is considerable activity between cells at the local site of the inflammatory response. Indeed, we will see in later chapters that direct interaction of cells that initiate the proinflammatory response with incoming lymphocytes trigger adaptive cell activity. Cell-to-cell contact gives the advantage of directly delivering cytokines and mediators, allowing functional lymphocyte development in the immediate area to target productive immune function. However, there is also a great need for systemic communication which can mediate responses across great distances, to direct cells and organs located distant to the site of tissue damage.

Table 2.2 Selected Cytokines and Functions

Cytokine	Cell Source	Cell Target	Primary Effects
IL-1α,β	Monocytes	T cells	Costimulatory molecule
	Macrophages	B cells	Activation (inflammation)
	Fibroblasts	Endothelial cells	Adhesion, fever
	Epithelial cells		Acute phase reactants
	Endothelial cells		
IL-2	T_H1 cells	T cells	Growth and activation
	NK cells	B cells	
		Monocytes	
IL-3	T cells	Bone marrow hematopoietic precursors	Growth and differentiation
	NK cells		
	Mast cells		
IL-4	T_H2 cells	Naive T cells	Differentiation to T_H2 cell
	Mast cells	T cells	Growth and activation
		B cells	Isotype switching to IgE
IL-5	T_H2 cells	B cells	Growth and activation
	Mast cells	Eosinophils	
IL-6	T cells	T cells	Costimulatory molecule
	Macrophages	B cells	Adhesion, fever
	Fibroblasts		Acute phase reactants
IL-8	Macrophages	Neutrophils	Chemotaxis and activation
	Epithelial cells		
	Platelets		
IL-10	T_H2 cells	Macrophages	Inhibits T_H1 development
		T cells	
IL-12	Macrophages; NK cells	Naive T cells	Differentiation into a T_H1 cell
IL-13	T cells	B cell	IgE switching
			B-cell growth
IL-17	T cells	Neutrophils, inflammatory cells	Inflammatory regulation
IFN-α,β	T cells	Monocytes	Activation
	NK cells	Endothelial cells	Increased class I and II MHC
		Macrophages	
IFN-γ	T_H1 cells	Monocytes	Activation
	NK cells	Endothelial cells	Increased class I and II MHC
		Macrophages	
TGF-β	T cells	T cells	Inhibits activation and growth
	Macrophages	Macrophages	

(*Continued*)

Table 2.2 (Continued)

Cytokine	Cell Source	Cell Target	Primary Effects
GM-CSF	T cells	Bone marrow progenitors	Growth and differentiation
	Macrophages		
	Endothelial cells		
	Fibroblasts		
TNF-α	Macrophages	T cells, B cells, endothelial cells	Costimulatory molecule
	T cells		Activation (inflammation)
			Adhesion, fever, acute phase reactants

IL, interleukin; IFN, interferon; TGF, transforming growth factor; GM-CSF, granulocyte-macrophage colony stimulating factor; TNF, tumor necrosis factor.

Table 2.3 Partial List of Chemokines Produced by Monocytes and Macrophages

Chemokine Class	Chemokine	Cells Affected
CC	MCP-1 (CCL2)	Monocytes, NK cells, T cells, dendritic cells
	MIP-1α (CCL3)	Monocytes, NK cells, T cells, dendritic cells
	MIP-1β (CCL4)	Monocytes, NK cells, T cells, dendritic cells
	Rantes (CCL5)	Eosinophils, basophils, monocytes, dendritic cells, T cells
	Eotaxin (CCL11)	T cells, eosinophils
CXC	IL-8 (CXCL8)	Neutrophils (naïve T cells)
	IP-10 (CXCL10)	Monocytes, NK cells, T cells

A special subclass of chemical mediators responsible for attracting cells to the area of inflammation is called **chemokines**. Chemokines assist in leukocyte migration into tissue (**diapedesis**). As a class, they are small polypeptides synthesized by a wide variety of cell types, all of which act through receptors that are members of the G-protein-coupled signal transducing family. All chemokines are related in amino acid sequence. Their receptors are integral membrane proteins characterized by a common physical shape containing seven membrane-spanning helices. Chemokines fall mainly into two distinct groups. The CC chemokines have two adjacent cysteine residues (hence the name "CC"). The CXC chemokines have an amino acid between two cysteine residues. Each chemokine reacts with one or more receptors and can affect multiple cell types (Table 2.3).

As the focal point of inflammation spreads, an amplification of cognate component signals occurs. Endothelial cells (cells that make up blood vessel walls) and incoming leukocytes generate and release

additional prostaglandins, leukotrienes, platelet activating factors, and enzymes. Factors draining back to the blood supply circulate to organs and initiate responses from tissues distant to the region of inflammation. For example, signals received by the hypothalamus can trigger increased body temperature (fever), while signals to the liver trigger synthesis of **acute phase proteins** including clotting factors and complement components. The liver also produces **c-reactive protein** (**CRP**), which enables increased activation of complement during times of infection.

The acute phase proteins, in combination with chemical mediators and cytokines released from cells at the site of infection, promote release of neutrophils from the bone marrow. Related chemokines released at the site of inflammation also attract myeloid-derived granulocytes which specifically function to combat infectious agents. This culminates in recruitment of neutrophils and **polymorphonuclear cells** (**PMNs**) to the site of tissue damage. Arriving neutrophils become engaged in the inflammatory process; neutrophils contain primary and secondary granules containing specific proteases to kill microorganisms. Activated neutrophils express high levels of antibody and complement receptors to allow increased phagocytosis of invading organisms. Activation of neutrophils leads to respiratory burst producing reactive oxygen and nitrogen intermediates, which directly kill invading pathogens. Neutrophilic myeloperoxidases also function to destroy pathogenic invaders.

The initiation of the proinflammatory responses is extremely potent to limit spread of microorganisms; however, full clearance of the infectious agent typically requires adaptive immune components. Overall, the productivity and success of the acute inflammatory response lies in the ability of the lymphatics to successfully drain fluids, cells, and debris to nearby lymph nodes, where phagocytic antigen presenting cells "show" digested pieces of the foreign protein to cells of the adaptive immune response. In essence, the next step is a "priming" of lymphocytes for specific activation. The activation of lymphocytes leads to release of antibodies, cytokines, and growth factors with specificity to target the causative agent of the inflammatory response. As a clinical manifestation, the triggering of adaptive components leads to lymph node swelling, a simple sign that the immune system is undergoing high activity. We will see in the next few chapters how activation of lymphocytes leads to directed amplification of the adaptive response,

which is required for continued protection against foreign agents. We will also see that when the inflammatory response remains persistent, the chronic inflammation can lead to permanent tissue damage.

SUMMARY

- A coordinated effort of cells and blood components is required to elicit the inflammatory response. Inflammation manifests as redness, swelling, heat, pain, and loss of function (rubor, tumor, calor, dolor, and functio laesa).
- The inflammatory process is initiated and controlled via chemical mediators, resulting in an influx and activation of inflammatory cells.
- Antibodies and complement components are critical for initiation of innate immune functions, working independently and in concert to eliminate microorganisms. By-products of the complement enzymatic cascade function to both heighten and direct later responses.
- Recognition of foreign agents leads to release of cytokines and chemokines to function locally to attract granulocytes and leukocytes to the site of inflammation. These factors also work systemically in release of acute phase proteins to further drive protective responses.
- The response culminates in draining of fluid, cells, and antigens to local lymph nodes where maturation and activation of adaptive lymphocytes occurs.

CHAPTER 3

The B Lymphocyte and the Humoral Response[1]

Chapter Focus: To discuss factors involved in generation and establishment of the humoral response. The structure of the immunoglobulin will be presented as a way to understand the biological functions balanced with the molecule's ability to recognize unique antigenic determinants. A discussion will examine each immunoglobulin isotype to detail how structural features confer biological properties, mediated in part through interactions with receptors on effector cell subsets. B lymphocyte development will be addressed as it relates to producing cells with capabilities to synthesize immunoglobulins, examining concepts of gene rearrangement to allow generation of unique antigen-binding structures.

B LYMPHOCYTES PRODUCE ANTIBODIES

In 1965, pathologist David Glick reported that removal of the bursa of Fabricius, a hematopoietic organ located near the cloaca of chickens, resulted in significant decrease in circulating antibodies. Orthologous lymphocytes in humans were determined to develop in the bone marrow. It is now understood that the B subset of lymphocytes is responsible for **humoral immunity**, defined by their expression of antibody molecules.

STRUCTURAL CHARACTERISTICS OF IMMUNOGLOBULINS

Immunogloblulins, interchangeably referred to as **antibodies**, share a common structure that allows them to bind to a nearly limitless number of specific antigens (including proteins, carbohydrates, glycoproteins, polysaccharides, nucleic acids, and lipids). Their structure confers multiple cellular processes, mediated by variable and constant region domains.

[1]Chapter contributed in collaboration with Keri C. Smith, PhD.

Introductory Immunology. DOI: http://dx.doi.org/10.1016/B978-0-12-420030-2.00003-2

The anatomy of the immunoglobulin in its monomeric form may be described as a "Y" shaped glycoprotein consisting of two identical heavy chains of 55 kDa paired with two identical light chains of 25 kDa (Figure 3.1). These chains are held together by one or more interchain disulfide bonds; enzymatic cleavage of the disulfide bonds results in two fragments—a homogenous portion that could be crystallized (fragment, crystalline; **Fc**) and another fragment that could bind antigen (**Fab_2**). These regions denote the effector antigen-binding functions of the antibody molecule. Within the heavy chain at the junction of the Fab_2 and Fc regions are short amino acid sequences that are rich in proline and cysteine. These are **hinge regions** that confer flexibility for optimized binding with antigen.

Each heavy chain pairs with one of two varieties of light chains, the κ or λ light chain, to comprise the Fab_2 portion of the antibody. Either chain variety, but not both, may be used in an individual antibody molecule—approximately 60% of human antibodies utilize the κ

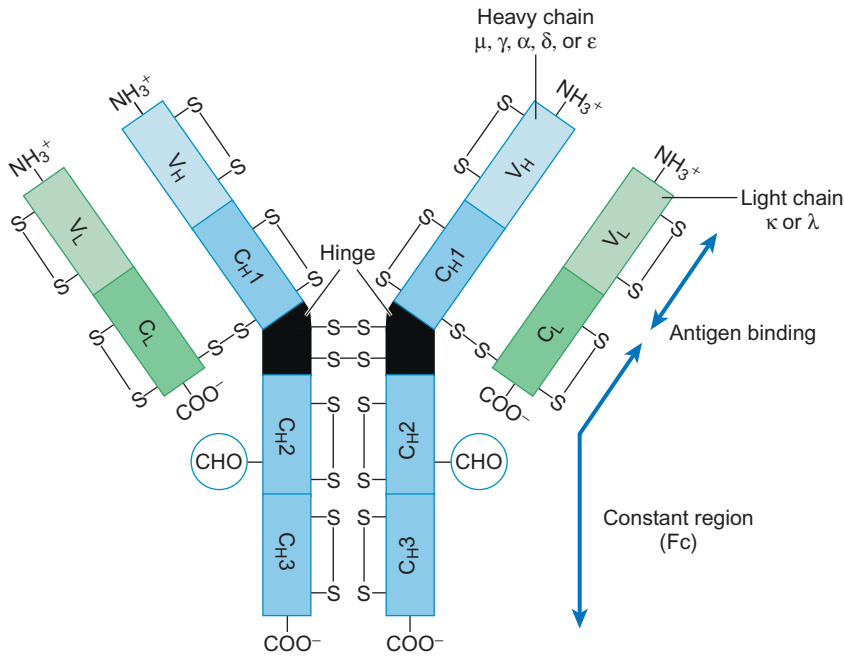


Figure 3.1 ***Anatomy of the immunoglobulin.*** *The basic structure of the antibody contains heavy chains and light chains, showing intra- and inter-disulfide bonds and the characteristic hinge region. The interactions between variable domains constitute the antigen-binding domain, while the constant regions confer specific biological properties of the molecule.*

chain, and the remainder contain the λ chain. In humans, five unique heavy chains are defined by differences in amino acid sequence. They are labeled according to Greek letter designations α, γ, μ, ε, and δ. The combination of one specific heavy chain with one light chain results in a specific antibody **isotype** now referred to as **IgA**, **IgG**, **IgM**, **IgE**, or **IgD**. The properties of these specific isotypes are summarized in Table 3.1. As with other members of the Ig superfamily (which include T cell receptors discussed in Chapter 4), each chain shares a similar tertiary structure, with characteristic "immunoglobulin folds" consisting of "β-barrel" antiparallel β-pleated sheets connected by loops of variable length stabilized by at least one disulfide bond. The overall structures of the specific subclasses of immunoglobulin folds confer biologic functions.

Table 3.1 Classes of Antibody Isotypes and Their Functional Properties

Isotype	Immunoglobulin Class				
	IgM	**IgD**	**IgG**	**IgE**	**IgA**
Structure	Pentamer	Monomer	Monomer	Monomer	Monomer, dimer
Heavy chain designation	μ	δ	γ	ε	α
Molecular weight (kDa)	970	184	146–165	188	160 × 2
Serum concentration (mg/mL)	1.5	0.03	0.5–10.0	<0.0001	0.5–3.0
Serum half-life (days)	5–10	3	7–23	2.5	6
J chain	Yes	No	No	No	Yes
Complement activation	Strong	No	Yes, except IgG4	No	No
Bacterial toxin neutralization	Yes	No	Yes	No	Yes
Antiviral activity	No	No	Yes	No	Yes
Binding to mast cells and basophils	No	No	No	Yes	No
Additional properties	Effective agglutinator of particulate antigens, bacterial opsonization	Found on surface of mature B cells, signaling via cytoplasmic tail	Antibody-dependent cell cytotoxicity	Mediation of allergic response, effective against parasitic worms	Monomer in secretory fluid, active as dimer on epithelial surfaces

The **variable domain**, comprised of the light chain and heavy chain heterodimers, is responsible for antigen binding. Each individual antibody clone expresses a unique variable domain. Within the 100–110 amino acids that make up this domain are regions of extreme sequence variability, called **hypervariable regions**. Each light chain and each heavy chain expresses three of these unique regions, which make up the **complementarity-determining region (CDR)**, so called because its protein structure complements antigen binding. The Fab portion contains two identical variable domains; therefore, each complete molecule is capable of simultaneously binding two identical antigens. The regions between them are less variable framework regions which form the structural support to allow antigen contact. Importantly, antigen binding occurs through noncovalent means (e.g., van der Waals forces, hydrogen bonding, hydrophobic bonding, and electrostatic interactions). Hence, the three-dimensional structure formed by the combination of the protein chains is essential for proper antigen binding.

The **constant domains** provide structural stability. Each light chain contains one constant domain, while heavy chains have three to four. These domains associate to form the Fc region of the antibody which, depending on isotype, mediates various downstream effects, including complement binding, opsonization, and phagocyte activation. Properties specific for the Fc region of each isotype are described in detail below.

IMMUNOGLOBULIN NOMENCLATURE

Though all immunoglobulins are remarkably similar in structure, changes in amino acid sequences can led to obvious differences in functions, structure, and antigen specificity (Figure 3.2). These differences are denoted by several different terms: The most structurally diverse are the **antibody isotypes** which are defined by their expression of different heavy chains (α, γ, μ, δ, or ε). The IgG and IgA isotypes are subdivided further into specific **subclasses**. These subclasses share 90% amino acid homology, but the differences in protein folding and disulfide bonding considerably affect their biologic properties. Within the same isotype and subclass, individuals can express unique allelic variants, called **allotypes**, of light and heavy chains. Allotypes generally consist of one amino acid substitution at a specific site. They are inherited in a codominant autosomal Mendelian pattern and have been used for forensic purposes. Large-scale studies associate certain

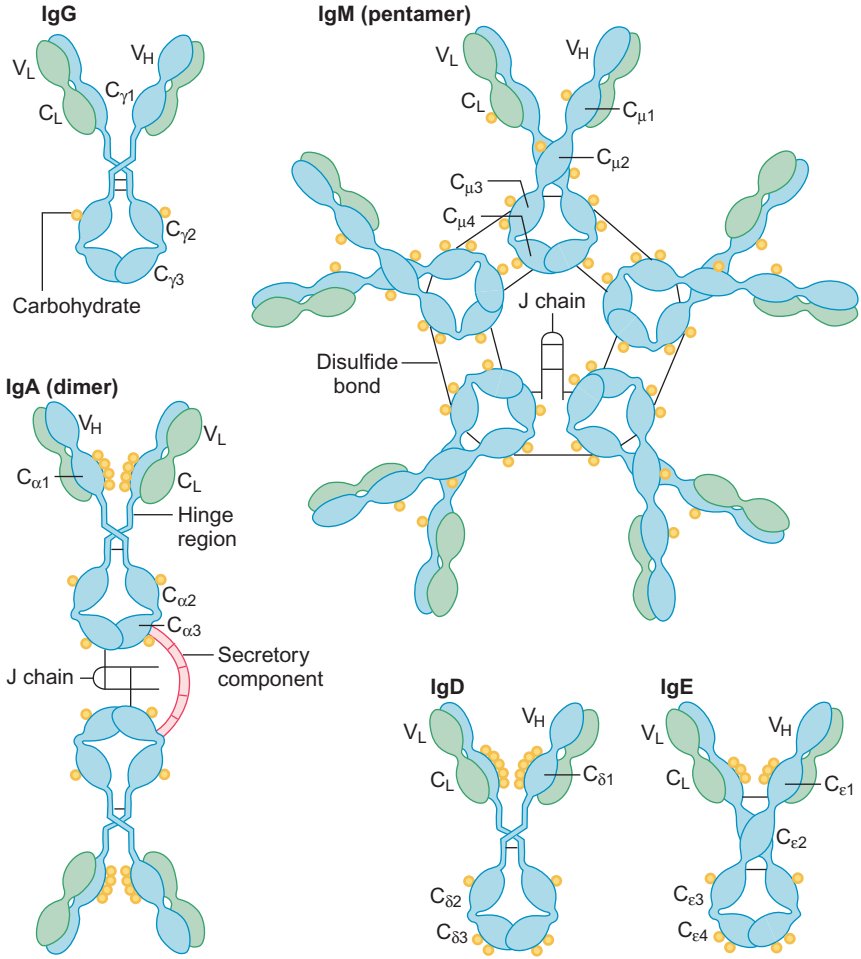


Figure 3.2 ***Classes of antibody isotypes.*** *Pictured are the five classes of antibody isotypes representing monomeric IgG, IgD, and IgE, pentameric IgM, and dimeric IgA with secretory component.*

allotypes with disease progression or with development of autoimmunity. Finally, the hypervariable regions of the light and heavy chains represent a signature unique to a single antibody, also known as an **idiotype**.

BIOLOGIC PROPERTIES OF ANTIBODY ISOTYPES

IgD

The IgD immunoglobulin is expressed primarily as a membrane-bound monomeric form on naïve B cells. Only small amounts of soluble IgD

can be detected in serum. It has a molecular weight of 180 kDa and contains a relatively flexible hinge region. The function of this antibody as a surface receptor is not entirely understood, though some evidence suggests that it may facilitate binding of antigen and play a role in affinity maturation. Expression of IgD is lost after response to antigen because of class-switch recombination events.

IgM

IgM can also be expressed in a membrane-bound form on mature, naïve B cells. Its expression is lost following class-switch recombination. However, due to alternative RNA processing mechanisms, IgM can also be produced in a secreted form by **plasma cells**, the activated form of B lymphocytes. IgM plays a very important role in primary antibacterial immune response and is the first antibody isotype secreted during an immune response. Elevated levels in adults indicate recent antigenic exposure.

The secreted, pentameric form of IgM is very large—900 kDa in size. Each monomer interacts with a small joining polypeptide, called a "J chain," to form a pentameric molecule consisting of five monomers. Because each original monomer has two antigen-binding sites, each pentameric complex is able to bind to 10 identical antigens simultaneously. Because of this, IgM has a greater **functional avidity** than monomeric IgG. However, due to lack of flexibility in its hinge regions and steric hindrance that occurs on larger antigens, its effective valency is lowered. IgM is secreted before affinity maturation takes place and generally has a lower affinity for antigen than IgG or IgA.

This high functional avidity due to the cross-linking of multiple antigens can be best demonstrated by the ability of IgM to aggregate or agglutinate particulate antigens such as bacteria or red blood cells. As such, IgM is very effective at neutralizing bacterial antigens early in the immune response. A clinical manifestation of IgM binding can be observed in assays used to determine blood type. Naturally occurring IgM antibodies reactive with red blood cell antigens of the ABO series are referred to as **isohemagglutinins**. Precipitation reactions occur when a specific isohemagluttinin is present in serum that comes in contact with the appropriate blood group antigens. The pentameric structure of IgM is particularly effective at mediating complement activity. Indeed, IgM is the most efficient isotype on a mole:mole basis for

complement activation of the classical pathway. The majority of IgM is located in intravascular spaces; its large size precludes traversing the maternal/placental barrier.

IgG

Monomeric secreted IgG is the smallest of the isotypes, with a heavy chain consisting of three constant domains. IgG is primarily secreted in the serum, where it can account for up to 15% of total protein in the human body. Due to its small size, IgG is capable of crossing endothelial cell barriers and is approximately equally distributed between extravascular and intravascular spaces. The small size of IgG also allows it to cross the placenta; maternal-derived IgG provides passive immunity to the neonate for up to 4 months after birth.

IgG is the primary immunoglobulin present in blood and extravascular spaces. It can effectively bind to bacterial toxins, viral receptors, or bacterial adhesions to act in a **neutralizing** capacity to prevent pathogen binding to host cell surfaces. Binding of IgG to antigen can also result in downstream activation of antigen presenting cells via coordinated interaction with specific surface receptors (FcγR). This process is known as **opsinization**, derived from the Greek phrase *opsonin* that means "to prepare for eating," as it allows for targeted uptake of antigen in a phagosome. The Fc portion of IgG plays an important role in **antibody-dependent cell-mediated cytotoxicity** (**ADCC**). In this instance, binding of antigen−IgG complex to FcγR expressed by natural killer cells helps to guide destructive granules to a target cell. IgG also plays an important role in the **activation of the complement cascade**, specifically, binding of multiple IgGs to antigen leads to the initiation of classical complement activity.

Differences in the amino acid sequences as well as in the number and location of disulfide bonds in the γ chain result in four different IgG subclasses. These subclasses, numbered according to prevalence in human serum, differ (Table 3.2). The IgG response to specific antigen is T cell dependent, meaning that it requires T helper cell-derived cytokines and physical interactions for subsequent class switching. IgG appears in serum within 2 weeks of primary antigen exposure, and it is very rapidly produced at even higher levels following a second antigen "boost." This anamnestic response is the basis of vaccination strategies that result in long-term immune protection against pathogens (see Chapter 9).

Table 3.2 Unique Biologic Properties of the Human IgG Subclasses				
	IgG1	**IgG2**	**IgG3**	**IgG4**
Occurrence (% of total IgG)	70	20	7	3
Half-life (days)	23	23	7	23
Complement binding	+	+	Strong	No
Placental passage	++	±	++	++
Receptor binding to monocytes	Strong	+	Strong	±

IgA

IgA is best known as the hallmark immunoglobulin of the mucosal immune response, and the dimeric, secretory form is found in sero-mucous secretions including saliva, tears, nasal fluids, sweat, colostrum, and secretions of the genitourinary and gastrointestinal tracts. The α chain consists of three constant regions, and like the μ chain, it also has a cysteine containing tailpiece that allows for the association of the polypeptide J chain and the formation of the dimeric structure. Of note, IgA is secreted on the basal side of mucosal surfaces by plasma cells in lamina propria. The assembled dimer binds via a polymeric Ig receptor on the basal surface of the mucosal epithelial cell, followed by receptor-mediated endocytosis and transport of IgA to its apical surface. The polymeric Ig receptor is partially cleaved from the IgA dimer, and the remaining attached portion is referred to as **secretory component**. Secretory component functions to bind the IgA to the mucous surface and protect the antibody against cleavage from digestive enzymes.

IgA is produced in very large quantities (as high as 3 g/day in the intestine) and can neutralize bacteria. It also has a high viricidal activity; its dimeric form can efficiently agglutinate viruses. IgA is not capable of activating the complement pathway, but it has been demonstrated to have bactericidal activity for gram negative organisms, though this requires the presence of endogenous lysozyme. IgA can be found in serum, where it exists as a 160 kDa monomer that neutralizes antigen in the blood and extravascular space. Finally, the transfer of IgA from mothers' milk to the intestinal tract of the infant provides passive immunization against pathogens in the neonate. IgA can be secreted as one of two subclasses, which differ in length of hinge region and in the number of disulfide bonds. In mucosal secretions, the balance between the IgA1 and IgA2 subclasses is approximately 1:1; however, the serum form of IgA is almost exclusively monomeric IgA1.

IgE

IgE molecules are normally only present in trace amounts in serum and have a very short half-life. IgE is secreted in a monomeric form and is a highly potent activator of mast cells—very little IgE is required to induce a very obvious downstream response! By itself, IgE does not activate complement or neutralize bacteria or viruses. Instead, it is secreted by plasma cells in response to antigen and is then bound via its Fc portion to FcεR expressed on mast cells. In this form, it can be retained on the cell surface in extravascular space for weeks to months. Mast cells may be considered a cellular form of an "armed bomb"; they contain granules loaded with histamine, heparin, and leukotrienes, and can rapidly synthesize and secrete prostaglandins and cytokines. Upon secondary encounter with antigen, cross-linking of two or more of the "preloaded" IgE–FcεR complexes triggers mast cell degranulation.

IgE is necessary for human health in the face of infection with specific parasites, notably helminths. Antigen stimulates IgE bound to mast cells; the degranulation of released mediators increases vascular permeability and local inflammation. This results in the recruitment of eosinophils from the blood to the site of the parasitic infection. Eosinophils can also bind to IgE attached to the surface of the parasite, then release the contents of their granules to destroy the worm via an ADCC-type mechanism. IgE is an important part of the "first line of defense" against pathogens that enter the body across epithelial barriers. A deleterious effect of IgE can occur when it binds to normally innocuous antigens, such as pollen, triggering mast cell degranulation associated with allergic responses.

KINETICS OF ANTIBODY RESPONSE

Antibody isotypes are found in higher concentrations during an active immune response. Typical immune responses in humans, as defined by detection of antibodies in serum specific for injected antigen, have four phases, the length of which depend upon whether the response is primary or secondary (memory, anamnestic). In the primary response, B lymphocytes need time to receive help from T cells. This period can last up to 2 weeks. IgM production occurs first (sometimes followed by IgG class switching). Next follows the exponential phase in which the concentration of antibody in serum increases exponentially, which

corresponds to the development of plasma cells secreting class-switched antibody. A steady state of antibody secretion and degradation is then maintained, and when the antigenic threat has been dealt with, the response enters the declining phase. Plasma cells die, but memory B cells remain. Upon restimulation, these memory cells generate the anamnestic immune response, characterized by a very short lag phase, a greater production of antibody in the exponential phase, and a longer period of steady-state antibody maintenance. Class-switched antibodies, including IgG, IgA, and IgE, are more likely to appear in this secondary response, and affinity maturation often occurs in the rapidly dividing plasma cell population. The capacity to generate a secondary response may persist for many years if not decades.

MEMBRANE-BOUND IMMUNOGLOBULIN

Heavy chains may contain a transmembrane domain that allows for the Ig to be expressed on the surface of B cells. They allow for antigen-specific binding and subsequent activation of B lymphocytes. Importantly, antigen binding by the antibody is not sufficient to induce a cellular activation signal. Further association of the Ig heavy chains with two transmembrane invariant protein chains, Igα and Igβ, is required to generate a cellular signal. Igα and Igβ contain cytoplasmic immune-receptor tyrosine-based activation motifs (ITAMs) that are phosphorylated following binding of the membrane-bound Ig to multivalent antigen.

DEVELOPMENT OF B CELLS

Hematopoietic stem cells in the bone marrow give rise to multipotent cells resulting in the development of a common lymphoid progenitor. This cell can produce the earliest committed B cell—the pro-B cell. The developmental stages of the B cells correspond to gene rearrangement events, ultimately culminating in the formation of cells expressing a vast number of unique receptors for antigen.

GENE RECOMBINATION

The antigen-binding capacity of the B cell receptor (BCR) is generated before the cell is exposed to antigen. Rearrangement of multiple genes is necessary to produce the high diversity needed for a broad immune response (Figure 3.3). Recombination of both heavy and light chain

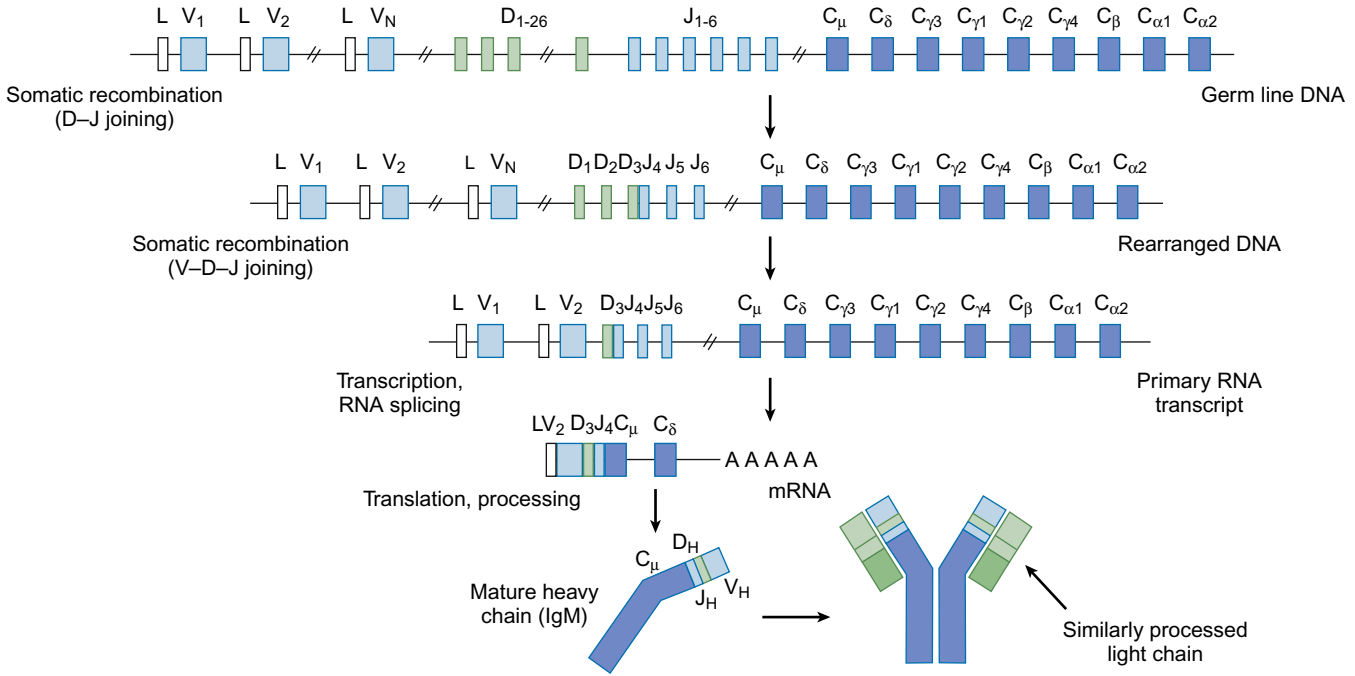


Figure 3.3 ***Genetic organization and recombination events.*** *Antibody diversity is generated by DNA recombination events that randomly fuse variable, diversity, and joining regions. The recombination is accomplished in a defined order by recombinase enzymes RAG-1 and RAG-2. The first events culminate in transcribing mRNA coding for IgM and IgD; differential translation determines whether mature polypeptide will be IgM or IgD. During plasma cell differentiation, the isotype may be changed where the same variable region is recombined with a different constant region sequence (not shown). L, leader sequence; V, variable; D, diversity; J, joining; C, constant region.*

genes is accomplished via the actions of the **V(D)J recombinase** enzyme complex. Germline DNA which encodes the heavy chain genes contains three different regions, referred to as V_H (variable), D_H (diversity), and J_H (joining), that contribute to production of the **variable domain**. A V(D)J recombinase mediates joining of gene segments by splicing unused segments out of the genome. The rearranged gene segments are then brought together and juxtaposed with the segments coding for the constant region of the heavy chain. The combining of genes is a "sloppy" process. Extra nucleotides are inserted by the enzyme deoxynucleotidyl transferase to fill spaces. This process, called "N region diversification" expands the potential pool of BCRs generated by effectively creating new codons between junctions. Rearrangment of the light chain variable domain occurs in the same fashion, though the germline DNA lacks D gene segments. The process of V(D)J rearrangement culminates in the generation of three CDRs in each variable region. Interestingly, two of the three CDRs are "hardwired" into the V gene segment—they are dependent upon the V segment selected during rearrangement. The third CDR consists of the junction of the V, J, and (in heavy chain) D segments and thus has higher variability. The combinatorial diversity that results from the rearrangement of the heavy chain (50 V_H genes X 26 D_H genes X 6 J_H genes) yields more than 7500 possible VDJ loci combinations. The light chain germline DNA sequences, which lack D regions, result in approximately 200 and 160 different VJ combinations, respectively.

DEVELOPMENT AND SELECTION OF MATURE B CELLS

The pro-B cell begins to mature with V(D)J recombination of the heavy chain genes. If this rearrangement is accomplished successfully, the complete Ig heavy chain is expressed by the cell as a "Pre-B receptor." Production of a functional heavy chain inhibits further rearrangement of the heavy chain genes (enforcing allelic exclusion). These large pre-B cells then proceed with recombination of their light chain genes, after which the cell expresses a complete IgM molecule on the cell surface and is identified as an immature B cell.

The immature B cell is then tested for autoreactivity. If the IgM expressed on the cell surface reacts with multivalent self-molecules (such as MHC expressed on stromal cells), the cell is considered

"defective" and must be removed from the cell repertoire. This is accomplished by triggering apoptosis in the self-reactive B cells (clonal deletion). Reactivity with self can also trigger receptor editing where the VDJ recombinase remains active and continues to rearrange light chain DNA until a nonautoreactive BCR is produced. If an immature B cell shows no reactivity with self-antigen, it migrates from the bone marrow. The mature B cell takes up residence in peripheral lymph tissues.

Some B cells weakly reactive to self-molecules may be allowed to exit the bone marrow. Under normal circumstances, these cells remain "ignorant" of their self-antigen, but under certain conditions (e.g., inflammation) may become active. Such "sleeper" B cells are thought to play a role in some autoimmune diseases. Together, the clonal deletion, receptor editing, and anergy induced within bone marrow form the mechanisms of central tolerance.

ACTIVATION AND DIFFERENTIATION OF B CELLS

Mature B cells in the periphery that have not yet responded to antigen are considered naïve. Response to antigen depends on the type of epitope recognized by the variable regions, as well as signaling through nearby membrane co-receptors (CD19 and CD21). BCRs specific for protein antigens require "help" from CD4 + T cells that recognize related antigenic determinants (**discussed in Chapter 4**). Secretion of cytokines by T cells then drives B cell maturation and proliferation. This process results in the generation of plasma cells—fully mature, "class-switched" B cells that secrete antibody and no longer express membrane-bound immunoglobulins.

Importantly, interaction of B cells with T cells drives the process of isotype, or "class" switching. **Isotype switching** is the process by which the antigen specificity of the antibody (located within the variable domains) remains the same, but the μ or δ heavy chain gene locus is excised and replaced with another heavy chain gene constant domain. Specific cytokine signals determine which heavy chain is substituted, and once the intervening DNA between recombination sites is deleted, the switch is irreversible. Interactions of B and T cells with antigen can also result in multiple rounds of mutation and selection for higher-affinity BCR in the germinal centers of peripheral lymph tissues.

Somatic hypermutation can result in amino acid replacements in the V regions of H and L chains, and the selection of cells with more tightly binding BCR to the antigen occurs, commonly known as **affinity maturation**.

While most of the B cells that proliferate in the germinal centers differentiate into plasma cells, a distinct population may follow a different path to become memory B cells. Upon reencounter with antigen, they can rapidly divide and produce high-affinity secreted antibody. Repeated doses of antigen, such as those that occur during immunization "boosters," result in the production of increased numbers of memory cells.

SUMMARY

- B lymphocytes produce immunoglobulins (antibodies) with specific biological functions that confer humoral immunity.
- During B cell development, rearrangement of the germline DNA generates antigen-binding diversity. Recombination events occur prior to detection of antigen.
- There are five sources of antibody diversity: (1) presence of multiple V gene segments; (2) combinatorial diversity of random recombination of V, D, and J segments; (3) junctional and insertional diversity altering V−D and D−J junctions; (4) co-expression of different H and L chain pairs; and (5) somatic hypermutation.
- Each immunoglobulin isotype confers unique biological properties, mediated through interactions with antigens as well as through receptors on effector cells. Isotype switching occurs after antigenic stimulation and requires T cell produced cytokines.
- Antibodies themselves are neutral; they can be either protective or destructive depending on multiple immune parameters.

CHAPTER 4

T Lymphocytes: Ringleaders of Adaptive Immune Function

Chapter Focus: To examine T lymphocytes as regulators of adaptive immune function. As such, they function as the primary effectors for cell-mediated immunity. A illustrative discussion will detail cellular development, moving from bone marrow precursors undergoing antigen receptor gene rearrangement through thymic selection and subsequent maturation events which permit antigen recognition. The process of antigen presentation by major histocompatability molecules will be discussed, followed by analysis of effector functions that allow T cells to regulate immunosurveillance toward infectious assault.

T LYMPHOCYTES: SPECIFIC AND LONG-LASTING IMMUNITY

The immune system must be able to recognize a large pool of antigens while maintaining the ability to distinguish between foreign and self. The **T lymphocytes** (**T cells**) confer response specificity using surface antigen receptors to facilitate recognition of foreign material. These adaptive lymphocytes can respond to a wide array of antigens; however, the speed of response is slower than innate functions due to intermediate steps required from the time of infection to the mounting of a protective response. In essence, the T cell acts as a master ringleader of cell-mediated immunity, a task accomplished by direct cellular contact, or via secreted cytokine factors.

THE T CELL RECEPTOR

The **T cell receptor** (**TCR**) is a transmembrane heterodimer composed of two disulfide-linked polypeptide chains (Figure 4.1). Each lymphocyte carries a TCR of only a single specificity. T lymphocytes can be stimulated by antigen to give rise to progeny with identical antigenic specificity. The vast majority of T lymphocytes express alpha (α) and beta (β) chains on their surface. Cells that express gamma (γ) and delta (δ) chains comprise only 5% of the normal circulating T cell population

Introductory Immunology. DOI: http://dx.doi.org/10.1016/B978-0-12-420030-2.00004-4

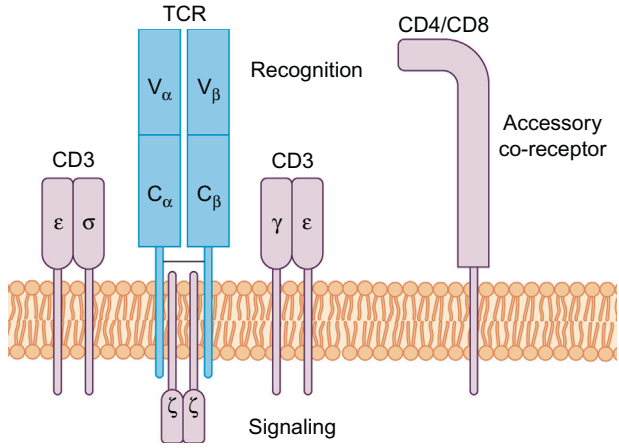


Figure 4.1 ***Structure of the TCR complex.*** *The TCR is made up of two covalently linked polypeptide chains. The predominant antigen-binding chains, α and β, are shown. Both transmembrane peptides exhibit a variable (antigen recognition) and a constant external domain connected via a disulfide bond. The TCR is always expressed with the associated CD3 complex, required for signal transduction. T-helper cells (T_H) express CD4, required for interaction with APCs, whereas CTLs express the CD8 coreceptor molecule.*

in healthy adults. Each chain (α, β, γ, or δ) represents a distinct protein with approximate molecular weight of 45 kDa. An individual T cell can express either an αβ or a γδ heterodimer as its receptor, but never both. The TCR is always expressed with the associated **CD3 complex**, comprised of multiple independently expressed units, required for signal transduction once presented antigen is encountered.

T CELL DEVELOPMENT

All cells of the lymphoid lineage are derived from the common lymphoid progenitor cell, which differentiates from bone marrow hematopoietic stem cells. T cell precursors migrate to the thymus where they develop and undergo thymic selection to eliminate auto-reactive cells (Figure 4.2). As with B cell development, the developmental stages of T cells correspond to gene rearrangement events, culminating in specificity for the TCRs. The TCR gene rearrangement is referred to as **V(D)J recombination**. As T cells develop, germ line sequences undergo recombination events of specific genes in the V (variable), D (diversity), and/or J (joining) regions (Figure 4.3). Similar to B cells, N region diversification can occur whereby extra nucleotides are inserted by the enzyme deoxynucleotidyl transferase; this expands the potential

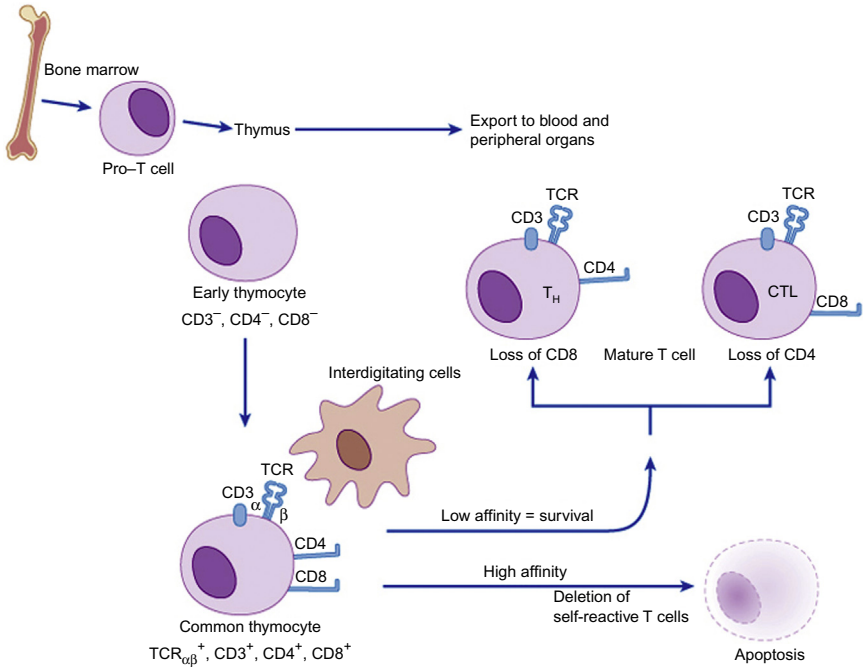


Figure 4.2 ***Main stages in thymic selection.*** *Pro-T cells migrating from the bone marrow enter the thymus, where they express rearranged TCR, CD3, CD4, and CD8 proteins. Positive selection occurs to eliminate self-reactive T cells. Maturing thymocytes lose either CD4 or CD8 surface molecules and are exported to peripheral tissue as CD4+ T_H cells or as CD8+ CTLs.*

pool of TCRs generated. The rate of T cell production in the thymus is highest in young individuals. The thymus shrinks during adulthood, suggesting that the complete T cell repertoire is primarily established prior to puberty.

The thymus plays an integral role in both education and regulation of T cell lineage, with development starting in the thymic cortex and ending in the medulla. Progenitor cells migrating into the thymus do not express the dominant **CD4** and **CD8** markers. Thus, these progenitor cells are termed "double-negative" thymocytes. The development step is rearrangement of the TCR, with commitment to the αβ T cell or γδ T cell phenotype. As previously described for B lymphocytes, T lymphocytes are also created with a vast array of antigenic specificities prior to contact with antigen. During TCR gene rearrangement, genes undergo recombination. The developing cells must express a functional TCR to survive. At this stage, the CD3 molecule is also

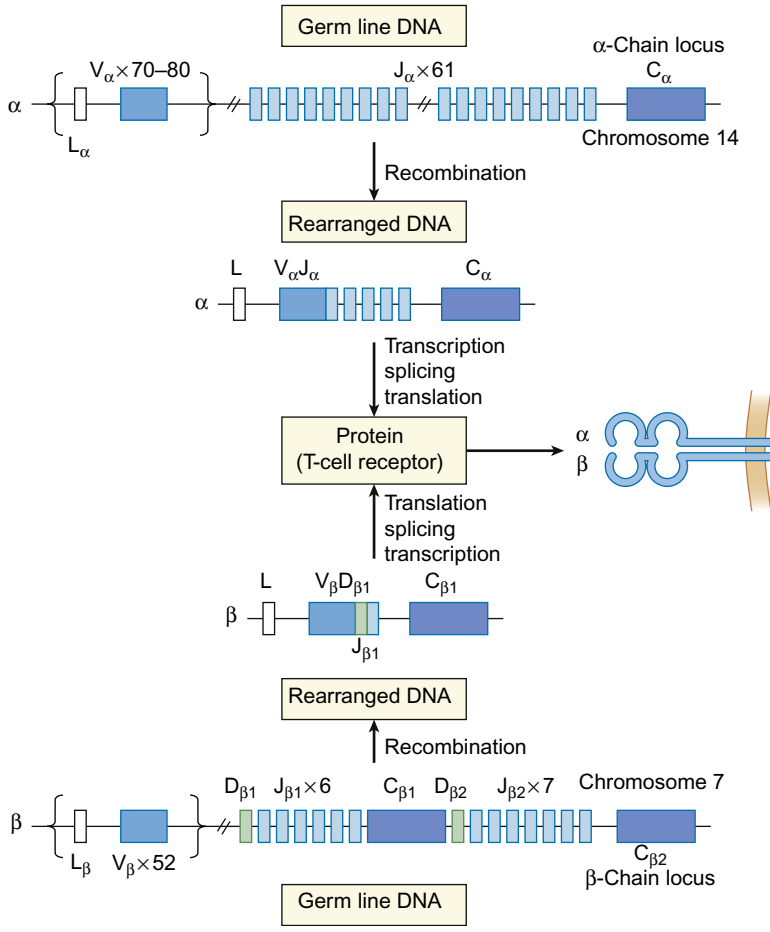


Figure 4.3 ***Gene rearrangement of TCR loci.*** *TCR diversity is generated by combinatorial joining of variable (V), joining (J), and diversity (D) genes and by N region diversification (nucleotides inserted by the enzyme deoxynucleotidyl transferase). The top and bottom rows show germ line arrangement of the V, D, J, and constant (C) gene segments at the TCR α and β loci. During T-cell development, variable sequences for each chain are assembled by DNA recombination. For the α chain (top), a Vα gene segment rearranges to a Jα gene segment to create a functional gene encoding the V domain. For the β chain (bottom), rearrangement of a Dβ, a Jβ, and a Vβ gene segment creates the functional V domain exon.*

expressed, allowing intracellular signal transduction. This signaling causes proliferation and expression of CD4 and CD8. Cells are now referred to as "double-positive" thymocytes.

Once the full αβ TCR is expressed on the thymocyte cell surface, the selection process that will shape the T cell repertoire begins. T cell recognition of antigen requires recognition of not only the

peptide epitope presented but also the antigen presentation molecule itself, the **major histocompatability complex** (**MHC**). The first selection process is positive selection, and the new αβ TCR that recognizes MHC and the peptide epitope is allowed to mature. The positive recognition of MHC class I or class II coordinates with development of CD4 and CD8 T cells, respectively. This means that a thymocyte that recognizes MHC class I will cease expression of CD4, leading to development of a CD8 T cell, and vice versa. Thus, at the end of positive selection, the αβ TCR expression increases, and the cell becomes a single positive thymocyte. The next selection process, negative selection, is where the cells are tested for reactivity against self-antigens. Those that survive finish the maturation process and are released into circulation.

ANTIGEN RECOGNITION BY T CELLS: REQUIREMENT OF MAJOR HISTOCOMPATABILITY MOLECULES

T cells only recognize antigens that are processed into short peptides and presented on the surface in an antigen presentation molecule, the major histocompatibility complex (MHC), which is referred to as **human leukocyte antigen** (**HLA**) in humans. **MHC class I** molecules are found on all nucleated cells, whereas **MHC class II** are only present on professional **antigen-presenting cells** (**APCs**; B cells, dendritic cells (DCs), and macrophages). The method of peptide processing determines to which MHC it will be loaded.

The HLA Locus

The HLA locus in humans is found on the short arm of chromosome 6 (Figure 4.4). The class I region consists of HLA-A, HLA-B, and HLA-C loci. The class II region consists of the D region, subdivided into HLA-DP, HLA-DQ, and HLA-DR subregions. A region between the class I and class II loci encodes for class III proteins with no structural similarity to either class I or class II molecules. The class III molecules include complement proteins, tumor necrosis factor, and lymphotoxin.

The highly polymorphic class I and class II MHC products are central to the ability of T cells to recognize foreign antigen and the ability to discriminate "self" from "non-self." MHC class I and class II molecules that are not possessed by an individual are seen as foreign antigens upon transplantation and are dealt with accordingly by the

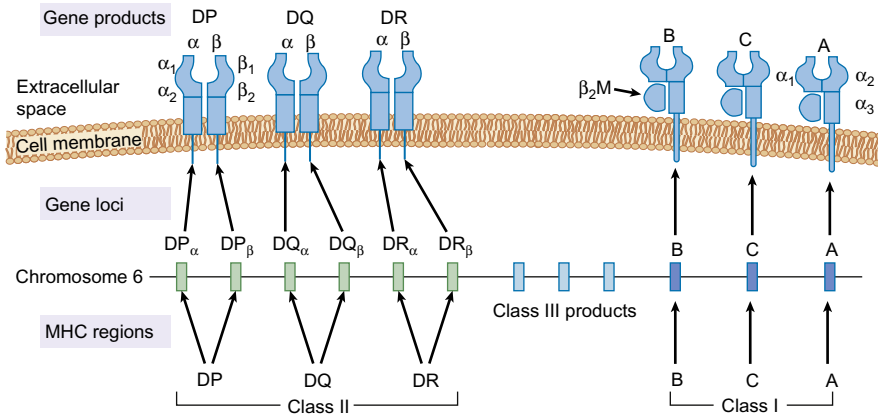


Figure 4.4 ***Genetic organization of the HLA locus and associated gene products.*** *The polymorphic human MHC genes of the HLA locus coding for class I and class II molecules are located on chromosome 6. Class I genes are designated as A, B, and C, each coding for a three-domain polypeptide (α1, α2, α3) that associates with invariant β_2-microglobulin (β_2M). Class II genes are DP, DQ, and DR, each coding for individual α and β chains that interact to provide binding sites for antigen presentation.*

recipient's immune system (discussed in Chapter 11). All MHC molecules show a high level of **allotypic polymorphism**, i.e., certain regions of the molecules differ from one person to another. The chance of two unrelated people having the same allotypes at all genes that encode MHC molecules is very small.

The MHC class I molecules are each somewhat different from one another with respect to amino acid sequence, and all three (HLA-A, HLA-B, and HLA-C) are codominantly expressed on all nucleated cells. "**Codominantly expressed**" means that each gene encoding these proteins from both parental chromosomes is expressed. The MHC class II molecules are a bit different in that expression includes homologous and heterologous αβ dimer mixtures, representing proteins from both parents. Both α and β subunit genes exhibit species-specific polymorphism. Homologous dimers match class II molecules expressed on either parental cell type, whereas heterologous dimers are unique to the F1 genotype and are functionally nonequivalent to parental class II molecules.

MHC Class I

MHC class I molecules bind **endogenous**-derived peptides from antigens processed in the cytosol (Figure 4.5), such as viral proteins

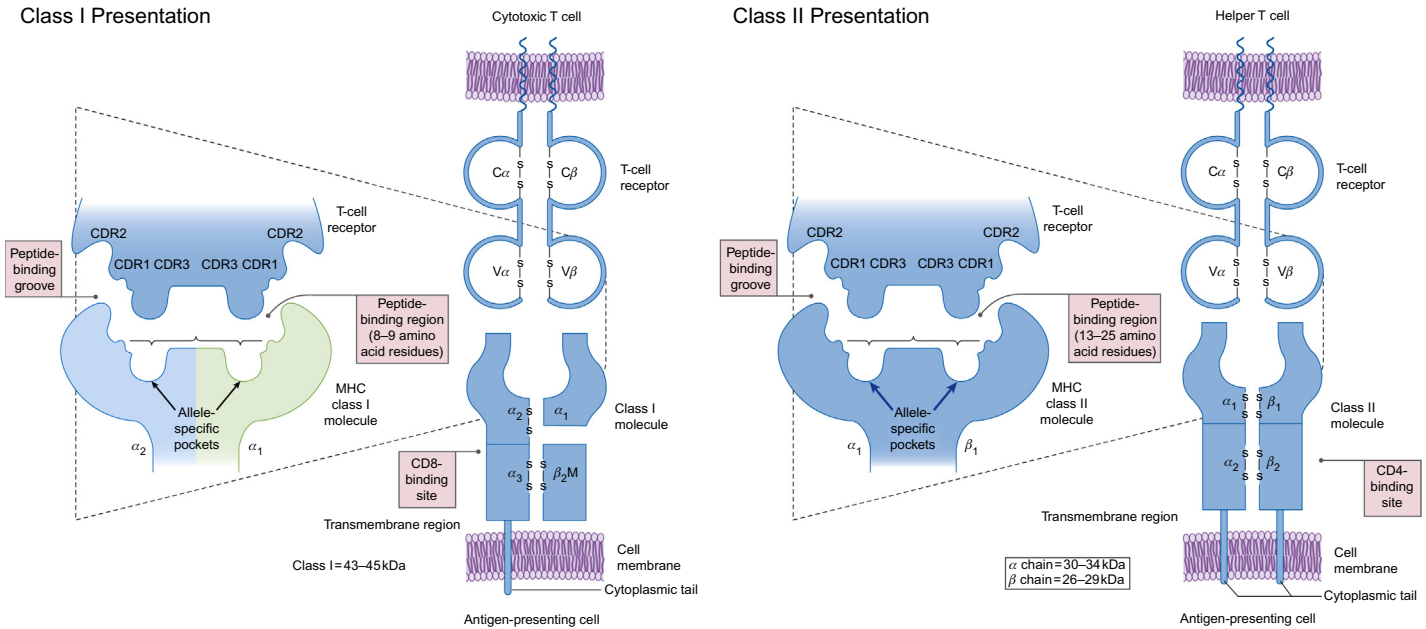


Figure 4.5 ***Endogenous and exogenous MHC presentation to T cells.*** *(Left) Endogenous pathway for antigen processing allows MHC class I molecules to interact with intracellular produced peptides degraded by proteasomes. The MHC class I transmembrane molecule is associated with the invariant β_2-microglobulin β_2M, giving structure to the extracellular domain for presentation of processed antigen to CTLs. Short peptide fragments (8–10 amino acids in length) noncovalently interact with the domains on the class I molecule and complementarity determining regions (CDRs) on the TCR, stabilized by the CD8 molecule. (Right) The exogenous pathway prepares processed antigens for presentation to CD4+ T cells via MHC class II-regulated mechanisms. Processed antigenic fragments (13–25 amino acids in length) interact with domains on the class II molecule within the peptide-binding groove, allowing presentation to CD4+ T-helper cells. Interactions with the TCR are stabilized by CD4 recognition of conserved regions on the class II molecule.*

produced within the host cell. Protein degradation occurs naturally by a complex called the proteasome. Antigens are degraded and transported to the endoplasmic reticulum (ER) by the **TAP** (**transporters associated with antigen processing**) protein, and the peptides are further processed and loaded onto the MHC class I. Once the peptide is bound to MHC class I, the complex is stabilized by a **β_2-macroglobulin** molecule and then exported to the cell membrane surface. The entire surface molecule can now be recognized by CD8 T cells (cytotoxic T cells) specific for the bound peptide.

MHC Class II

MHC class II molecules bind **exogenous**-derived peptides from antigens processed in organelles (Figure 4.5). Any extracellular antigen, such as whole bacteria, engulfed by APCs via phagocytosis or endocytosis is enclosed in an intracellular vesicle. Activation of the APCs leads to acidification and activation of proteases to degrade the antigen into peptide fragments. The vesicle containing peptides is fused with another vesicle containing MHC class II proteins. Upon fusion, the peptide is loaded on to MHC class II molecules, and the entire complex migrates to the cell membrane surface, where peptide specific CD4 T cells (helper T cells) can recognize it.

T LYMPHOCYTE FUNCTIONS

To generate an active immune response, a small number of B and T cell clones that bind antigen with high affinity undergo activation, proliferation, and differentiation. Some of these cells become **effector T cells** that express activities that help to eliminate the pathogen. Others become **memory cells** that can give rise to secondary responses as described below.

Complete cellular activation requires a series of interactions (Figure 4.6). Naïve T cells that migrate out of the thymus circulate through the peripheral lymphoid organs, searching for cognate antigen recognized via specific surface molecules. These naïve T cells carry surface receptors to allow targeted migration to lymph nodes, where they make contact with resident DCs. The DCs represent a population of cells that specifically promote recognition of small linear peptide epitopes, functioning as APCs to promote further development of naïve T cells. Activation requires three signals. The first is binding of the TCR

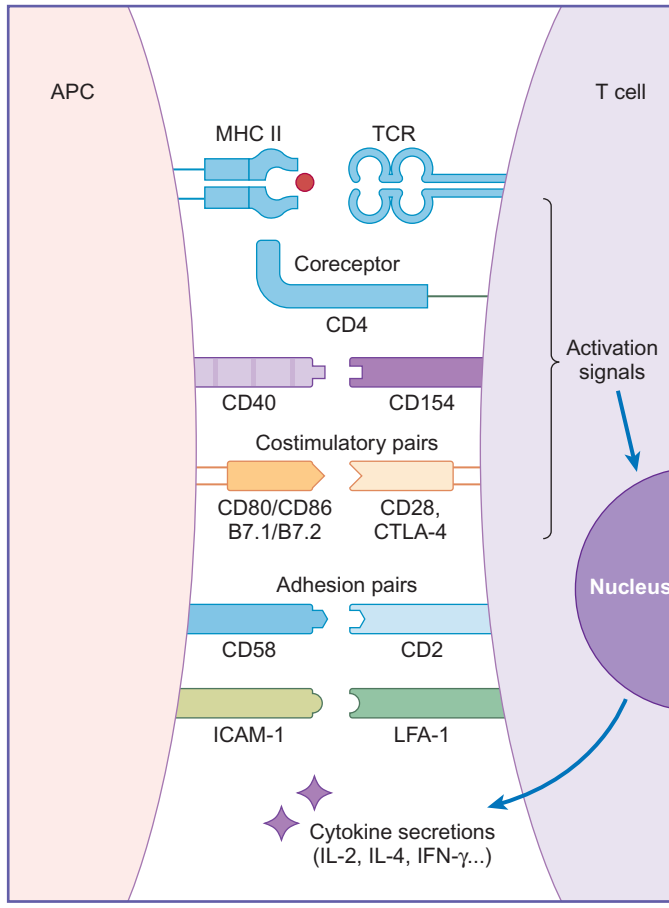


Figure 4.6 ***Interactions between the T cell and APC.*** *Activation of lymphocytes requires multiple signals which include binding of the antigen-specific receptor to the antigen, interaction with stabilizing surface molecules, and exposure to cytokines or secondary costimulatory signals. For T-helper cells shown, recognition of the presented antigen is enhanced by CD4, which attaches to nonpolymorphic regions on the MHC class II molecule. Further stabilization is accomplished by integrin interactions between molecules on the T cell with ligands on the surface of the APC. Costimulatory molecules present on both the T cell and the APC are critical for T-cell function and activation.*

to the peptide/MHC. The second is binding of costimulatory molecules (CD28 on T cells to CD86 or CD80 on DCs). The third signal includes cytokines produced by the DCs during encounter with the naïve T cell. It is this third signal that is responsible for T cell differentiation. Once activated, the effector T cells proliferate, develop specific subtype features, and express receptors to allow them to migrate to sites where activity is needed.

CD4+ T-Helper Cells

CD4 T cells recognize antigenic epitopes presented by MHC class II molecules. Upon activation by APCs, the CD4 T cells proliferate and differentiate into several subtypes, each with specific functional capabilities (Figure 4.7).

T-helper type 1 (T_H1) cells are effector CD4 T cells that differentiate when IL-12 is given as the third signal. T_H1 cells produce primarily IFN-γ cytokine, which is a primary cytokine used to activate macrophages to promote intracellular killing of pathogens. The **T-helper type 2 (T_H2)** cells are effector CD4 T cells that differentiated in the presence of IL-4 and produce vast quantities of IL-4, IL-5, and IL-13. T_H2 cells provide help to promote antibody production, specifically IgE, to target helminthes and parasites. Indeed, the original name for IL-4 was B cell growth factor. It should be noted that other major subsets of T cells exist; formation of these subsets is also under cytokine regulation. For example, **T-helper 17 (T_H17)** cells are differentiated under TGF-β1 and IL-6; they produce high levels of IL-17 and enhance neutrophilic response.

One major function of CD4 T cells is to help activate B cells and promote antibody production. It is now hypothesized that the main effector CD4 cell responsible for this process in the lymphoid follicles

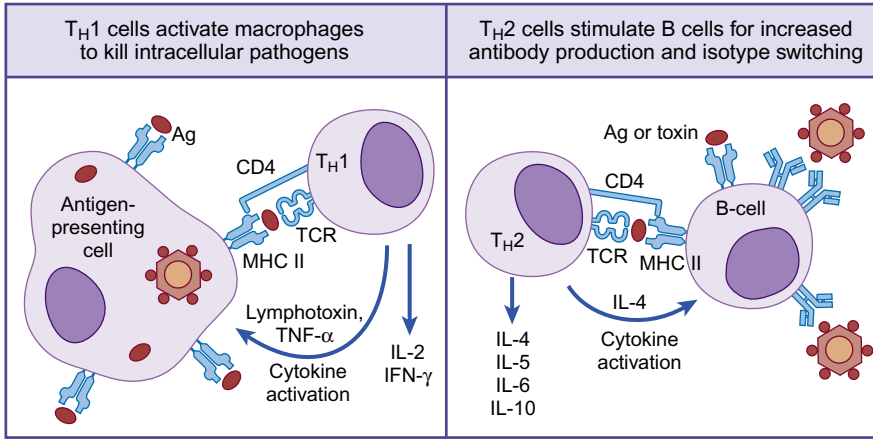


Figure 4.7 ***Effector functions of T-helper cells.*** *T_H1 cells activate macrophages through production of cytokines, leading to assisted destruction of intracellular microorganisms. T_H2 cells drive B-cell differentiation to stimulate B cells to proliferate, to secrete immunoglobulins, and to undergo isotype switching events. IFN, interferon; IL, interleukin; TNF-α, tumor necrosis factor alpha.*

is the **T follicular helper cell** ($\mathbf{T_{FH}}$). The T_{FH} cell differentiates under presence of IL-6 and can secrete a wide variety of cytokines, thus enabling stimulation of production of antibodies associated with both T_H1 (IgG2a) and T_H2 (IgE, IgG1) immune responses.

Regulatory CD4 T cells (**Tregs**) are a unique subset of lymphocytes. T cells produce FoxP3, a transcription factor, and phenotypically express CD25 molecules on their cell surface. These regulatory T cells affect effector functions by several methods that include direct cell-to-cell contact, increased expression of surface molecules, increased responsiveness to growth factors, and altered production of regulatory cytokines (such as TGF-β1 and IL-10). Finally, other phenotypic populations of T-helper cells exist ($\mathbf{T_H9}$, $\mathbf{T_H22}$) which also control unique facets of immune response.

EVENTS INVOLVED IN T LYMPHOCYTE ACTIVATION

Immune response must be regulated to allow sufficient activity to protect the host without excessive or inappropriate responses (hypersensitivities; discussed in Chapter 8) that may lead to disease states. Receptor recognition of antigen mediates transcription of cytokine genes by a complex sequence of molecular events. Absence of a secondary signal can lead to cellular inactivation.

The professional presenting cells that provide costimulation are DCs, macrophages, and B cells. Of these cell types, DCs deliver the best costimulatory signals for activation of naïve T cells. Presenting cells use the membrane molecules of the B7 family to deliver costimulatory signals through interaction with their ligand on T cell membranes, CD28; two isoforms of the B7 family (B7.1 and B7.2; also called CD80 and CD86) act to regulate cytokine responses. For example, CD80 binding upregulates T_H1 cytokines (IL-2, IFN-γ), while CD86 binding upregulates T_H2 response (IL-4, IL-5, IL-6, and IL-10). Interactions with CD28 are critical, in that binding to CD28 on the lymphocyte leads to IL-2 production, a major T cell growth factor, while inhibition of binding leads to development of tolerance. Reactivity leads to differential intracellular regulation of transcription factors. Interaction of the B7 molecules with other T cell surface antigens, such as CD152 (CTLA-4), leads to suppressive signals and tolerance/anergy, and the induction of memory cell formation (Figure 4.8).

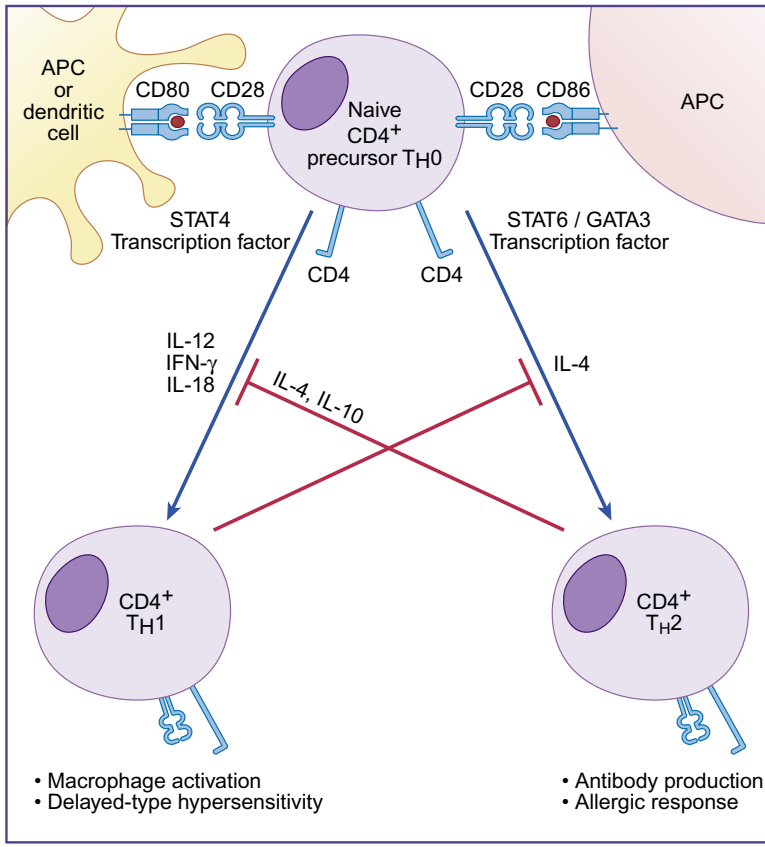


Figure 4.8 ***Development of T-helper cell phenotypes.*** *T_H1 and T_H2 cells derive from precursor cells under the influence of local cytokines. Each secretes a phenotypic subset of cytokines that drive effector responses. Secreted cytokines modulate response to the other subset and inhibit alternate functional development. Not shown are the T_H17 and T regulatory subsets, which mature under the influence of cytokine IL-17 and TGF-β.*

ROLE OF T CELLS IN B CELL ACTIVATION

B cells are capable of using their surface immunoglobulin to engulf antigen, after which they can process and present antigenic fragments to T cells. This promotes direct elicitation of cytokines from T cells for stimulation, resulting in activation and plasma cell development (Figure 4.9). Clonal expansion of B cells under influence of T cell cytokines leads to plasma cell development, isotype class switching, and production of memory responses. Specifically, IL-4 secreted from T_H2 cells acts as a B cell growth factor, and IL-6 assists in delivering signals for maturation of the antibody response. Under certain circumstances, B cells can also respond and proliferate through **T-independent**

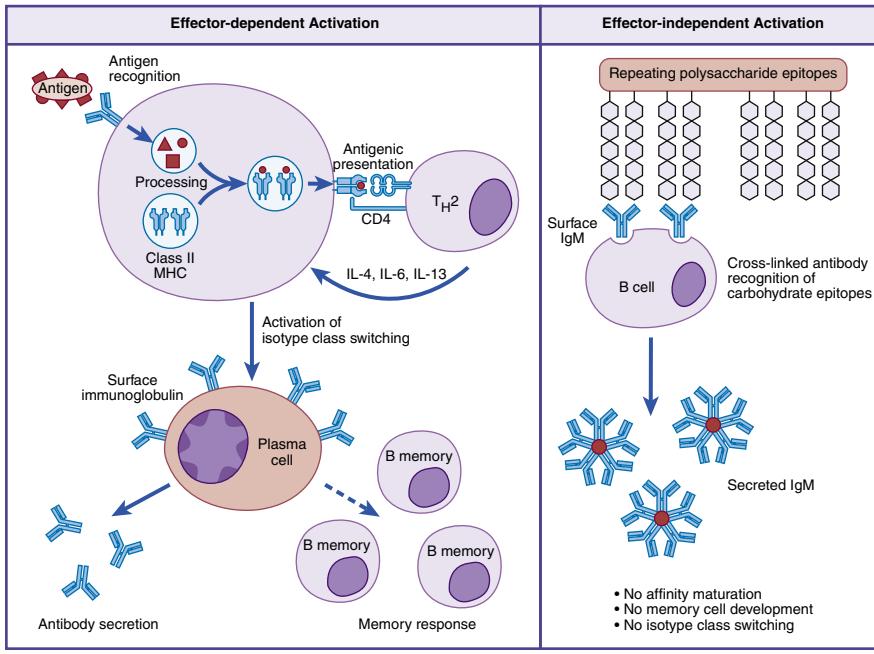


Figure 4.9 ***B cell response to T-dependent and T-independent antigens.*** *(Left) T_H2 cells specifically recognize class II-presented antigenic determinants and drive B-cell activation, leading to isotype class switching and antibody secretion. (Right) Alternatively, T-independent responses to repeating carbohydrate epitopes stimulate antibody production but do not lead to maturation of the antibody response.*

mechanisms, usually involving antigens with long repeating epitopes that allow cross-linking of immunoglobulin receptors on the surface of the B cell. A common example occurs with bacterial capsid antigens containing repeating carbohydrate (polysaccharide) epitopes. In the case of T-independent activation, there is no accompanying maturation of response; antibody production is primarily limited to IgM isotypes.

CYTOTOXIC T CELL EFFECTORS

$CD8^+$ T Cells

CD8 T cells recognize antigen presented on MHC class I molecules and become **cytotoxic CD8 T cells (CTLs)** when activated. While CD8 T cells also produce various cytokines, their main activity is to eliminate infected host cells. Activation of CD8 T cells requires additional costimulatory signals. This can occur with or without CD4 T cells help. In some instances with infected DCs, the infection creates enough inflammation for their production of cytokines and costimulatory signals that are sufficient to

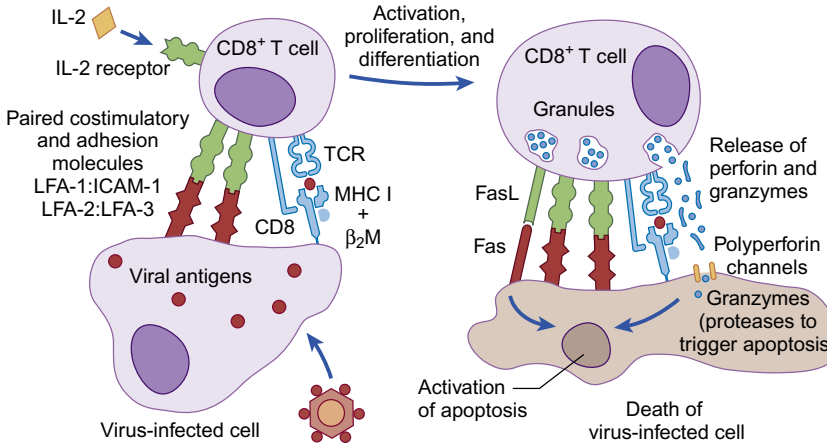


Figure 4.10 ***Effector function of CTLs.*** *CTLs recognize virally infected target cells which express foreign antigens complexed with MHC class I molecules. Responses are mediated through IL-2 and the IL-2 receptor (CD25), and strengthened through interactions between the CTL and the infected target cell. Cytotoxic effector molecules produced (perforins, granzymes) initiate destruction of the target cell and deliver apoptotic signals through Fas and FasL on cellular surfaces. LFA, lymphocyte function-associated antigen; ICAM, intracellular adhesion molecule.*

activate CD8 T cells in the absence of CD4 T cells. However, most CD8 T cell activation requires CD4 T cells to sufficiently help activate and upregulate costimulatory signals required for optimized target cell destruction.

Activated CD8 T cell kills target cells by apoptosis (Figure 4.10). One major method is through release of cytotoxic granules. Binding of the TCR to MHC class I triggers synthesis of **perforin** and **granzymes**, which are stored within the cytosol. The granules are released at the point of contact, allowing specific targeting and limited bystander death. Perforin assembles on target membranes, allowing delivery of granzymes into the target cell. Granzymes are a group of serine proteases that activates caspases, leading to cell death. Direct cell-to-cell contact is also critical for functionality. CD8 cells can induce apoptosis by ligation of **Fas** and **Fas ligand**, which are expressed on lymphocytes and on infected target cells. Activated CD8 T cells also produce several cytokines that contribute to host defense, including IFN-γ, TNF-α, and lymphotoxin-α. IFN-γ inhibits viral replication while increasing expression of MHC class I, improving the chance that an infected cell will be recognized.

INNATE LYMPHOCYTES AND SUPERANTIGENS

Up to now, the lymphocytes described are all involved in the adaptive immune response. This means that upon introduction of a new

antigen, such as during the initial infection or vaccination, the lymphocytic response requires approximately 2 weeks to fully activate the appropriate naïve cell, undergo clonal expansion, and migration cells to the site of infection to enact activity. However, there are lymphocytes that operate in the innate capacity and appear at the site of infection or inflammation within 1–2 days of infection. These innate lymphocytes make up a minor population of lymphocytes that recognize only a small number of unique antigens.

γδ T Cells

The antigen receptors of some T cells are comprised of alternative γ and δ polypeptides rather than the common α and β chain TCRs described above. These γδ T cells constitute 10–15% of the human blood T cell population and are abundant in the gut epithelia, the lungs, and the skin. They appear to be important in immune responses to epithelial pathogens. Unlike conventional αβ T cells, the γδ T cells do not go through positive or negative selection, and are released into circulation upon successful rearrangement of their TCR. Generally, γδ cells lack CD4, although some express CD8. Unlike αβ T cells, γδ T cells generally do not recognize antigen presented on MHC molecules but recognize antigenic targets directly, similar to an antibody. Their function is not well known but is hypothesized to recognize changes in heat shock proteins, MHC class Ib molecules, and phospholipids in an infected cell. In this way they may suffice to rapidly mount responses against a subset of phylogenetically conserved ligands, thus representing an efficient early defense against pathogenic organisms.

Natural Killer T Cells

Natural killer T (NKT) cells are a heterogeneous group of T cells that share properties of both T cells and natural killer (NK) cells. The majority of these cells recognize an antigen-presenting molecule (CD1) that binds self- and foreign lipids and glycolipids (Figure 4.11). CD1 molecules are non-MHC restricted and nonpolymorphic. However, although distinct from MHC class I and II, they show a similar structure to MHC class I, having three extracellular domains and expressed in association with β2-microglobulin. They constitute only 0.2% of all peripheral blood T cells. It is now generally accepted that the term "NKT cells" refers primarily to CD1 restricted T cells coexpressing a heavily biased, semiinvariant TCRα chain that is paired with one of three TCRβ chains. Upon activation,

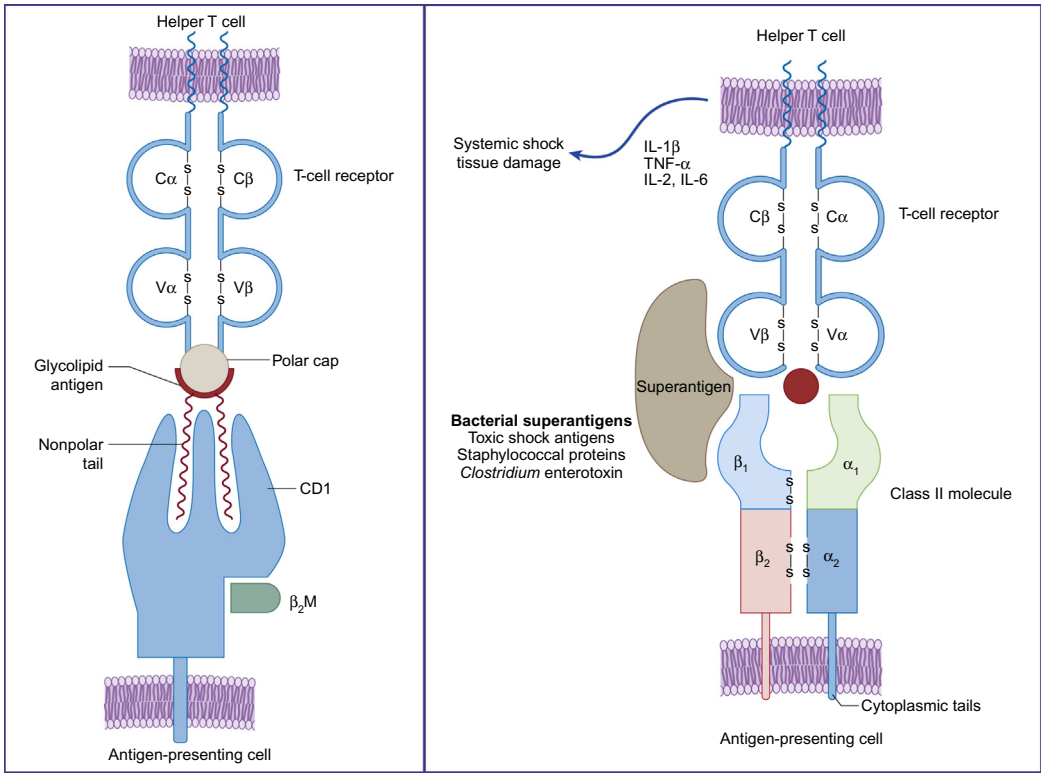


Figure 4.11 ***(Left) Nonclassical lipid antigen presentation by CD1 molecules.*** *The peptide groove of the CD1 surface molecule is lined with nonpolar, hydrophobic side chains which allow binding of antigen in a deep, narrow hydrophobic pocket to enable presentation to the TCR. (****Right****)* ***Superantigens.*** *T cells of various antigenic specificities are activated when bacterial superantigens cross-link MHC class II molecules with common TCR Vβ regions. This causes activation in the absence of specific peptide filling the MHC molecule.*

these cells secrete several cytokines, including IFN-γ, IL-4, and IL-10, and perform direct killing of target cells. NKT cells should not be confused with NK cells.

Superantigens

A superantigen is a molecule that is able to elicit T lymphocyte responses by circumventing normal antigen processing and presentation functions. Superantigens are defined by their ability to stimulate a large fraction of T cells via interaction with the TCR Vβ domain (Figure 4.11). Superantigens are predominantly bacterial in origin, such as staphylococcal enterotoxin and toxin-1 responsible for toxic shock syndrome. The superantigen directly bridges the TCR with the MHC class II molecule, causing T cells to divide and differentiate into effector cells to release cytokines (IL-2, TNF-α, and IL-1β). Because the number of T cells that share Vβ domains is high (up to 10% of all T cells), large numbers of cells may be activated, regardless of antigen specificity. This leads to massive systemic disruption and clinical features similar to septic shock.

SUMMARY

- The T lymphocytes are regulators of adaptive function, serving as primary effectors for cell-mediated immunity. Antigenic specificity is dictated by means of the TCR heterodimer receptor, derived from recombination of gene segments.
- Recognition of presented antigen in the context of major histocompatability molecules and costimulatory molecules allows differentiation of effector processes.
- CD4+ T-helper lymphocyte cells recognize exogenous antigen presented in the context of MHC class II molecules. Different subclasses of T-helper cells secrete unique subsets of cytokines that assist in functional activity.
- CD8+ T lymphocytes, also called cytotoxic T cells (CTLs), recognize endogenous antigen presented in the context of MHC class I molecules. CTLs kill target cells directly by inducing apoptosis via released preformed proteins.
- Other mechanisms for T cell activation have evolved, most likely in response to pathogens that circumvent typical pathways required for adaptive immune protection.

CHAPTER 5

How We Defend Against Infectious Agents

Chapter Focus: To examine the process of defense against different types of pathogens, with an element focused on organism classes commonly encountered. Opportunistic infections are discussed, related to the diminished response induced when individuals are immunocompromised. General mechanisms organisms use to evade immune function will also be addressed.

IMMUNE HOMEOSTASIS AND PATHOGENIC ORGANISMS

The human host represents an immense microbiome with hundreds of trillions of symbiotic microorganisms living on or in our bodies. Yet our bodies are exquisitely adapted to handle constant assault by different classes of pathogens and opportunistic agents. Indeed, we are able to balance normal commensal flora with the ability to specifically distinguish agents that are harmful to daily living, a facet which antibiotics do not share. Indeed, the immune system is able to balance responses to its normal microbiome with the need to control and eliminate infectious agents that cause disease. This is done at the innate level of response, with activity dependent on physical barriers and cellular recognition of pathogenic motifs. It is also accomplished at the adaptive level, with reactivity directed toward specific foreign antigens present on pathogenic invaders.

MAJOR IMMUNE DEFENSE MECHANISMS AGAINST PATHOGENS

The course of response against typical acute infections can be subdivided into distinct stages. Initially, the level of infectious agent is low, beginning with breach of a mechanical barrier (e.g., skin, mucosal surface). Once inside the host, the pathogen encounters a microenvironment for suitable replication. The agent replicates, releasing antigens that trigger innate immune function, generally characterized as nonspecific. Preformed effector molecules recognize microorganisms within the

Introductory Immunology. DOI: http://dx.doi.org/10.1016/B978-0-12-420030-2.00005-6

first 4 h of infection and assist in limiting expansion of the organism. Complement components and released chemokines attract professional phagocytes (macrophages and polymorphonuclear cells (PMNs)) and natural killer (NK) cells to the site of infection, to assist in activation of these cells. After 4 or 5 days, antigen-specific lymphocytes (B and T cells) undergo clonal expansion, enabling directed control and eventual clearance of the infectious agent. The host is left with residual effector cells and antibodies, as well as immunological memory to provide lasting protection against reinfection.

A wide variety of pathogenic microorganisms exist. They may be globally classified into groups: bacterial, mycobacterial, viral, protozoal, parasitic worms, and fungal. The host defense is based upon availability of resources to combat a localized pathogen (Figure 5.1).

PHYSICAL BARRIERS TO INFECTION

Four major categories of physical barriers exist to limit entry and control expansion of foreign pathogens. Defensive roles may be **anatomic** (skin, mucous membranes), **physiologic** (temperature, low pH, chemical mediators), **phagocytic** (digestion of microorganisms), or **inflammatory** (vascular fluid leakage).

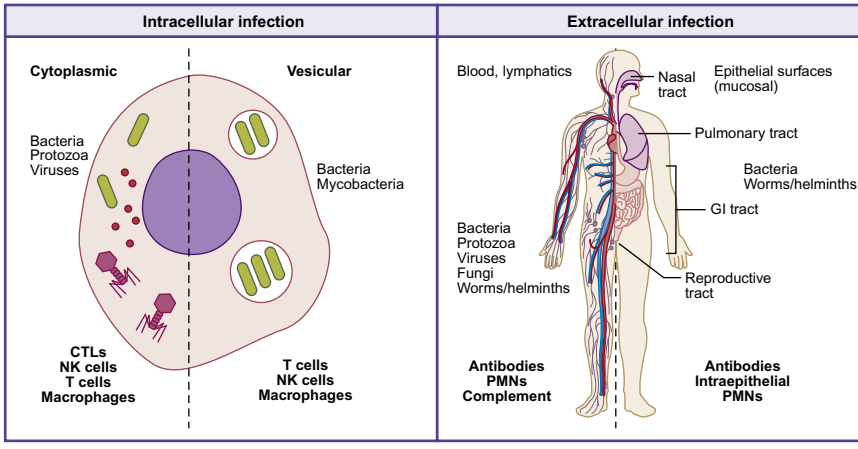


Figure 5.1 ***Functional immune response is dependent on organism location within the host.*** *Effective immune responses are directed against intracellular organisms, residing in cytoplasmic or vesicular space, or against extracellular organisms residing at mucosal surfaces or present in blood, lymph, or tissue.*

Anatomic Barrier

The skin has the thin outer epidermis and the thicker underlying dermis to impede entry and provide an effective barrier against microorganisms. Sebum, produced in sebaceous glands, is made of lactic acid and fatty acids which effectively reduce skin pH to between 3 and 5 to inhibit organism growth. Mucous membranes are covered by cilia which trap organisms and propel them out of the body.

Physiologic Barrier

The physiologic barrier includes factors such as temperature, low pH, and chemical mediators. Many organisms cannot survive or multiply in elevated body temperature. Soluble proteins play a major role in innate responses. Lysozymes can interact with bacterial cell walls; interferons α and β are natural inhibitors of viral growth; complement components use both specific and nonspecific immune factors to convert inactive forms to active moieties that damage membranes of pathogens. Low pH in the stomach and gastric environment discourages bacterial growth.

Phagocytic and Endocytic Barriers

Blood monocytes, tissue macrophages, and neutrophils phagocytose and kill microorganisms via multiple complex digestion mechanisms. Bacteria become attached to cell membranes and are ingested into phagocytic vesicles. Phagosomes fuse with lysosomes where lysosomal enzymes digest captured organisms.

Inflammatory Barriers

Initial localized tissue damage by invading organisms results in release of chemotactic factors (complement components, fibrinopeptides) to signal changes in nearby vasculature allowing **extravasation** of polymorphonuclear cells to the injury site. Innate immune recognition utilizes preformed effector molecules to recognize broad structural motifs that are highly conserved within microbial species. Engagement of innate components leads to triggering of signal pathways to promote inflammation, ensuring that invading pathogens remain in check while the specific immune response becomes engaged.

Virtually all pathogens have an extracellular phase where they are vulnerable to antibody-mediated effector mechanisms. If an extracellular agent resides on epithelial cell surfaces, antibodies such as IgA and

nonspecific inflammatory cells may be sufficient for combating infection. If the agent resides within interstitial spaces, in blood or in lymph, then protection may also include macrophage phagocytosis and neutralization responses. Intracellular agents require a different response to be effective. For cytoplasmic agents, T lymphocytes and NK cells, as well as T-cell-dependent macrophage activation, are usually necessary to kill the organism.

Pathogens can damage host tissue by direct and indirect mechanisms. Organisms may directly damage tissue by release of exotoxins that act on the surface of host cells or via released endotoxins that trigger local production of damaging cytokines. Pathogens may also directly destroy the cells they infect or force indirect damage through actions of the adaptive immune response. Pathological damage may occur due to excess deposition of antibody:antigen complexes, or through bystander killing effects during overactive specific response toward infected host target cells (discussed in Chapter 8).

BACTERIAL INFECTIONS

Bacterial infections begin with a breach of a mechanical barrier, after which released bacterial factors during replication initiate a cascade of events (Figure 5.2). Initially, infection may be resisted by antibody-mediated immune mechanisms, including neutralization of bacterial toxins. However, the role of complement in response to bacterial infection must be stressed. Major biological components of the complement system include activation of phagocytes, direct cytolysis of target cells, and coating (opsonization) of microorganisms for uptake by cells expressing complement receptors.

Complement is a system of more than 30 serum and cell surface proteins involved in inflammation and immunity. In conjunction with specific antibodies, complement components act as a primary defense system against bacterial (and viral) infections. Most of the complement proteins are "acute-phase proteins" produced by liver hepatocytes and found in the serum. Complement components can increase in concentration two- to threefold during infection. The sequential activation of complement proteins, called the **complement cascade**, is initiated by protein−protein interactions. At each step, the number of molecules activated increases, amplifying the reaction. Many complement

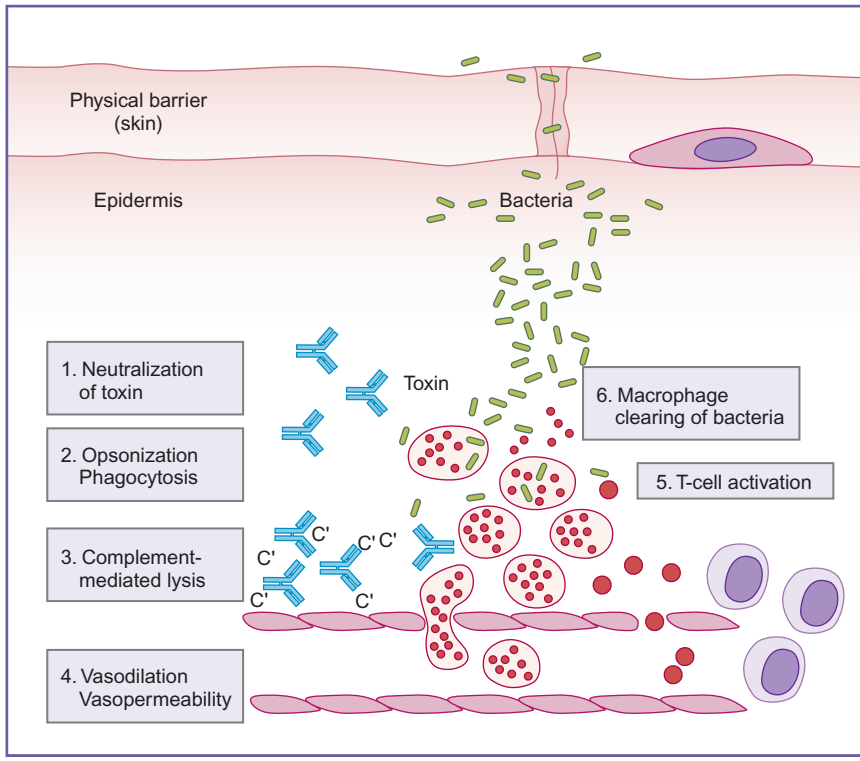


Figure 5.2 ***Immune response to bacterial infections.*** *Immune defenses against bacterial agents include antibodies for neutralization of toxins, opsonization of organisms for targeted destruction, and activation of complement for direct lysis. Vasodilation of blood vessels allows entry of cells to sites of infection to assist in control of infection.*

proteins are present as zymogens (inactive precursors) which are activated either by conformational changes or by proteolytic cleavage by other complement proteins. Activation of these zymogens results in specific serine protease activity capable of cleaving other complement proteins, producing the complement cascade (refer to Figures 2.2 and 2.3).

Several complement polypeptide cleavage fragments are involved in primary biological functions of immunity. Their main function begins when antibodies recognize pathogenic determinants and form the basis of a physical structure to which complement components interact. Specifically, complement components interact with the Fc portion of IgM and IgG binding to the surface of bacteria. This initiates a cascade of events whereby a membrane attack complex (MAC) is built upon the cellular surface, assembling as a pore channel in the lipid

bilayer membrane and causing osmotic cell lysis. MAC formation requires prior activation by either the classical or alternative pathways and utilizes the proteins C5b, C6, C7, C8, and C9.

Related complement components (C3b or inactivated C3b; iC3b) can bind directly to pathogens. Interaction with complement receptors on the surface of macrophages, monocytes, and neutrophils leads to enhanced phagocytosis and targeted destruction of organisms. Proteolytic degradation of C3 and C5 leads to production of leukocyte chemotactic factors referred to as **anaphylatoxins**. For example, C3a is chemotactic for eosinophils. C5a is a much more potent chemokine, attracting neutrophils, monocytes and macrophages, and eosinophils. Interaction of C3a, C4a, or C5a with mast cells and basophils leads to release of histamine, serotonin, and other vasoactive amines, resulting in increased vascular permeability, causing inflammation and smooth muscle contraction. The vasodilatation and vasopermeability result in an influx of professional phagocytes and acute polymorphonuclear infiltrates.

Neutrophils are typically the first infiltrating cell type to the site of inflammation (Figure 5.3). Activated endothelial cells increase expression of E-selectin and P-selectin which are recognized by neutrophil surface mucins (PSGL-1 or sialyl Lewis^x on glycoproteins or glycolipids) and induce neutrophil rolling along the endothelium. **Chemoattractants** (e.g., IL-8) can further trigger firm adhesion and diapedesis. Subsequent chemotaxis can also be induced fibrinopeptides and leukotrienes. Activated neutrophils express high-affinity Fc receptors and complement receptors to allow increased phagocytosis of invading organisms. Activation of neutrophils leads to respiratory burst, producing reactive oxygen and nitrogen intermediates, as well as release of primary and secondary granules containing proteases, phospholipases, elastases, and collagenases. Pus, a yellowish white opaque creamy matter produced by the process of suppuration, consists of innumerable neutrophils and tissue debris.

Macrophages involved in innate immunity include **alveolar macrophages** in the lung, **histocytes** in connective tissue, **Kupffer cells** in liver, **mesangial cells** in kidney, **microglial cells** in brain, and **osteoclasts** in bone. Chemokine mediators such as macrophage inflammatory protein-1α (MIP-1α) and MIP-1β attract monocytes to the site of pathogenic infection. Like the neutrophil, monocytes express surface ligands

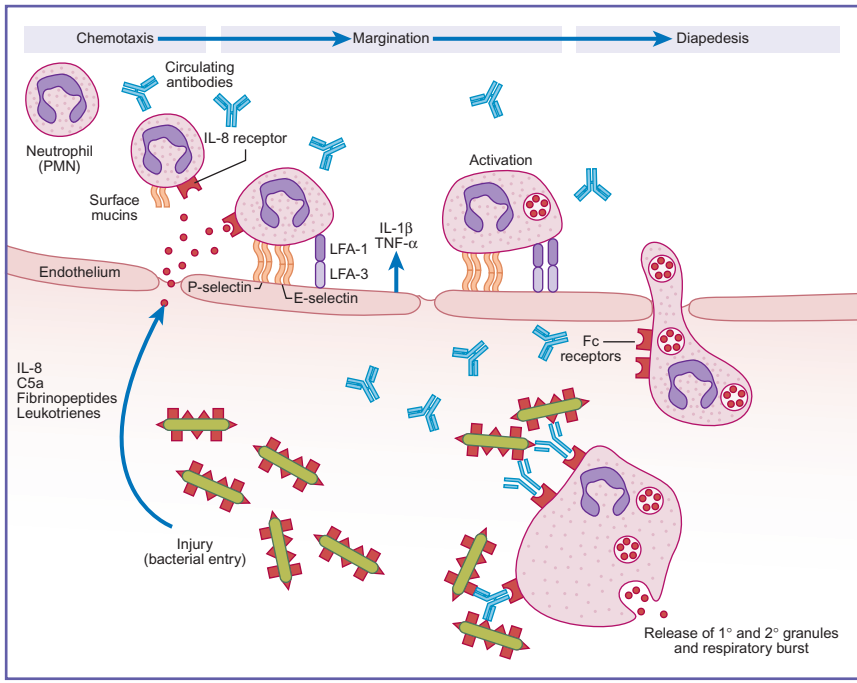


Figure 5.3 ***Events associated with neutrophil transendothelial migration.*** *Bacteria entering through a breach in the mechanical barrier (skin) trigger release of chemotactic factors that upregulate selectins on endothelial beds. Circulating polymorphonuclear cells interact via weak binding surface mucins; interactions are enhanced in the presence of stimulating cytokines, leading to margination, and diapedesis. Neutrophils shown enter tissue and undergo respiratory burst to release primary and secondary granules upon cross-linking of Fc receptors with antibodies recognizing bacterial epitopes. IL, interleukin; TNF, tumor necrosis factor.*

(integrins) which recognize ligands (cell adhesion molecules) on endothelial cells. This interaction mediates cell rolling, firm adhesion, and **diapedesis**. The entire process is referred to as **extravasation**. Activated tissue macrophages secrete proinflammatory mediators in response to bacteria and bacterial products, including IL-1, IL-6, IL-8, IL-12, and TNF-α. TNF-α is an inducer of a local inflammatory response to contain infections. IL-1, IL-6, and TNF-α have a critical role in inducing the acute-phase response in the liver and induce fever, which favors effective host defense in several ways. IL-12 may also activate NK cells.

Microbial Motifs Detected Through Pattern-Recognition Receptors

Monocytes and macrophages express receptors that recognize broad structural motifs called **pathogen-associated molecular patterns**

(**PAMPs**) that are highly conserved within microbial species. Such receptors are also referred to as **pattern-recognition receptors** (**PRRs**). Engagement of these receptors leads to immediate triggering of signal pathways that promote phagocytosis, an event which requires actin and myosin polymerization. Multiple factors assist in preparing the particulate for engulfment and targeting for destruction, including various opsonins comprised of complement components. Examples of the PRRs include receptors that recognize common bacterial carbohydrate elements, such as lipopolysaccharide (LPS), mannose, and glucans (glycogen polysaccharides). A unique family of membrane-bound receptors, termed the **Toll-like receptors** (**TLRs**), play a critical role in recognition of bacterial components (cell wall membranes, LPS, flagellin, CpG oligodeoxynucleotides); interaction with any of these receptors initiates proinflammatory responses that function locally and systemically (Table 5.1). In the case of overwhelming infection, this class of PRRs is actively engaged, contributing to presentation of sepsis, clinically manifested as an overwhelming proinflammatory cascade.

The process of phagocytosis compartmentalizes the invading pathogen into an intracellular vacuole referred to as a **phagosome**. The phagosome fuses with intracellular lysosomes, forming a **phagolysosome** vacuole. The lysosome is extremely acidic in nature and contains powerful lytic enzymes; fusion with the phagosome allows directed delivery for targeted destruction of invading organisms. In addition, phagocytosis triggers a mechanism known as the **respiratory burst**, leading to production of toxic metabolites that assist in the killing process. The most critical of these toxic metabolites are nitric oxide, hydrogen peroxides, and superoxide anions.

Table 5.1 TLRs and Their Ligands

TLR	Ligand
TLR1, 2, 6	Lipopeptides
TLR3	dsRNA
TLR4	Gram-negative LPS
TLR5	Flagellin
TLR7, 8	Single-strand RNA
TLR9	Unmethylated CpG DNA

Listed are important TLRs and specific ligands involved in pathogen recognition. At least 15 different TLRs have been identified, with ligand motifs identified for most of them.

During the chronic stage of the infection cell-mediated immunity (CMI) is activated. T cells reacting with bacterial antigens may infiltrate the site of infection, become activated, and release lymphokines that further attract and activate macrophages. Likewise, NK cells enter the infected region and assist in macrophage activation. The activated macrophages phagocytose and degrade necrotic bacteria and tissue, preparing the lesion for healing. The polymorphonuclear cells, especially neutrophils, are an excellent example of the first line of innate defense against bacterial agents.

Important factors released by macrophages in response to bacterial antigens include cytokines that exert both local and systemic function. Locally, IL-1, TNF-α, and IL-8 cause inflammation and activate vascular endothelial cells to increase permeability and allow more immune cells to enter infected area. TNF-α will also destroy local tissue to limit growth of bacteria. In addition, IL-6 can stimulate an increase in B-cell maturation and antibody production, and IL-12 will lead to activation of NK cells and priming of T cells toward a T_H1 response. Systemically, IL-1α, IL-1β, TNF-α, IL-6, and IL-8 all contribute to elevated body temperature (fever) and production of acute-phase proteins.

MYCOBACTERIAL INFECTIONS

Mycobacterial infections such as tuberculosis and leprosy are extremely complex (Figure 5.4). The mycobacteria have evolved to inhibit normal macrophage killing mechanisms (e.g., phagosome–lysosome fusion) and survive within the "disarmed" professional phagocyte. T_{DTH}-initiated cellular responses are the main mechanisms involved in an active immune response, including granulomatous hypersensitivity, to wall off and contain organisms, but only after infection has become established. Both helper and cytotoxic T cells are responsible for control of active infection, working through recognition of mycobacterial antigens and glycolipids released by infected cells. This leads to release of cytokines and chemokines that recruit additional immune effectors. A local environment is established to contain infection, resulting in granuloma pathology. Healing of the infected center may occur, with limited necrosis of the infected tissue. If the infection persists, an active caseous ("cheesy" appearing) granuloma may form with a necrotic nidus comprised of infected and active macrophages. The granuloma is usually circumscribed by responding T cells, with host-mediated

destructive response occurring inside the area of organism containment. The presence of giant cells (activated syncytial multinucleated epithelioid cells) is characteristic of late-stage response.

At one time it was thought that the tissue lesions of the disease tuberculosis required the effect of delayed hypersensitivity. The term hypersensitivity was coined because animals with cellular immune reactivity to tubercle bacilli developed greater tissue lesions after reinoculation of bacilli than did animals injected for the first time. The granulomatous lesions seen in tuberculosis depend upon primary innate functions as well as acquired immune mechanisms; lesions are not the cause of disease but an unfortunate effect of protective mechanisms. In the lung, extensive damage with accompanying caseous granulomatous pathology can ultimately result in respiratory failure. The granulomatous immune response produces the lesion, but the mycobacterium causes the disease.

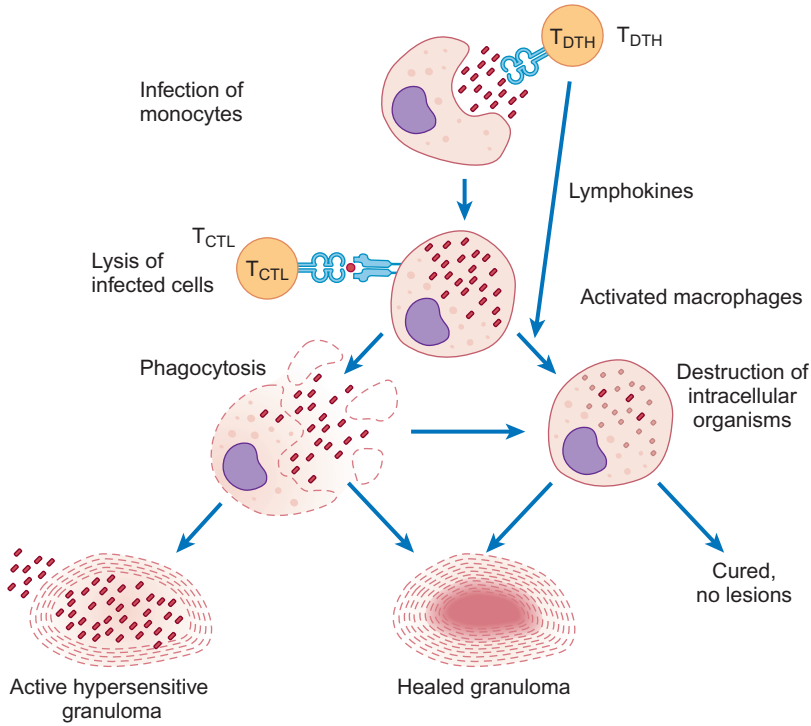


Figure 5.4 ***Immune response to mycobacterial infections.*** *Immunity against mycobacteria is initiated by phagocytic macrophages, the preferred host for the infectious agent. The overall outcome and associated pathologies are dependent upon level of activation by cellular delayed type hypersensitive (T_{DTH}) response.*

VIRAL INFECTIONS

Immune responses to viral agents are dependent upon location of the virus within the host (Figure 5.5). Antibodies play a critical role during the extracellular life cycle of the virus. Antibodies can bind to virus-forming complexes to inactivate virions and allow them to be cleared effectively by professional phagocytes. Humoral responses can prevent the entry of virus particles into cells by interfering with the ability of the virus to attach to a host cell, and secretory IgA can prevent the establishment of viral infections of mucous membranes. Once viral infection is established within cells, it is no longer susceptible to the effects of antibody. Upon entry to cells, immune resistance to viral infections is primarily T-cell mediated. To be effective in attacking intracellular organisms, an immune mechanism must have the capacity

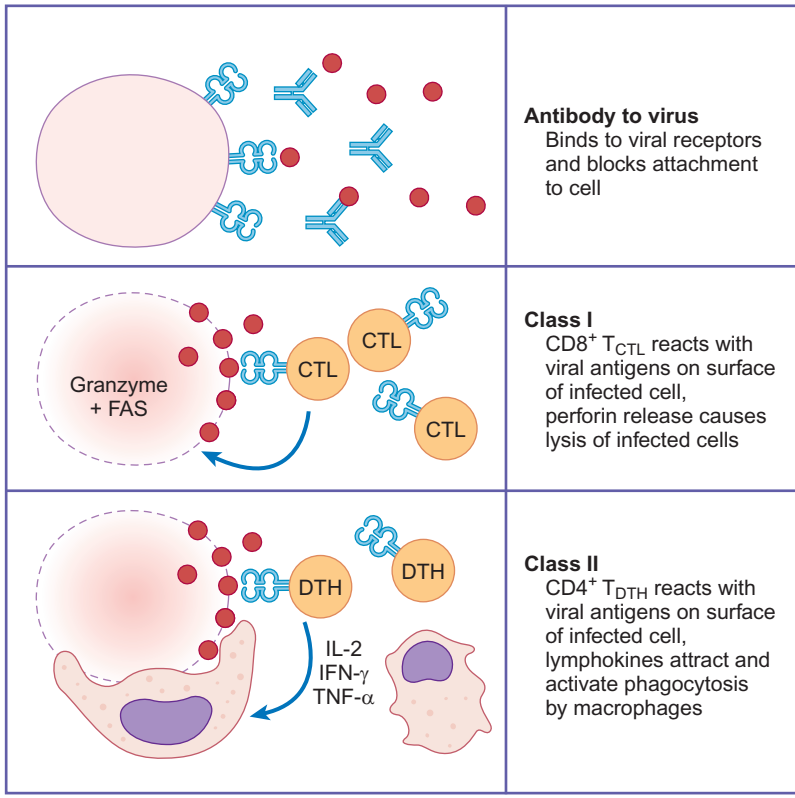


Figure 5.5 ***Immune response to viral infections.*** *Immunity against viral infections is threefold, with contributions by antibodies, cytotoxic T cells, and T-helper cells.*

to react with cells in solid tissue. This is a property of cell-mediated reactions, in particular cytotoxic T lymphocyte (CTL), but not of antibody-mediated reactions.

Most nucleated cells have an inherent, but limited, mechanism to downregulate viral replication through self-production of IFN-α and IFN-β. However, these nonspecific interferon responses are not sufficient to eliminate the virus. NK cells are an early component of the host response to viral infection. NK cells nonspecifically recognize and kill virally infected targets. NK cells provide a link between innate and adaptive immunity as they produce multiple immunomodulatory cytokines (e.g., IFN-γ, TNF-α, transforming growth factor-β (TGF-β), and IL-10). In addition, NK cells release IFN-γ (physically different from the other interferons) and IL-12, molecules which both activate macrophages and help to prime T cells for an effective antiviral T_H1 response.

At some stage of the infection process, viral-infected cells will express viral antigens on their cell surface in combination with class I molecules. Specific sensitized $CD8^+$ CTL cells recognize presented viral antigens and destroy the virus-infected cells (and therefore limit viral replication) through release of factors which include granzymes, perforins, and interferons. Lethal signals may also be delivered through Fas/Fas-ligand mediated mechanisms. Adverse effects occur if the cell expressing the viral antigens is important functionally, as is the case for certain viral infections of the central nervous system. If the virus-infected target is a macrophage, lymphocyte T-helper cells exhibiting delayed-type hypersensitivity (T_{DTH}) functions can activate the macrophages to kill their intracellular viruses; lymphokine-activated macrophages produce a variety of enzymes and cytokines that can inactivate viruses. The critical nature of T-cell-mediated response to viral infections is evident in patients defective in their CMI.

HUMAN IMMUNODEFICIENCY VIRUS

The human immunodeficiency virus-1 (HIV-1) is a member of the Lentivirus family and is the retrovirus most commonly associated with HIV infection in the United States and Europe. It has a high clinical importance and enormous social and economic impact throughout the world. As a retrovirus it requires reverse transcription of its

single-stranded RNA genome to a double-stranded DNA intermediate for integration into the host cell genome. The HIV virus infects CD4 positive cells, including T-helper lymphocytes, macrophages, and other cell types. The gp120 virus surface antigen binds with high affinity to the cell surface CD4, promoting fusion between virus and cell membranes. Coreceptor chemokine receptors CXCR4 and CCR5 may also play a role in internalization, as well as immunoglobulins that assist in antibody-dependent uptake. The natural history of HIV depicts the outcome of HIV infection which leads to destruction of lymphocytes and development of acquired immune deficiency syndrome (AIDS) (Figure 5.6).

PARASITIC INFECTIONS (HELMINTHS)

Host responses to parasitic worm infections are generally more complex because the pathogen is larger and not able to be engulfed by phagocytes (Figure 5.7). The helminths typically undergo life cycle changes as

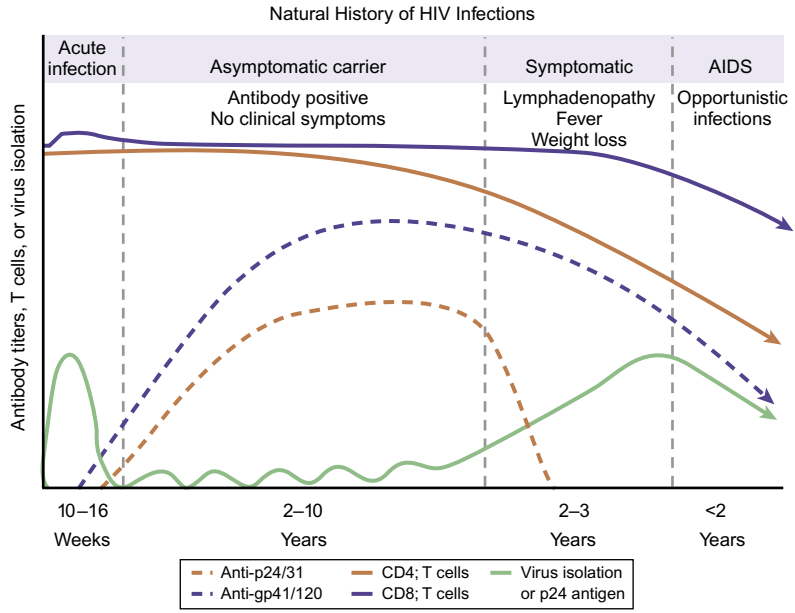


Figure 5.6 ***The natural history of HIV.*** *Infection of CD4+ lymphocytes, and other cell types, leads to virus production and cytolysis or long-term latent infection which progresses from primary infection through late symptomatic infection (AIDS). Accompanying this process are profound defects in T helper and cytotoxic cell activity, with concomitant development of opportunistic infections.*

they adapt for life in the host. Worms are located in the intestinal tract and/or tissues. Tapeworms, which exist in only the intestinal lumen, promote no protective immunologic response. On the other hand, worms with larval forms that invade tissue typically stimulate an immune response. The tissue reaction to *Ascaris* and *Trichinella* consists of an intense infiltrate of polymorphonuclear leukocytes, with a

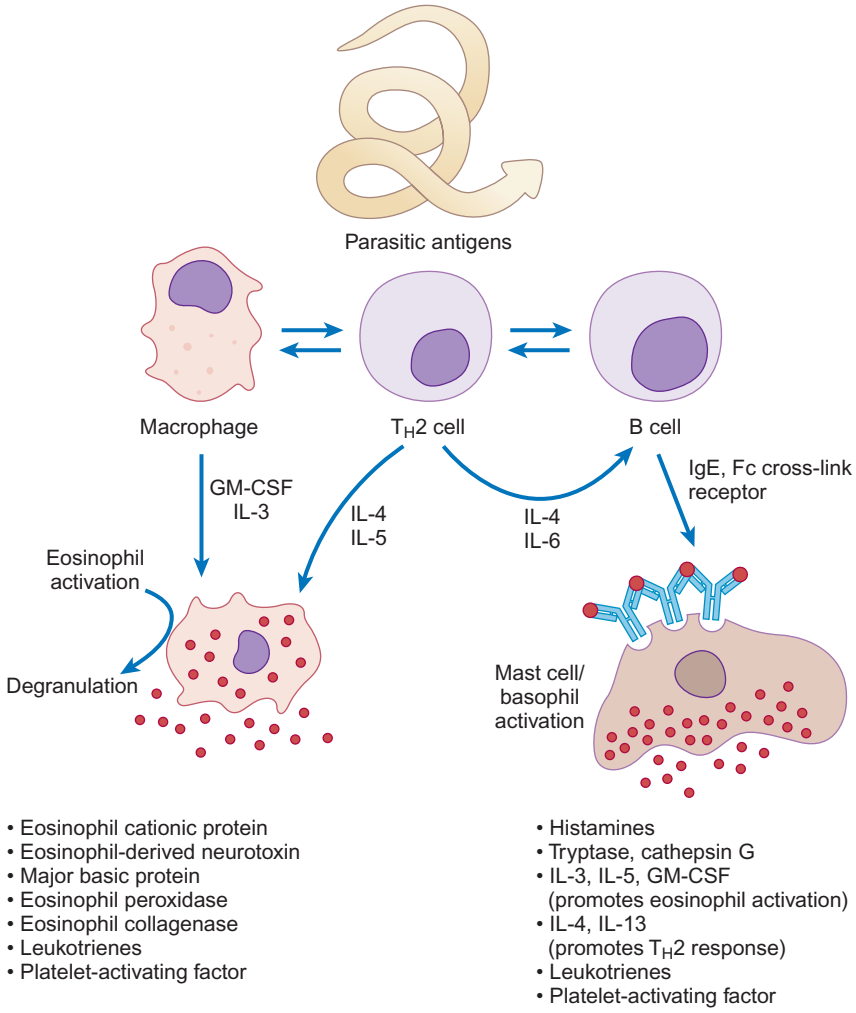


Figure 5.7 ***Response to parasitic worms.*** *Immune activity against parasitic worms is directed by T-helper 2 cells, driving activation of eosinophils, basophils, and mast cells to release inflammatory mediators to limit parasitic activity and kill invading organisms.*

predominance of eosinophils. Therefore, a variety of antigens that are life cycle stage dependent are displayed in changing tissue environments. Numerous cells play a role, depending on the location of the organism. Antigens on surface of organisms, or released into local environment, may stimulate T cells and macrophages to interact with B cells to secrete specific antibodies. IL-5, a T-cell-derived factor, is instrumental in stimulation of eosinophils; the eosinophils act by associating with specific antibody to kill worms by antibody-dependent cell cytotoxic (ADCC) mechanisms or by releasing enzymes from granules to exert controlling effects on mast cells.

Antigen reacting with IgE antibody bound to intestinal mast cells stimulates release of inflammatory mediators, such as histamine, proteases, leukotrienes, prostaglandins, and serotonin. These agents cause an increase in the vascular permeability of the mucosa, exposing worms to serum immune components, and stimulate increased mucus production and increased peristalsis. These activities are associated with expulsion of parasitic worms from the gastrointestinal tract through formation of a physical barrier to limit adherence and interactions with the mucosal surface.

Eosinophil granules contain basic proteins which are toxic to worms. Eosinophils may be directed to attack helminths by cytophilic antibodies that bridge the eosinophil through the Fc region and the helminth by specific Fab-binding ADCC. Anaphylactic antibodies (IgE) are frequently associated with helminth infections, and intradermal injection of worm extracts elicits wheal-and-flare reactions. Children infested with *Ascaris lumbricoides* have attacks of urticaria, asthma, and other anaphylactic or atopic reactions presumably associated with dissemination of *Ascaris* antigens.

FUNGAL INFECTIONS

Cellular immunity appears to be the most important immunologic factor in resistance to fungal infections, although humoral antibody certainly also plays a role in protection. T_H1 type responses are protective via release of IFN-γ. By contrast, T_H2 responses (IL-4 and IL-10) typically correlate with disease exacerbation and pathology. The importance of cellular reactions is indicated by the intense

mononuclear infiltrate and granulomatous reactions that occur in tissues infected with fungi and by the fact that fungal infections are frequently associated with depressed immune reactivity of the delayed hypersensitivity type (opportunistic infections). For example, the condition of chronic mucocutaneous candidiasis caused by persistent or recurrent infection by *Candida albicans* usually only manifests in patients with a general depression of cellular immune reactions. As a general rule, fungi appear to be resistant to the effects of antibody, and CMI is needed for effective resistance.

EVASION OF IMMUNE RESPONSE

The ultimate evolution of the host–parasite relationship is not a "cure" of an infection and complete elimination of the parasite, but rather a mutual coexistence without deleterious effects imparted to the host. In many human infections, the infectious agent is never fully destroyed and the disease enters a latent state. Infectious organisms have developed "ingenious" ways to avoid immune defense mechanisms. Organisms may locate in niches (privileged sites) not accessible to immune effector mechanisms (protective niche) or hide themselves by acquiring host molecules (masking). They may change surface antigens (antigenic modulation), hide within cells, and produce factors which inhibit the immune response (immunosuppression) or fool the immune system into responding with an ineffective effector mechanism (immune deviation).

Bacteria have evolved to evade different aspects of the phagocyte-mediated killing, as outlined in Figure 5.8. Viral entities also subvert immune responses usually through the presence of virally encoded proteins. Some of these proteins block effector functions of antibody binding, block complement mediated pathways, inhibit activation of infected cells, and can downregulate MHC class I antigens to escape CTL killing. The Herpes virus produces a factor that inhibits inflammatory responses by blocking effects of cytokines through receptor mimicking, and another that blocks proper antigen presentation and processing. Another example is the Epstein–Barr virus, which encodes a cytokine homolog of IL-10 to immunosuppress through activation of T_H2 rather than T_H1 responses.

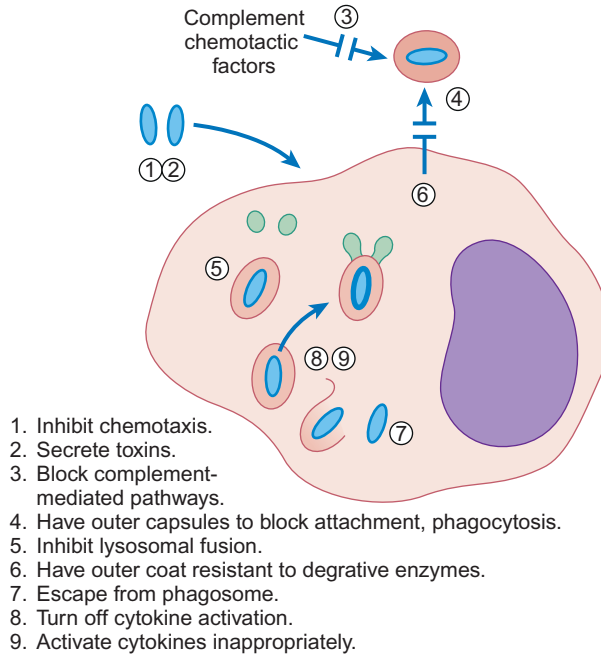


Figure 5.8 ***Mechanisms of infectious organisms to avoid immune defenses.*** *Organisms evade immune responses through various mechanisms including location in protective niches, acquisition of host molecules, alteration of surface antigens, and producing factors to inhibit or redirect effective immune response.*

SUMMARY

- The immune response to initial infection is divided into phases. The first is an early innate and nonspecific response, where preformed effector cells and molecules recognize microorganisms. The next phase is again primarily a nonspecific encounter with the organism, characterized by recruitment of professional phagocytes and NK cells to the site of infection. The final phase involves antigen-specific effector cells (B and T lymphocytes) which undergo clonal expansion; these cells provide memory responses in case of reinfection.
- The host defense is based upon availability of resources to combat a localized pathogen. This is contingent upon the life cycle of the pathogen and the extent of exposure to soluble factors or cellular processes.
- The immune mechanisms against classes of pathogens are dependent upon properties of the infectious agent. The immune system has

evolved to induce multiple arms of response to combat the broad array of organisms seeking to colonize the body.

- Virtually all classes of infectious agents have devised ways to avoid host defenses. These mechanisms include: nonaccessibility in protective niches, antigenic modulation of surface molecules, and release of factors to either suppress the immune response or cause immune deviation and ineffective response to the pathogen.

CHAPTER 6

Basic Disorders of Immune Function

Chapter Focus: To detail concepts associated with mechanisms underlying immunodeficiency, focused on causes related to primary (genetic) components for clinical manifestation. Deficiencies in cell phenotypes (lymphocytes, NK, and phagocytic cells) as well as innate components will be discussed, along with immune-based treatment options for patients with congenital immunodeficiency. Finally, information will be presented regarding relative immunodeficiency as a predisposition for infection.

IMMUNODEFICIENCY DISORDERS

Immunodeficiency disorders are a diverse group of illnesses that result from one or more abnormalities of the immune system. The abnormalities can involve absence or malfunction of blood cells (lymphocytes, granulocytes, monocytes) or soluble molecules (antibodies, complement components) which result from an inherited genetic trait (primary) or from an unrelated illness or treatment (secondary). The principal manifestation of immunodeficiency is an increased susceptibility to pathogens as documented by increased frequency or severity of infection, prolonged duration of infection with development of an unexpected complication or unusual manifestation, or infection with organisms of low pathogenicity.

GENETIC BASIS FOR PRIMARY IMMUNODEFICIENCY

The altered genetic component gives rise to deficiencies in proteins and cellular functions. These are often defects in one particular component, leading to disruption of a pathway that culminates in effective immune function. Figure 6.1 depicts the general classes of immune deficiencies, subdividing defects in innate and adaptive mechanisms. General mechanisms and associated clinical disorders are discussed below.

Introductory Immunology. DOI: http://dx.doi.org/10.1016/B978-0-12-420030-2.00006-8

<table>
<tr><th colspan="3">Immunodeficiency</th></tr>
<tr><th>B-cell deficiencies</th><th></th><th>T-cell deficiencies</th></tr>
<tr><td>Recurrent bacterial infections

Bruton's agammaglobulinemia—defect in B-cell development
Common variable–hypogammaglobulinemia—defect in plasma cell differentiation
Hyper-IgM syndrome—defect in class switching</td><td>B- and T-cell deficiencies
Severe combined immunodeficiency</td><td>Severe viral, fungal, and protozoal infections

Bare lymphocyte syndrome—lack of class II MHC
Omenn syndrome—defect in TCR gene rearrangement
DiGeorge syndrome—thymic aplasia</td></tr>
<tr><th>Phagocytic cell deficiencies</th><th></th><th>Complement deficiencies</th></tr>
<tr><td>Recurrent bacterial infections

Chronic granulomatous disease—lack of respiratory burst
Leukocyte adhesion deficiency—lack of PMN extravasation into tissue
Chediak–Higashi syndrome—defect in neutrophil microtubule function and related phagosome/lysosome fusion</td><td></td><td>Recurrent bacterial infections
Defects in immunocomplex clearance

C1, C2, or C4 deficiency—defects in clearing immunocomplexes
C3 or C5 deficiency—block in alternative and classical pathways
C6, C7, C8, or C9—defect in MAC assembly and function</td></tr>
</table>

Figure 6.1 ***Primary immunodeficiencies.*** *Manifestation of immunodeficiency is dependent upon the etiology of response. B-cell deficiency is marked by recurrent infections with encapsulated bacteria. T-cell deficiency manifests as recurrent viral, fungal, or protozoal infections. Phagocytic deficiency with associated inability to engulf and destroy pathogens usually appears with recurrent bacterial infections. Complement disorders demonstrate defects in activation patterns of the classical, alternate, and/or lectin-binding pathways and related host defense mechanisms.*

INNATE DEFICIENCIES

Deficiencies in innate components are at the heart of many immune disorders. Any functional defect in function of phagocytic cells, dendritic cells, neutrophils, or NK cells results in the inability to rapidly control invading pathogens. Likewise, defects in proteins involved in early pathways of the complement cascade limit subsequent events that control responses postinfection.

Chronic Granulomatous Disease

Chronic granulomatous disease (CGD) represents a defect in phagocytic cells normally associated with engulfment and subsequent respiratory oxidative activity, superoxide production, and hydrogen peroxide creation that is essential for killing phagocytosed bacteria. Leukocytes from individual with CGD demonstrate defects due to genetic abnormalities that limit intracellular enzymatic function and effectiveness of the phagolysosome. In many cases, there is no dysfunction in chemotactic response, and it is typical to observe high leukocyte counts in

infected people with this disorder. However, bactericidal functions are defective and increased frequency of cellular response is ineffective. Of note, very effective laboratory tests are available to diagnose CGD, including the nitroblue tetrazolium dye test, dihydrorhodamine test, and the chemiluminescence assay, all of which measure levels of hydrogen peroxide and subsequent superoxide production.

Leukocyte Adhesion Deficiency

The leukocyte adhesion deficiencies (LADs) are autosomal recessive disorders that are characterized by recurrent bacterial and fungal infections. In these patients, leukocyte adhesion-dependent functions are impaired or absent. The molecular basis of the defect is absent or deficient expression of the glycoprotein β2 integrins CD11 or CD18 which participate in leukocyte adhesion to cells and parenchyma. Another variant of the LAD syndrome occurs when carbohydrate ligands are defective on neutrophils for binding to cytokine-activated endothelium, thus inhibiting their movement into tissue. Of interest, these individuals usually display impaired wound healing as well.

Chediak–Higashi Syndrome

Chediak–Higashi syndrome represents a defect in neutrophilic microtubule function. Essentially, this dysfunction presents through an inability of the lysosome and phagosome to fuse post ingestion of microorganisms. Lysosomal trafficking is impaired, resulting in high incidence of recurrent pyogenic infections.

Complement Disorders

The complement system comprises distinct serum proteins that effect multiple biological functions. In addition to direct bactericidal function, the proteins and their enzymatically produced products regulate cellular chemotaxis, phagocytic functions through opsonization, vascular modulation, and direct activation of multiple cell phenotypes. Genetic deficiencies or mutations of any complement cascade zymogen lead to impaired host defense.

Complement factor C3 deficiency is a rare disorder exemplifying the serious nature of complement dysfunction. C3 is the pivotal complement component for classical and alternative pathway activity. Without C3, the anaphylatoxins C3a and C5a are not released and phagocytic cells are not chemotactically driven to the site of infection.

The end result is that bacteria are not effectively opsonized, and polymorphonuclear leukocytes and monocytes are not driven to increase phagocytosis. General dysfunction of the C5–C9 membrane attack complex is impaired. Individuals are especially susceptible to pyogenic infections, as well as to meningococcal and gonococcal infections. **Hereditary angioedema** represents another specific disorder of the complement cascade, related to pathways that control C1 turnover. In this case, continued production of C1 generates excess vasoactive mediators C3a and C5a, causing capillary permeability and edematous activity.

Innate Pattern-Recognition Receptor Disorders

The Toll-like receptors (TLRs) represent innate receptors that regulate response to motifs found on pathogenic invaders. As such, TLRs initiate the first line of defense to trigger cytokine responses that dictate effective lymphocytic involvement. Although much is still to be understood regarding this subclass of pattern-recognition receptors (PRRs), it is clear that absence or defects in recognition of pathogen associated molecular patterns (PAMPs) impair recruitment of leukocytes to sites of infection and reduce natural augmentation of antimicrobial or antiviral activity. Furthermore, lack of engagement of the PRRs renders dendritic cells defective in their ability to migrate to draining lymph nodes, where they effectively present antigen to T cells.

ADAPTIVE IMMUNE DISORDERS

Genetic abnormalities are also at the base of the lymphocyte immunodeficiencies. Both B and T lymphocytes begin their journey in the bone marrow; any underlying genetic abnormality caused by absent or altered enzymes can arrest maturation at defined stages of hematopoietic development. Improper genetic control for rearrangements of the antigen receptor genes will result in lack of mature cells required for effective adaptive immune function.

X-Linked (Bruton's) Agammaglobulinemia (X-LA)

By virtue of their lack of mature B cells, patients with X-linked (Bruton's) agammaglobulinemia (X-LA) have no circulating plasma cells to secrete immunoglobulins. As such, these individuals are extremely susceptible to infection with multiple classes of pathogenic organisms. The molecular basis has been linked directly to a defective cytoplasmic tyrosine kinase, which prevents B-lymphocyte maturation

and eventual production of immunoglobulins. Recurrent bacterial and pyogenic infections begin early in life (between 6 and 9 months of age) when maternal antibodies decrease due to natural decay. Individuals diagnosed with X-LA are susceptible to infections which include agents that cause pneumonia, sinusitis, otitis, and meningitis. Sepsis may also result from infection with capsular coated organisms, such as pneumococci and streptococci, which require IgG isotype antibodies for opsonization and subsequent phagocytosis for targeted destruction.

Selective IgA Deficiency

Selective IgA deficiency is the most common form of immunodeficiency. Aptly named, individuals with this deficiency lack immunoglobulin A (IgA), which is a critical isotype to protect against infections of mucous membranes lining the mouth, airways, and digestive tract. Gastrointestinal infection and malabsorption issues are typically prominent, which is expected since IgA coats mucosal surfaces. Clinical definition includes undetectable serum IgA in the presence of normal serum levels of IgG and IgM. The mechanisms for failure of IgA to be secreted are based in genetic lesions; in many cases the α-heavy chain gene remains intact yet a problem remains with both IgA production and secretion.

Common Variable Immunodeficiency

Patients with common variable immunodeficiency possess malfunctioned B cells that demonstrate a developmental block in plasma cell differentiation. This results in an essential failure to secrete immunoglobulins. They also lack effective T-helper cell function. The group is typically diagnosed after the second decade of life (15–35 years of age) when they begin to show signs of recurrent bacterial infections. Concurrently, there is a decreased immunoglobulin load and associated impairment in humoral responses. Cellular immunity is usually normal. The defect(s) have been linked to proteins involved in activation and regulation of isotype switching. Clinical presentation includes persistent lung infections, as well as presence of intestinal pathogens cleared normally by healthy individuals. Upon immunization, only low levels of IgM isotype antibody are produced with no IgG produced even after multiple immunizations.

Hyper IgM Disorders

Hyper IgM (HIM) is an immunodeficiency in which high levels of circulating IgM are present at the expense of other antibody isotypes. In males, the abnormal gene in the X-linked type (HIM-1) is due to abnormal production of a required ligand (CD40L) present on T cells that modulates interaction between the cognate CD40 ligand on B cells. This interaction is required for cellular activation as well as for proper isotype-switching events. It should be noted that females may also exhibit a form of hyper IgM, termed HIM-2, which correlates with defective activation-induced deaminase or uracil DNA glycosylase.

DiGeorge Syndrome

DiGeorge syndrome represents a severe T-cell deficiency and is characterized clinically by an absent thymus as well as by associated developmental abnormalities in the newborn. As expected with defective T cells, there is an increased susceptibility to opportunistic infections, most notably fungal infections in the very young. Infants are typically seen with normal numbers of B lymphocytes, and presence of serum immunoglobulins. However, these infants, fail to mount effective antibody response due to lack of T lymphocyte helper cell activity.

Wiskott–Aldrich Syndrome

Wiskott–Aldrich syndrome is a rare X-linked disorder where mutations in the key regulator of actin polymerization (WAS protein) have been identified. Mutations give rise to deficiencies in signaling, cell locomotion, and immune synapse formation. The immune deficiency leads to decreased antibody production and the inability of T cells to become polarized, thus allowing diagnostic placement as a combined immunodeficiency.

Severe Combined Immunodeficiency

There are many types of combined lymphocytic disorders in which deficiency in both B- and T-cell populations result in susceptibility to recurrent life-threatening infection. The classically defined form of severe combined immunodeficiency (SCID) is X-linked, but autosomal recessive forms also exist. Phenotypic analysis reveals an absence of lymphocytes that bear mature cell surface molecules and/or functional antigen receptors. Often, lymphocytes also exhibit an inability to respond to mitogenic stimulation. Frequently, there are small numbers of B cells and few serum immunoglobulins. The molecular defect

in X-linked SCID is a mutation in the gene that codes for the gamma chain of the IL-2 receptor; this chain shares functions with multiple cytokine receptors, thus impairing T-cell maturation and proliferation and basically eliminating T-cell functionality. The SCID defect is due to the fact that the defective IL-2γ chain not only renders the IL-2 receptor dysfunctional, but also renders the receptors for IL-4, IL-7, IL-15, and IL-21 dysfunctional as well.

There are several other forms of SCID involving genetic lesions (all autosomally inherited) in multiple critical genes needed for adaptive immune function. For example, **Omenn syndrome** is an autosomal recessive SCID where the recombination activating genes (RAG1 and RAG2) involved in antigen receptor DNA rearrangement render that protein dysfunctional, adversely affecting circulating levels and functionality of both B cells and T cells. Two other disorders that represent T-cell deficiencies should be included in the overall discussion. **Ataxia telangiectasia** is an autosomal recessive disorder where thymic dysfunction does not permit T-cell development. Low numbers of both CD4 + and CD8 + cells ensue. **Bare lymphocyte syndrome** also has a mechanism directly affecting CD4 + cells; lack of Class II histocompatability molecules prevents positive selection for T-helper cell phenotype during maturation in the thymus.

TREATMENT OF IMMUNODEFICIENCY DISEASES

The effective treatment of immunodeficiency is in large part dependent upon identification of the underlying disorder. A major challenge of primary immunodeficiency is to translate genetic and molecular discoveries into new therapies for patients. In general, all disorders require general supportive care with constant guard against infectious assault. In many cases a bone marrow transplant is an effective means of replacing hematopoietic stem cells, although complications may arise due to transplantation-related issues. Cytokine therapy is effective in selected defects where specific lack of a component is identified. The same holds true for complement defects; if the factor missing is known it can be therapeutically administered to recover function.

Clinical success has been seen with many other palliative treatments. B-lymphocyte disorders are primarily treated with **intravenous immunoglobulin** (**IVIG**). This mechanism of passive antibody transfer restores

regular immune function, although continued administration is required every 3–4 weeks due to the half-life of the IgG isotype given. IVIG treatment is also effective in disorders of other cell phenotypes that ultimately affect antibody production. Regarding phagocytic disorders, supplementation with cytokines such as interferon-γ may be highly effective, especially in the case of chronic granulomatous disorders.

A final word is that there is a common thread of hope that physicians will adopt a universal neonatal screening process to identify patients with primary immunodeficiency. This would allow early diagnosis and effective treatments prior to presentation with life-threatening infections or establishment of cancer due to lack of natural protective immunosurveillance.

IMMUNODEFICIENCY AS A PREDISPOSITION TO DISEASE

Immunodeficiency is closely associated with expected changes due to lack of functional cells required for common surveillance functions of the host. As such, over time there is a high incidence of both cancers (viral induced) and autoimmune dysregulation, especially in individuals that manifest defects in lymphocytic populations. However, the principal manifestation of immunodeficiency is an increased susceptibility to infection as documented by increased frequency or severity of infection, prolonged duration of infection with development of an unexpected complication or unusual manifestation, or infection with organisms of low pathogenicity.

Events of stress impact immune function. Certainly, acute stress elicits physiological responses that prime or enhance immunity in preparation for injury or an infectious threat. However, chronic stress and distress (on the order of weeks to months to years) result in immune dysregulation and direct immunosuppression. In chronic stress, it has been shown that the physiological response persists long after cessation of the stressor (changes in diet, sleep, exercise, environment, or neuroendocrine reactivity). Shifts in T-helper cell function occur in situations of prolonged stress, predominantly controlled by neuroendocrine factors regulated by the hypothalamus–pituitary–adrenal axis. Thus, we become more susceptible to infectious agents. Psychological and emotional stressors, in addition to factors regulating physiological parameters, are also included in this regulation.

Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome

The interaction between the **human immunodeficiency virus** (**HIV**) and the immune system is complex and represents a secondary immunodeficiency related to viral destruction of lymphocytes. The manifestation of the infection results in **acquired immunodeficiency syndrome** (**AIDS**) which is directly related to decreased CD4 + T-cell numbers and function (reviewed in Chapter 5). In turn, this increases susceptibility to infection by intracellular pathogens, viruses, and fungi. It also causes a decrease in surveillance activity against certain tumors. Because CD4+ T cells play a central role as helper cells and mediators of delayed-type hypersensitivity, subsequent loss of this population will leave the individual exposed to attack by many opportunistic agents.

SUMMARY

- Inherited gene defects are primary causes of immunodeficiency. Defective function may occur in both innate and adaptive immune system cells.
- The lack of immune effector functions generally results in increased incidence of infection. However, lack of regular immune surveillance can also lead to increase in cancers as well as autoimmune dysfunctions.
- Diagnostic tests augment medical history and physical exam. A process to screen newborns for primary immunodeficiencies would allow early diagnosis and effective treatments prior to presentation with life-threatening infections.
- Therapeutic intervention depends on the nature of the immune deficiency, with most successful treatments relying on corrective replacement of defective or absent immune function.

CHAPTER 7

Autoimmunity: Regulation of Response to Self

Chapter Focus: To discuss concepts associated with mechanisms underlying development of autoimmunity. Elements underlying autoimmune dysfunction will be examined, moving from basic concepts of tolerance to specific mechanisms involved in major clinical disorders when tolerance to self-antigens is lost. The goals are to present development of autoimmune reactivity so that clinical disease and outward symptoms are understood as related to underlying immune mechanisms, and to identify molecular targets involved in the host self-recognition response. A discussion will also include therapeutics that target immune parameters, as well as laboratory tests for specific autoimmune disorders.

HOMEOSTASIS, IMMUNE REGULATION, AND AUTOIMMUNITY

The regulation of immune function and overall immune homeostasis is under control of multiple factors that include genetic and environmental components. HLA allotypes, antigen dose, and existing cytokine milieu can all influence responses to commonly encountered antigens. **Autoimmunity** represents manifestation of a specific adaptive response to self-antigens. Paul Ehrlich (1854–1915) realized that reactivity toward one's own self-tissue was a pathologically destructive process, which he referred to as "horror autotoxicus." We now realize that the autoimmune process is a complex interaction where specific adaptive responses are mounted against self-antigens due to loss or circumvention of tolerance-related mechanisms. Autoimmune diseases therefore result from the dysregulation of immune processes and pathways that are involved in normal immune function, with resulting pathological damage to self-tissue.

TOLERANCE TO SELF

At the basis of autoimmunity is the loss of control of self-reactive T lymphocytes (Figure 7.1). Induction of **tolerance** in immature lymphocytes is critical for elimination of self-reactive cells. Tolerance begins

Introductory Immunology. DOI: http://dx.doi.org/10.1016/B978-0-12-420030-2.00007-X

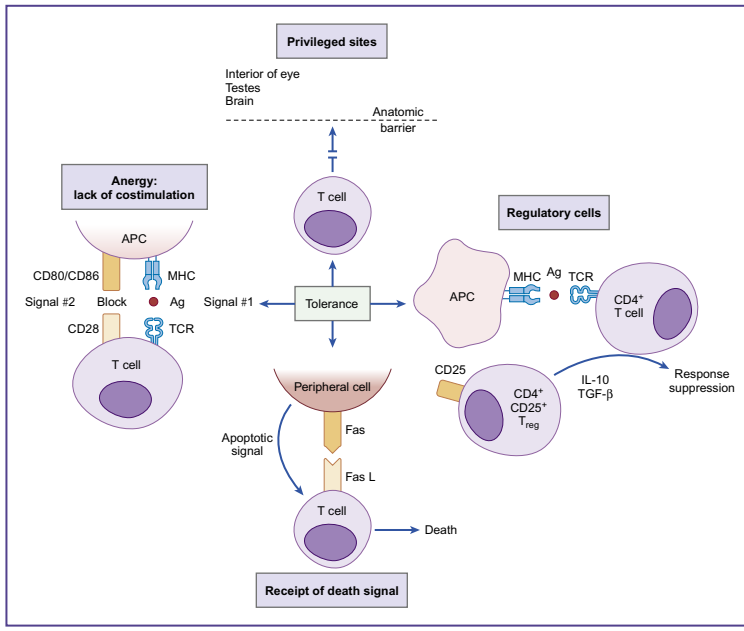


Figure 7.1 ***Mechanisms of tolerance.*** *Tolerance can occur by means including apoptosis of reactive cells, development of anergic response to antigen through loss of secondary signals, regulation of response as a result of antigen excess, and active suppression by regulatory T cells.*

during development. **Central tolerance** is provided during lymphocytic maturation and involves the physical destruction of self-reactive cells. For T lymphocytes, negative selection via apoptosis in the thymus eliminates the majority of cells that bear high reactivity to self-peptides that can be accommodated into the presenting grooves of the HLA molecules. The elimination of self-reactive T cells involves a series of interactive events with different cell phenotypes throughout compartments in the thymus. Basically, there is a balance in the relative strength in interaction with HLA molecules which naturally express self-peptides (in the absence of infection). B lymphocytes also undergo central tolerance, under a more complicated scenario, which takes place during developmental stages in the bone marrow.

It is nearly impossible to completely eliminate all reactive cells in the body. **Peripheral tolerance** is a way to ensure that cells do not react to self-antigens in the secondary lymphoid tissues. Mature cells leaving the thymus may be tolerized, subject to antigenic factors that

affect immunogenicity. Factors that induce tolerance under normal conditions include extremely high- or low-dose antigen, weak binding to histocompatability molecules, and route of administration (e.g., oral antigens are well tolerated). Peripheral tolerance occurs primarily via **anergic** reactions. Induction of "normal" T-cell reactivity requires multiple signals, including antigen recognition by the antigen receptor as well as secondary signals from adhesion and costimulatory molecules on the cell surface. If specific costimulatory signals (CD80 or CD86) on the surface of the presenting cell are absent, then cognate molecules (CD28) on the T cell are not engaged, which leads to a nonreactive, anergic state. In addition, a population of CD4+ T cells, called **T-regulatory cells** or **Tregs**, can actively anergize lymphocytes in the periphery that escape primary selection. This is done through focused release of cytokines which downregulate activities.

Tolerance may also occur in immunologically privileged sites, such as the eye, brain, and testes, usually due to local secretion of immunosuppressive factors (e.g., TGF-β). Of interest, if hidden ("cryptic") antigens are exposed during trauma or tissue damage, they now can potentially serve to initiate responses to overcome toleragenic states. The developing fetus in the uterus is a unique example of a privileged site where recognition to foreign antigen by maternally reactive lymphocytes is suppressed.

Immature B cells in the bone marrow undergo apoptosis upon binding of self-antigens, by activation-induced cell death mechanisms. Alternatively, the B cell may undergo receptor editing to change the binding specificity of the surface immunoglobulin, thus rendering the cell no longer self-reactive. Once in the peripheral tissue, B cells may undergo an anergic response dependent upon the level of specific antigen. Low-dose soluble monomeric antigens do not permit receptor cross-linking on the surface of the B cell, sending signals to clonally inactivate the B cell. Excessively high antigen dose can also result in anergic response due to overwhelming recognition in the absence of sufficient T-cell costimulation. Finally, self-reactive B cells that escape elimination or induction of anergy may be incapable of activation due to lack of T cells available to help initiate development of autoimmune response. Although at times, T cells can later become activated to bacterial antigens with cross-reactivity to self-molecules (called **molecular**

mimicry); when this occurs naturally existing tolerization states may be negated.

ETIOLOGY OF AUTOIMMUNE DISEASE

It is clear that a combination of environmental and genetic components are risk factors for autoimmune disease. Genetic factors have been identified that implicate polymorphisms in cytokine genes or their receptors, defective apoptosis genes, and complement component deficiencies. Infections or exogenous agents that cause physical damage are also likely to play important roles. The mechanisms underlying all autoimmune diseases are not fully elucidated; however, polymorphisms of MHC class II genes (alleles of HLA-DR and/or HLA-DQ) are associated with increased susceptibility to autoimmune diseases. Possible mechanisms for a loss of tolerance include (1) a lack of Fas-Fas ligand mediated deletion of autoreactive T cells in the thymus during development, (2) loss of T-regulatory or T-cell cytokine-mediated suppressor function, (3) cross-reactivity between exogenous and self-antigens (molecular mimicry), (4) excessive B-cell function due to polyclonal activation by exogenous factors (viral or bacterial origin), (5) abnormal expression of MHC class II molecules by cells that normally do not express these surface molecules, and (6) release of sequestered self-antigens from privileged sites thus priming for responses to antigens not previously seen by the immune system.

As a rule, autoimmune disease symptoms vary greatly between individuals. Periods of extreme reactivity are often interspersed with asymptomatic periods of remission. Initial autoimmunity symptoms often include fatigue, rashes, general or localized pain, and low-grade fever. A classic indicator of autoimmunity is inflammation, represented as immune reactivity present at both local and systemic levels.

Autoimmune diseases can be classified as organ specific or systemic in nature (Table 7.1). Three major types of mechanisms are recognized as causing different autoimmune disorders (Figure 7.2). Two of these mechanisms involve autoantibodies directed against self-antigens; for both, classical complement pathway activation exacerbates local damage and inflammatory responses. In the first case, autoantibodies may be directed against a specific self-component, such as a surface molecule. Examples include antibodies against the acetylcholine receptor

Table 7.1 Autoimmunity and Disease

Autoimmune Disease	Mechanism	Pathology
Autoimmune hemolytic anemia	Autoantibodies to RBC antigens	Lysis of RBCs and anemia
Autoimmune thrombocytopenia purpura	Autoantibodies to platelet integrin	Bleeding, abnormal platelet function
Myasthenia gravis	Autoantibodies to acetylcholine receptor in neuromuscular junction	Blockage of neuromuscular junction transmission and muscle weakness
Graves' disease	Autoantibodies to receptor for thyroid-stimulating hormone	Stimulation of increased release of thyroid hormone (hyperthyroidism)
Hashimoto's thyroiditis	Autoantibodies and autoreactive T cells to thyroglobulin and thyroid microsomal antigens	Destruction of thyroid gland (hypothyroidism)
Type I diabetes (insulin-dependent diabetes mellitus)	Autoantibodies and autoreactive T cells to pancreatic islet cells	Destruction of islet cells and failure of insulin production
Goodpasture's syndrome	Autoantibodies to type IV collagen	Glomerulonephritis
Rheumatic fever	Autoantibodies to cardiac myosin (cross-reactive to streptococcal cell wall component)	Myocarditis
Pemphigus vulgaris	Autoantibodies to epidermal components (cadherin, desmoglein)	Acantholytic dermatosis, skin blistering
Multiple sclerosis	T-cell response against myelin basic protein	Demyelination, marked by patches of hardened tissue in the brain or the spinal cord; partial or complete paralysis and jerking muscle tremor
Systemic lupus erythematosus	Circulating immunocomplexes deposited in skin, kidneys, etc., formed by autoantibodies to nuclear antigens (antinuclear antibodies), including anti-DNA	Glomerulitis, arthritis, vasculitis, skin rash
Rheumatoid arthritis	Autoantibodies to IgG (rheumatoid factors); deposition of immunocomplexes in synovium of joints and elsewhere; infiltrating autoreactive T cells in synovium	Joint inflammation, destruction of cartilage and bone
Celiac disease	Antibodies made to gliadin (gluten), cross-reactive to tissue transglutaminase	Gluten-sensitive enteropathy, villous destruction, and gastrointestinal manifestations
Scleroderma (systemic sclerosis)	Antibodies to topoisomerases, polymerases, and fibrillarin	Skin-related fibrosis, damage to related arteries
Ankylosing spondylitis	CD4+ cells, possible activity to self-antigens (arthritogenic peptides, molecular mimicry, or aberrant forms of B27)	Rheumatic disease of joints and spine
Sjögren's syndrome	CD4+ cells, possible activity to self-antigens (M3 muscarinic acetylcholine receptor)	Lymphocytic mediated destruction of lachrymal and salivary glands

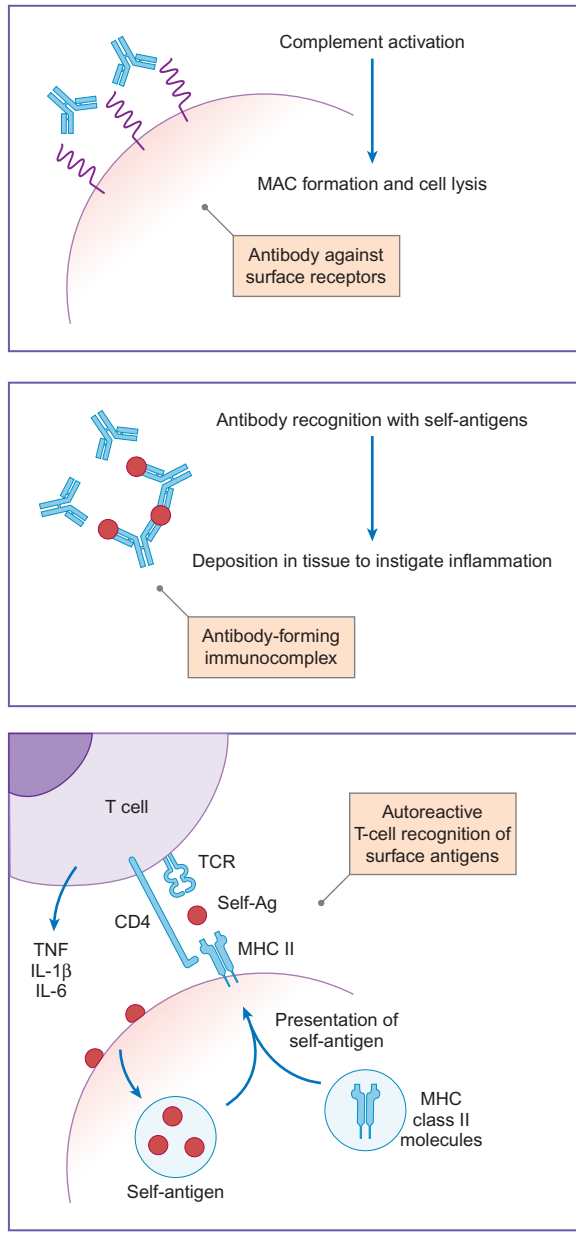


Figure 7.2 ***Major mechanisms of autoimmunity and disease.***

producing myasthenia gravis or antithyroid-stimulating hormone receptor antibodies producing Graves' disease. Autoantibodies may also bind with antigens present in the blood, forming antigen–antibody

(immune) complexes that deposit in organs or vascular beds, thus inciting an inflammatory response. This occurs in lupus glomerulonephritis where complexes of anti-DNA antibodies and bound DNA accumulate in the kidney. The third mechanism is that of autoreactive T cells that recognize organ-specific self-antigens, leading to direct tissue damage. In many cases, autoreactive T cells coexist with autoantibody responses, leading to exacerbation of disease and organ damage.

ROLE OF AUTOANTIBODIES AND SELF-REACTIVE T LYMPHOCYTES IN AUTOIMMUNE DISORDERS

The major autoimmune disorders and accompanying clinical presentations are listed in Table 7.1. What is critical to realize is that clinical presentation of autoimmune disorders is the culmination of multiple stages of misdirected or dysregulated immune function. Historically, autoimmune diseases were characterized solely by the presence of autoantibodies, or the presence of T cells reactive to identified antigens. However, researchers have come to appreciate that the architecture of the disorder is based on a combination of both innate and adaptive components. So while there may be a predominately dysfunctional arm of immune reactivity, it is more likely that multiple effector pathways and cell phenotypes are affected. The more common autoimmune disorders are discussed below.

Autoimmune hemolytic anemia is characterized by autoantibodies against antigens found on red blood cells (RBCs). Two types of antibodies exist. The "warm-reactive" autoantibodies are of the IgG isotype and react with the rhesus antigens (Rh antigens) on the cell surface at body temperature. This results in RBC opsonization and subsequent macrophage phagocytosis. There are also cold-reactive IgM antibodies that react with a different surface antigen (the I antigen), activating complement and mediating lytic events at lower temperatures.

Myasthenia gravis is a disorder where autoantibodies against the alpha chain of the nicotinic acetylcholine receptor in the neuromuscular junction act as an antagonist, resulting in dysregulation of muscle activity. This leads to clinical symptoms of muscle weakness, diplopia, dysarthria, and dysphagia. Of interest, since the isotype of the immunoglobulin is an IgG, it is possible for maternal antibodies to be transferred across the placenta to generate symptoms in the newborn.

Graves' disease patients demonstrate symptoms of hyperthyroidism, which include exacerbated weight loss, increased metabolism, palpitations, and fatigue. A classic physical symptom is ophthalmopathy and changes in eye socket orbit. In this disorder, autoantibodies against the thyrotropin stimulating hormone receptor act as an agonist, leading to thyroid overactivation. As discussed above with myasthenia, maternal antibodies can be transmitted to the fetus, resulting in transient neonatal hyperthyroidism.

Systemic lupus erythematosus is another disorder that is primarily autoantibody in origin. This disease is characterized by systemic autoimmunity and multiorgan involvement. In this case, there is high production of autoantibodies against nuclear components. Reactivity during tissue damage leads to creation of circulating immune complexes which deposit in tissues (skin, joints, kidneys) and heighten development of hypersensitive pathology leading to excess inflammation and chronic tissue damage.

Hashimoto's thyroiditis is a mixed disorder that is immune characterized by the presence of both autoantibodies and autoreactive T cells to thyroglobulin and thyroid microsomal antigens. Autodestruction of the thyroid gland leads to hypothyroidism and associated symptoms (fatigue, goiter, weight gain). T-helper type 1 cells are involved in disease manifestation.

Celiac disease is defined by classical mucosal change of the small intestine, triggered by gluten in the diet. The patient exhibits a "failure to thrive" with constant diarrhea, weight loss, and deficiencies due to inadequate nutrient uptake. Antibody reacts to gliadin, a protein found in wheat. However, the pathology is due to cross-reactive destructive response to self-transglutaminase and endomysium proteins.

Rheumatoid arthritis is "mixed" phenotypic autoimmune disorder. It was historically identified by presence of **rheumatoid factor**, which is a group of antibodies directed against the constant portion of the IgG isotype molecule. More recently, it has been shown that antibodies are also made to citrullinated peptides, but it is as yet unknown if this is diagnostic or mechanistic. T lymphocytes are involved in clinical manifestation of symptoms and are readily identifiable as infiltrates in synovial spaces. Overall, the immune complex formation and associated T-cell activation lead to activation of innate components. Subsequent

synovial inflammation ensues, culminating in destruction of cartilage and development of bone erosion.

Multiple sclerosis is an immune-mediated disease in genetically susceptible individuals where demyelination and axonal injury and destruction leads to slower nerve conduction. This leads to neurological dysfunction. The heart of the dysregulation is based in a T lymphocyte-mediated response where demyelination is accompanied by inflammation. Lesions throughout the brain and spinal cord appear and heal over time. The possible mechanisms associated with axonal loss during disease states are associated with presence of activated CD4+ T cells targeted to myelin proteins. It is thought that they migrate to the central nervous system and assist in activation of macrophages and B cells. This culminates in the secretion of proinflammatory cytokines, as well as antibodies, that continue to exacerbate the degenerative process.

Type I diabetes mellitus is characterized in a manner different from the disorders listed above. In this case, autoreactive CD8 + cytotoxic T lymphocytes are found reactive to pancreatic islet cells. Subsequent targeted islet cell destruction leads to a failure of insulin production. Of note, although autoantibodies to insulin and islet cell antigens may also be present during disease states, they are probably diagnostic rather than causative of the disorder.

Sjögren's syndrome is a chronic disorder in which leukocytes destroy the exocrine glands, specifically the salivary and lacrimal glands that produce saliva and tears. Although the exact antigenic targets affected remain unknown, the high prevalence of disorder in specific HLA populations indicates that the pathology is due to a mixed response whereby T lymphocytes provide assistance in the induction of self-reactive antibodies. The pathology may be secondary to an underlying connective tissue disease.

LABORATORY TESTS FOR AUTOIMMUNITY

The diagnostic criteria for autoimmunity is based on presentation of symptoms, usually with patient presentation of at least one (or a series) of clinical impairments that can be identified along a graded scale. Many times, the evaluation of disorder is accompanied by presence of a positive laboratory test, with persistence of immune markers present over multiple time periods (Table 7.2). The majority of tests rely on the presence of

Table 7.2 Some Clinical Diagnostic Tests for Autoimmune Disorders

General Tests for Autoimmunity	
C-reactive protein	Increased erythrocyte sedimentation rate
Autoantibody titers (anti-DNA, antinuclear components)	Presence of rheumatoid factor
Presence of specific HLA antigenic alleles	
Specific Antibody Tests for Autoimmunity	**Indicated Disorder**[a]
Antithyroglobulin antibody	Autoimmune thyroid disease Hashimoto's thyroiditis
Antithyroglobulin stimulating hormone receptor antibody	Graves' disease
Anti-gliadin, anti-transglutaminase, or anti-enodomysium antibodies	Celiac disease
Anti-mitochondrial antibody	Autoimmune liver disorder
Anti-islet cell antibody	Insulin-dependent diabetes mellitus (type I)
Anti-myelin basic protein antibody	Multiple sclerosis
Anti-acetylcholine receptor antibody	Myasthenia gravis
Anti-desmoglein 1 or 3 antibodies	Pemphigus (bullous dermatosis)
Antinuclear antibodies	Systemic lupus erythematosus Rheumatoid arthritis Scleroderma Sjögren's syndrome
Anti-dsDNA antibody Anti-phospholipid/cardiolipin antibody	Systemic lupus erythematosus
Anti-cyclic citrullinated peptide antibody Presence of rheumatoid factor	Rheumatoid arthritis

[a]*Overlapping diagnostic identification for specific disorders.*

autoantibodies. As such, many tests contain overlap with common autoreactive antigens, thus limiting their use as a sole diagnostic identifier for any particular disorder. For example, autoantibodies against nuclear components are found in multiple diseases. As such, they may be useful in identification of disorder, but only when combined with clinical criteria. Combination of these tests with histology and pathology allows a more accurate assignment of specific disease state.

TARGETED THERAPEUTICS

As our understanding of the pathogenesis of autoimmune disorders increases, so does the potential to utilize targeted therapeutics to mediate specific immune events related to clinical development of tissue damage. For example, TNF-α inhibitors are now becoming increasingly useful for the treatment of rheumatoid arthritis, ankylosing

spondylitis, psoriasis, and inflammatory bowel diseases. Likewise, antibody-based therapeutics that interfere with TNF signaling are tremendously powerful tools to reduce inflammatory responses. Antibodies can also be targeted to limit active B-cell populations; anti-CD20 antibody (a prominent B-cell surface antigen) is a very useful tool for the treatment of rheumatoid arthritis. In a similar manner, antibody biologics that bind the cytotoxic T-lymphocyte antigen 4 (CTLA-4; CD152) have been shown to downregulate IL-2 producing CD4+ T-helper populations. Other therapeutics target innate functions to limit inflammatory profiles; beta-interferon (IFN-β) is useful to treat multiple sclerosis, and inhibition of type I interferons may eventually be used to treat systemic lupus erythematosus.

SUMMARY

- Autoimmunity represents a failure of effective tolerance to self-antigens.
- Development of autoimmunity results from failure to effectively eliminate self-reactive lymphocytes and a failure to contain those lymphocytes after they enter peripheral tissues.
- Genetic and environmental factors play a role in the etiology of disease.
- Mechanisms of disease include autoantibodies that are directed against specific self-components, deposition of circulating antibody–antigen complexes, and deleterious responses by autoreactive T cells.

CHAPTER 8

The Immune Hypersensitivities

Chapter Focus: To investigate concepts associated with disorders classified as immune hypersensitivity. Underlying mechanisms will discuss the basis of response that culminates in clinical symptoms, focused on the primary cellular and molecular components for the basis of pathology development. The discussion will include allergic (Type I), cytotoxic (Type II), immune complex (Type III), and delayed-type (Type IV) hypersensitive responses, concentrating on how a vigorous immune response contributes toward tissue damage.

THE HYPERSENSITIVE DISORDERS

Immunological diseases can be grouped into two large categories of deficiency and dysfunction. As previously discussed, immune deficiency disorders are the result of the absence (congenital or acquired) of one or more immune system elements. In contrast, disorders due to immune dysfunction happen when a particular subset of immune responses occur which are detrimental to the host. This response may be against a foreign antigen or self-antigen and is usually defined as inappropriate regulation of an effector response. This happens in the absence of protection against pathogenic organisms. Notwithstanding, the host is adversely affected. A healthy immune system occurs as a result of balance between innate and adaptive immunity, cellular and humoral immunity, inflammatory and regulatory networks and small biochemical mediators (cytokines). Gell and Coombs understood the nature of this imbalance as related to pathology, and in the late 1960s and early 1970s classified these dysfunctional immune responses into categories called **hypersensitivity diseases**.

The term **hypersensitivity** therefore refers to a definable immune response that leads to deleterious host reactions rather than protection against disease. Hypersensitivities are a major cause of clinical disease. Although the mechanisms can be defined for each subclass, in reality, there is considerable overlap in the underlying causes that contribute to the hypersensitive responses and how they adversely affect tissues in

Introductory Immunology. DOI: http://dx.doi.org/10.1016/B978-0-12-420030-2.00008-1

the body. The hypersensitivity reactions fall into four classes based on their mechanisms and the ability to passively transfer response through antibodies or through T lymphocytes. These responses include inappropriate antigenic response, excessive magnitude of response, prolonged duration of response, and innocent bystander effector reactions leading to tissue damage. The major mechanisms are detailed below.

TYPE I HYPERSENSITIVITY: IgE-MEDIATED IMMEDIATE HYPERSENSITIVITY

The **Type I hypersensitivity** is due to aberrant production and activity of IgE against normally nonpathogenic antigens (commonly called **allergens**) (Figure 8.1). This **allergic hypersensitivity** is also called **immediate hypersensitivity** because of the speed of reaction development. Mast cells and basophils ordinarily have high-affinity IgE receptors that are constitutively filled with that immunoglobulin isotype. Antigenic exposure results in a cross-linking of cell-bound IgE with the

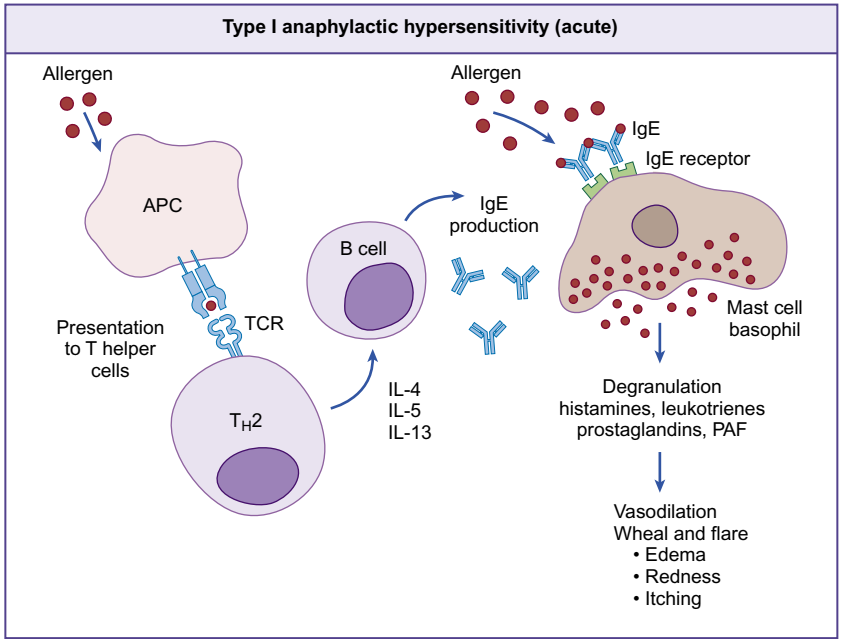


Figure 8.1 ***Type I hypersensitivity*** *(also called immediate hypersensitivity) is due to aberrant production and activity of IgE against normally nonpathogenic antigens (commonly called allergy). The IgE binds to mast cells via high-affinity IgE receptors. Subsequent antigen exposure results in cross-linking of mast cell-bound IgE. Activated mast cells release preformed mediators and synthesize new mediators, which are responsible for allergic symptoms.*

allergen, followed by nearly immediate activation of those cells. This results in a quick release of preformed mediators (e.g., histamine, leukotrienes, etc.) and an immediate resynthesis of new mediators (i.e., chemotaxins, cytokines). The activity of these mediators is responsible for the signs and symptoms of allergic diseases. Note that the IgE-associated responses differ from the mechanisms of anaphylatoxins (complement factors C3a, C4a, and C5a) which trigger mast cell degranulation in the absence of IgE.

For any Type I reaction to occur, there must be a preexisting IgE population specific for the allergen. By definition, a mature B-cell response to the antigen has already developed, in part with CD4+ T-helper 2 cytokines, such as IL-4 and IL-13 which promotes generation of plasma cell immunoglobulin production of the IgE isotype.

The prototype disorders for this hypersensitivity include allergic rhinitis and seasonal allergies, as well as allergic asthma. The typical allergens include pollens, fungal spores, and common dust mite and other household antigens. Immunotherapy for severe reacting individuals is usually through diversion and development of immune response other than those related to IgE synthesis. Pharmaceuticals have been developed to inhibit mast cell degranulation, thus alleviating many common symptoms. In severe cases where allergens are systemic, anaphylactic shock may occur due to immediate vasoactive mediator activity. This is characterized by a sudden and sharp drop in blood pressure, urticaria, and breathing difficulties caused by exposure to a foreign substance (such as bee venom, immediate drug reactivity, or food allergies). **Systemic allergic anaphylaxis** is life threatening; emergency treatment includes epinephrine injections, used as a heart stimulant, vasoconstrictor, and bronchial relaxant.

TYPE II HYPERSENSITIVITY: ANTIBODY-MEDIATED CYTOTOXIC HYPERSENSITIVITY

The **Type II hypersensitivity** is due to antibody reactivity against cell membrane-associated antigen that results in cytolysis (Figure 8.2). The mechanism may involve complement (**cytotoxic antibody**) or effector lymphocytes that bind to target cell-associated antibody and effect cytolysis via a complement-independent pathway (**antibody-dependent cellular cytotoxicity, ADCC**). The end result of the antibody response is cytolysis.

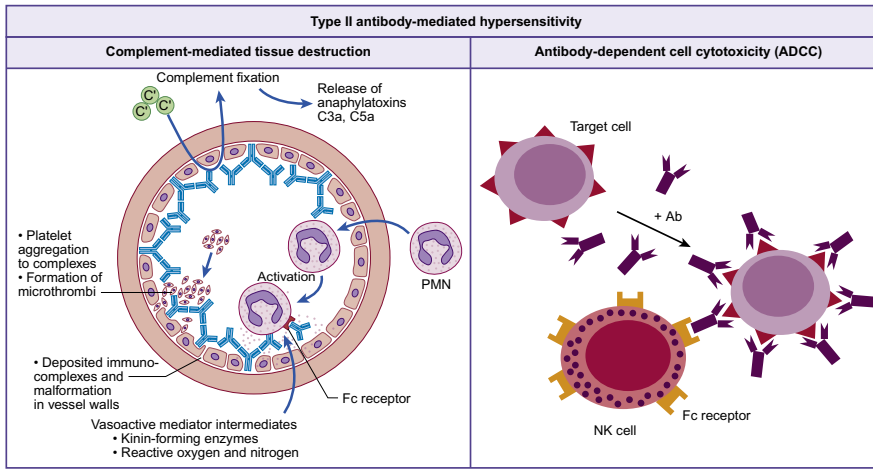


Figure 8.2 ***Type II hypersensitivity*** *is due to antibody directed against cell membrane-associated antigen that results in cytolysis. The mechanism may involve complement (cytotoxic antibody) that binds to target cell-associated antibody, subsequently effecting cytolysis (depicted) or via a complement-independent pathway of ADCC involving NK cells and targeted cell destruction (not shown).*

In complement-mediated Type II hypersensitivity, IgG isotype antibody recognition of cell surface epitopes leads to assembly of the complement C5–C9 **membrane attack complex** and subsequent lysis of the cell. This reaction is the underlying mechanism in multiple disease states, including that seen in autoimmune hemolytic anemia and Rh incompatibility leading to erythroblastosis fetalis. In rare cases, pharmaceutical agents, such as penicillin or chlorpromazine, can bind cells, forming a novel antigenic surface complex that provokes antibody production and Type II cytotoxic reactions.

A second mechanism for Type II reactions is characterized by ADCC induced by natural killer (NK) cells recognizing IgG attached to target cells bearing antigen. The constant portion of the antibody (Fc region) is bound by Fc receptors on the NK cell, leading to perforin release and NK cell-mediated lysis. Neutrophils, eosinophils, and macrophages may also participate in ADCC. ADCC may be involved in the pathophysiology of certain virus-induced immunological diseases, such as those seen during active response to retroviral infection.

Cytotoxic antibodies mediate many immunologically based hemolytic anemias while ADCC may be involved in the pathophysiology of certain virus-induced immunological diseases. Prototype disorders include many autoimmune-related diseases which bear evidence of

tissue destruction. Goodpasture syndrome represents autoantibody against basement membrane collagen type IV; deposition and accompanying complement activation leads to damage in both kidney and lung tissues. Mediators of acute inflammation generated at the tissue form end-product membrane attack complexes which cause cell lysis and death. The reaction takes hours to a day, with chronic strain on the body if the flared reaction continues unabated. Other disorders include idiopathic thrombocytopenic purpura (platelet destruction) and pemphigoid reactions resulting in skin blisters. And both myasthenia gravis (muscle weakness) and Graves' disease (hyperthyroidism) exhibit autoantibody-mediated cytotoxic events, although these disease processes are considerably more complex in nature and overlaps with other hypersensitivity mechanisms.

TYPE III HYPERSENSITIVITY: IMMUNE COMPLEX-MEDIATED HYPERSENSITIVITY

The **Type III hypersensitivity** results from soluble antigen–antibody immune complex deposition and subsequent events that activate complement to summon polymorphonuclear leukocytes (Figure 8.3). The antigens may be self or foreign (i.e., microbial). Such complexes are deposited on membrane surfaces of various organs (e.g., kidney, lung, synovium). The by-products of complement activation (C3a, C5a) are chemotaxins for acute inflammatory cells, resulting in infiltration by polymorphonuclear cells. Lysosomal enzymes are released which result in tissue injury. Platelet aggregation occurs, resulting in microthrombus formation in the vasculature. This type of hypersensitivity was classically characterized as the **Arthus reaction**, identified by high degree of neutrophils and mast cell infiltrates, vasoactive amine release, erythema and edema in response to intradermal injection of antigen.

These immune reactions result in the Type III inflammatory injury readily seen in diseases such as rheumatoid arthritis, systemic lupus erythematosus, and post-infectious arthritis. It is also evident during post-streptococcal glomerulonephritis, where damage severely affects kidney function.

An interesting example of the Type III reactions is that referred to as **serum sickness**, which historically arose when antisera made in animals were repeatedly given to humans to neutralize toxins. Immune

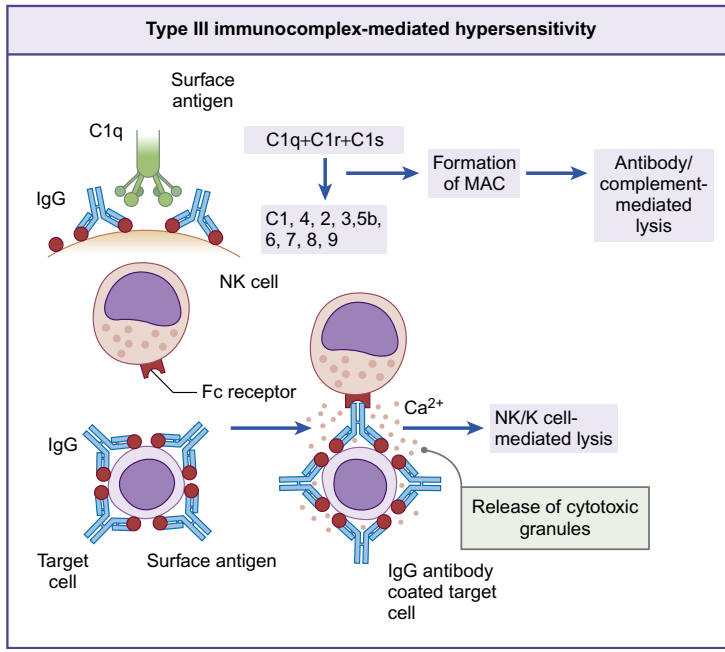


Figure 8.3 ***Type III hypersensitivity*** *results from soluble antigen–antibody immune complexes that activate complement. The antigens may be self or foreign. Complexes are deposited on membrane surfaces of organs. The by-products of complement activation are chemotaxins for acute inflammatory cells.*

complexes would form *in vivo* after 1–3 days, when host B cells recognized circulating foreign (heterologous) antibodies, and specific antibodies were produced that targeted those foreign epitopes. Subsequent particulates deposited in vascular beds, leading to pathologies. Symptoms included anaphylactoid purpura, rashes, fever, myalgia, and arthralgia. This type of reaction is still possible when intravenous immunoglobulin is administered therapeutically, although matching homologous human proteins limits reactivity. Of note, individuals can exhibit similar pathologies to drugs; complexes of antibodies directed toward chemotherapeutic agents (e.g., Penicillin) may lead to immune complexes that deposit in vascular beds and result in vasculitis, destruction of endothelium, and edema.

TYPE IV HYPERSENSITIVITY: DELAYED-TYPE (CELL-MEDIATED) HYPERSENSITIVITY

The **Type IV hypersensitivity** (also called **delayed-type hypersensitivity, DTH**) involves T cell–antigen interactions that cause activation and

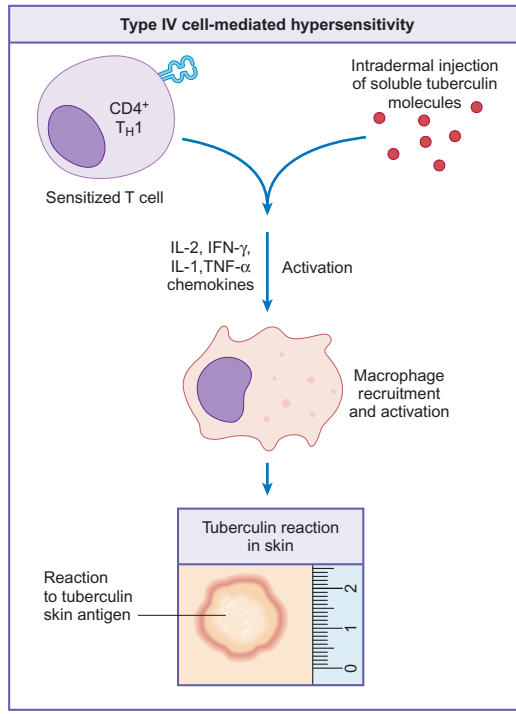


Figure 8.4 ***Type IV hypersensitivity*** *(also called DTH) involves macrophage–T cell–antigen interactions that cause activation, cytokine secretion, and potential granuloma formation. Shown here is the response to soluble tuberculin antigens. Although CD4+ T cells are depicted, they may give help to cytotoxic CD8+ T cells to further the response. The tissue injury is primarily due to the vigorous immune response rather than the inciting antigen.*

cytokine secretion (Figure 8.4). This type of hypersensitivity requires sensitized lymphocytes that respond 24–48 h after exposure to soluble antigen. DTH reactions may involve T-helper cells (CD4+) or cytotoxic T cells (CD8+ CTLs), rather than antibodies. Diseases such as tuberculosis, leprosy, and sarcoidosis as well as contact dermatitis are all clinical examples where tissue injury is primarily due to the vigorous immune response to released antigens rather than damage due to the inciting pathogen itself. In these examples, sustained release of antigen and continued activation of sensitized T cells results in amplified tissue damage. Persistent infections may provoke excessive macrophage activation and granulomatous responses, leading to extended fibrosis and necrosis of tissue.

A classic DTH reaction is exemplified in the tuberculin skin test (Mantoux reaction). An individual sensitized to tuberculosis through

exposure or infection develops CD4+ lymphocytes specific for mycobacterial antigens. Intradermal injection of purified protein derivative from mycobacteria (PPD) results in activation of sensitized CD4+ T cells. This is followed by secretion of cytokines which cause macrophage recruitment and activation. The final outcome is a localized reactivity manifested by erythema and induration. Of interest, individuals who are HIV positive with low CD4+ cell counts will not mount significant DTH responses during the tuberculin skin test, providing further evidence for the importance of this hypersensitive reaction in protective immunity.

The Type IV reactions also can be identified in pathologies resulting from viral infection. Here, the CD8+ cells react to antigens presented via class I MHC molecules or to antigens that are cell surface associated. Cytotoxic cells recognize the presented antigen and lyse the infected target. Bystander killing may occur due to over-aggressive responses, such as those seen during smallpox, measles, and herpes infections, as well as in contact dermatitis.

ALTERNATE HYPERSENSITIVITY CLASSIFICATIONS

Many investigators have revisited these four classifications, especially as the molecular knowledge of immunology has increased, and our understanding of the clinical manifestation of disease has grown. A fifth classification encompasses pathology leading to the granulomatous response, in which encapsulation and isolation of specific pathogens leads to tissue pathology. These events are driven by innate immunity or "foreign body" responses that lead to aggressive CD4+ Th1 or Th2 type responses. The outcome of cytokines produced is dependent in large part upon presentation of the particular persisting antigen.

SUMMARY

- Robust dysfunctional immune responses may often lead to tissue damage detrimental to the host.
- Allergic hypersensitivity (Type I) is an immediate hypersensitivity due to aberrant activity of IgE against normally nonpathogenic allergens, mediated through mast cell and basophil high-affinity IgE receptors.

- Cytotoxic hypersensitivity (Type II) results in tissue injury due to antigens recognized by IgG and IgM antibodies. Complement deposition triggers formation of the membrane attack complex, causing lytic damage.
- Immune complex hypersensitivity (Type III) represents an immune complex disorder in which reactivity *in vivo* leads to deposition of immune particulates. Complement by-products initiate chemotaxic influx of acute inflammatory cells, which release enzymes that result in tissue injury.
- Delayed-type hypersensitivity (DTH, Type IV) involves T-helper cells (CD4+) or cytotoxic T cells (CD8+ CTLs), rather than antibodies. This is a cell-mediated event directed at released antigens in a sensitized individual.

CHAPTER 9

Vaccines and Immunotherapy

Chapter Focus: To emphasize immunological principles as they relate to vaccination. The goal is to develop a perspective of active and passive immunization for vaccination against infectious agents of multiple classes. A discussion will demonstrate how principles in immunology combine with biotechnology to advance the field of vaccinology. Information regarding immunotherapeutics is presented as a way to provide homeostasis of normal immune function.

PRINCIPLES OF VACCINATION

The definition of "immunity" is centered on protection against infectious disease. This may be conferred most readily by immune responses generated through immunization or previous infection. Edward Jenner, an English country doctor, observed that people infected with cowpox virus often develop less severe smallpox disease. Consequently, he inoculated a boy with cowpox virus obtained from hand sores of a milkmaid. Six weeks later, after the boy recovered from cowpox, he was reinoculated with virulent smallpox virus. The boy survived. Thus was born the process of **vaccination**. Since that time, similar methodologies have been successfully adopted for **immunization** against a multitude of diseases.

Although unknown at the time, Jenner's vaccination represented **cross-reactivity** of common antigens present on the cowpox virus with molecules present on the smallpox virus. Antibodies raised against the avirulent form were also able to neutralize virulent infection. It has since been shown that development of specific antibodies is a powerful tool to provide long-lasting immunologic protection against infectious agents. Indeed, it is now appreciated that there are a wide variety of responses triggered via immunization; specific pathways can now be targeted to elicit the arm of the immune response most critical for protection against distinct pathogens (Table 9.1). Major advances in vaccine design are taking place. Improvements in methodologies to produce nonvirulent antigenic substances for use as vaccine antigens will dictate future successes in the immunization arena. These include

Introductory Immunology. DOI: http://dx.doi.org/10.1016/B978-0-12-420030-2.00009-3

Table 9.1 Example Vaccine Classes and Their Targets		
Type of Vaccine	**Components**	**Examples**
Live attenuated	• Viral or bacterial organism with reduced pathogenicity	Oral polio, varicella, measles–mumps–rubella, bacillus Calmette–Guerin (BCG)
Killed–inactivated	• Whole killed organism	Inactivated polio, typhoid
Subunit Recombinant subunit	• Inactivated or modified toxins, purified components • Gene-derived proteins produced in another organism	Diphtheria, tetanus, influenza Hepatitis B toxoid, human papillomavirus
Conjugate/ polyvalent	• Combined components isolated or genetically modified from multiple strains	*Haemophilus influenzae* type B, *Streptococcus pneumonia*, *Neisseria meningitidis*

novel ways to manufacture toxoids and synthetic peptides, improvements in recombinant DNA technology to allow live avirulent viral and bacterial agents to express other pathogen genes, development of DNA based vaccines, and new methods of conjugation to achieve superior immunogenicity for both polysaccharide and protein antigens.

BASIC CONCEPTS OF PROTECTIVE IMMUNIZATION

The objective of immunization is to generate high levels of memory cells using vaccination methods (Figure 9.1). A **primary immune response** refers to lymphocyte activation events following first recognition of the foreign material, following which a memory response is generated. Immunological memory represents a pool of circulating long-lived cells which remain present and available for action long after initial response activities wane. If the antigen is reencountered at a later time, a **secondary immune response** occurs where memory cells are engaged and activated. This secondary response is faster, more focused, and more effective than the original encounter.

Development of disease is a complex equation that links rapidity of response as a critical component related to disease outcome. The incubation period during establishment of infection is important because it dictates how much time is available to mount an immune response prior to disease initiation. A long pathogen incubation time results in an extended period where immune events can mature. This naturally leads to induction of relatively stronger immune response, with significant development of immunological memory. In this case, a secondary

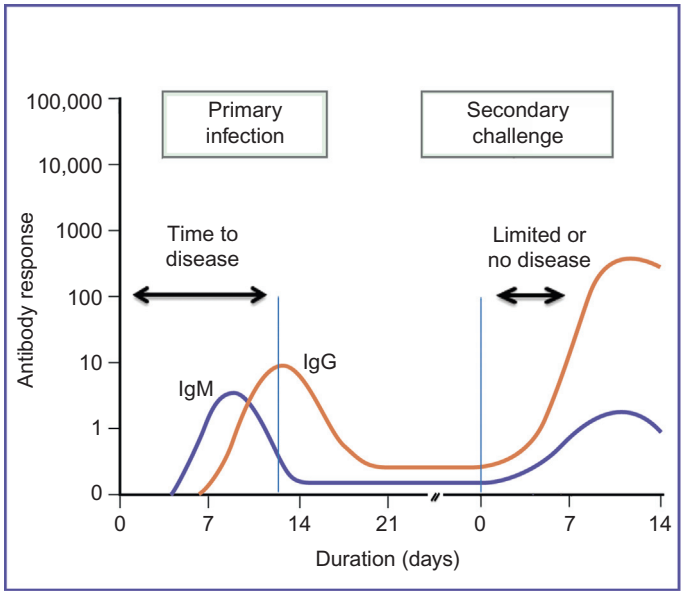


Figure 9.1 ***Primary and secondary immune responses.*** *A primary infection generally leads to a stronger and more rapid elicitation upon secondary exposure. However, if the incubation time is short during primary exposure, the development of secondary responses is impaired. Vaccination permits preexisting responses to dominate, which is critical during short infection incubation periods.*

exposure results in protection to disease due to preestablished memory response. However, when there is only a limited incubation period with a short time existing prior to disease induction, there exists only a limited time for induction of immune response. When this happens, secondary exposure does not necessarily elicit protection against disease. Vaccines are especially useful in this example, as there is a shorter window of opportunity for fighting pathogens. Induction of protection can be achieved with a vaccine provided one is able to sustain high, long-term reactive antibody **titers**. This is clinically achievable by giving several immunizations in a shorter time frame to raise sustained titers of specific antibody response.

TYPES OF IMMUNIZATIONS

Immunizations may be active or passive. Active immunization is a result of direct exposure to the antigen, allowing the host to generate protective immunity. The objective is to provide long-lasting immunity

against future exposures. **Active immunity** may be naturally acquired through infection (and subsequent recovery) or artificially through vaccination. **Passive immunity** also provides protection for the host, but is conceived by administration of humoral and/or cellular factors that provide immunity for the host. In this case, the host does not actively make a protective response. The objective of passive immunity is therefore to provide immediate but temporary protection against an imminent or ongoing threat.

AGE AND TIMING OF IMMUNIZATIONS

Vaccines are given to prevent life-threatening infections. It is therefore critical to relate factors such as patient age, demographics, geographical location, and pathogen incidence with the vaccines being administered (Table 9.2). For example, neonate or pediatric vaccines typically target pathogens that rapidly outpace the infant's ability to respond effectively. The newborn is naturally delayed in development of immune responses; especially in production of immunoglobulin isotypes (Figure 9.2). Newborns are immunocompetent but immune immature; fetuses make IgM, but not IgG until birth. Maternal IgG provides protection against bacterial agents through the first months of life. While the total amount of immunoglobulin in newborn serum is at a level close to that of a normal adult, almost all of it is of maternal origin. The half-life of IgG is 2–3 weeks; only 10% of maternal antibodies remain by 4 months of age, and only 3% by 6 months. Fortunately, at 2–3 weeks postpartum, supplemental antibodies of the IgA, IgM, and IgG isotypes are delivered through colostrum and breast milk.

Children under 2 years of age remain immunologically disadvantaged, with limited ability to produce antibodies of only the IgM isotype to bacterial capsular polysaccharides (T-independent antigens). Vaccines are designed to work with the newborn's developing immune system to elicit opsonizing antibodies of the IgG isotype. One trick is to chemically link a polysaccharide molecule, or a hapten, to a carrier protein to enlist a strong T-helper cell response and induce accompanying antibody isotype switching.

On the other end of the age spectrum, the elderly (>60 years of age) exhibit reduced capacity to mount primary responses to most

Table 9.2 United States Schedule for Active Immunization of Children and Adults

Vaccine (Birth to Year 18)	**Age (Dosing Dates for Vaccination)**
Hepatitis B	Birth, 1–2 months, 6 months to year 1
Rotavirus	2 months, 4 months
Diphtheria, tetanus, acellular pertussis (DTP), *Haemophilus influenzae* type b (Hib), pneumococcal conjugate (PCV13)	2 months, 4 months, 6 months, additional doses after year 1
Poliovirus	2 months, 4 months, 6–18 months, and after year 4
Influenza	Annual vaccination after month 6
Mumps, measles, rubella (MMR) Varicella	1 year, additional dose after year 4
Hepatitis A	1 year, additional dose before year 2
Influenza (childhood)	Annual vaccination after month 6
Human papillomavirus	3 doses in early teenage years
Meningococcal	11 years, boost in year 16
Vaccine (Year 19 Plus)	**Age (Dosing Dates for Vaccination)**
Influenza (adult)	1 dose annually, begin year 19
Tetanus, diphtheria, pertussis	1 dose annually, begin year 19, boost each decade
Varicella	2 doses recommended over lifetime
Human papillomavirus	Boosting of individuals of high risk or immunocompromised
Pneumococcal conjugate (PCV13) Meningococcal Hepatitis A, Hepatitis B *Haemophilus influenzae* type b (Hib)	Boosting of individuals of high risk
Zoster	Year 60 or older
An expanded recommended immunization schedule maintained by the Centers for Disease Control and Prevention may be found at http://www.cdc.gov/vaccines/schedules/index.html.	

antigens. Extreme age is a determinant for immune regulation; immune senescence occurs where the majority of memory responses remain available but poor primary (naïve) response results in increased susceptibility to organisms and strains never before encountered. Of interest, the immune senescent individual retains a strong response to bacterial polysaccharides. The goal in elderly individuals is therefore to induce high levels of specific responses. It may be necessary to repeat vaccinations at more frequent intervals (years, rather than decades) to maintain high functional response.

In healthy individuals, multiple doses may be required to induce immunoglobulin isotype switching and to attain high levels of antibody titer sufficient for long-lasting protection. The critical factor is

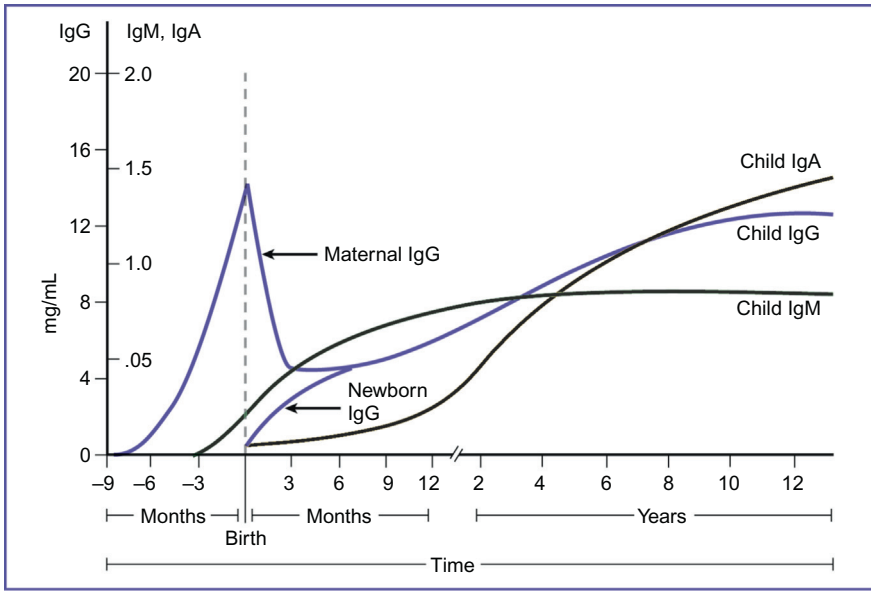


Figure 9.2 ***Changes in relative antibody titer and isotype in the newborn.***

knowledge of which "arm" of immune response is required to optimize protective response against any particular infectious agent or pathogenic factor. Induction of B-cell responses for antibody production is a successful method for toxin or viral neutralization. Antibodies are also tremendously effective for opsonizing bacteria to prepare them for phagocytosis, as well as for targeted pathogen killing when combined with complement components. Vaccines that drive T-cell development are effective against both intracellular and extracellular agents and to give help to monocytes to stimulate inflammatory responses critical for destroying invading pathogens. Cytotoxic lymphocytes are necessary for targeted destruction of intracellular pathogens, such as viral agents, in which a lethal hit delivered to infected target cells is required to limit spread of infection. Adjuvants, discussed below, are essential for directing the immune response in a way that will benefit protective outcomes.

Vaccines are especially effective for use in selected populations. These include military personnel, as well as travelers to high-risk areas, who may be exposed to pathogens not typically encountered in local environments. Similarly veterinarians and animal handlers should consider

vaccination against pathogens found in the workplace. College students should be vaccinated against sexually transmitted diseases and against agents that are easily spread in high-contact areas. Obviously, health care workers and physicians should be vaccinated against pathogens transferred through accidental blood product exposure. Also, although not completely obvious, it is recommended that those in high-risk lifestyles (high HIV demographic; IV drug users), as well as those that are immunocompromised, should undertake regular review of vaccines for prophylactic protection. Finally, while not as common, therapeutic vaccines may be used to treat infected individuals with slow growing pathogens. These include vaccines which may be used as a targeted therapeutic to treat diseases such as rabies and hepatitis.

A short, but frank discussion of relative vaccine safety is warranted. Vaccines are a safe and proven mechanism to induce strong protective immunity. They can elicit minor temporary inflammation following administration, however, effects are limited in duration and quickly dissipate (2–3 days) post injection. In some cases, elevated levels of hypersensitivity can occur, and there are rare cases of arthritis and arthralgia in individuals prone to these reactions. The Food and Drug Administration set strict rules for removal of toxic products during formulation. Reports of deaths in infants or elicitation of neurological impairment are false. Of interest, an erroneous report linked infant vaccines to developmental disorders (autism); yet, data to back this report was not substantiated.

While increased hygiene is protective, vaccines are critical to keep disease at bay. **Herd immunity** is a social concept that relates vaccination of a significant portion of a population (or "herd") to a measure of protection for individuals who have not developed immunity; vaccines protect us as well as those around us due to subsequent limitation of infection spread. Multiple vaccinations work; the child's immune system is robust and cannot be overwhelmed by multiple immunizations. Indeed, a child is exposed to more antigens in daily routine activities than through all the vaccines combined during childhood. Care should be taken with live vaccines, even if the organisms are attenuated. They should not readily be given to immunocompromised patients or to those with severe immune disorders. Likewise, patients undergoing concurrent immunosuppressive therapy, or even pregnant women, should avoid being vaccinated with live organisms.

Immunologic adjuvants are excipient components added to vaccines to potentiate immune responses. In essence, they function to direct an antigenic response toward the desired immune outcome and allow the vaccine to be a more effective prophylactic candidate. In general, adjuvants are capable of assisting generation of immunity, through stabilization of the antigen delivered and direct triggering of cellular responses.

The only adjuvants approved for use in the United States are the mineral salts **(aluminum salts; alum)** and an oil-based emulsion capable of stabilizing functional delivery of the antigen. However, many novel molecules are under active research for inclusion as approved adjuvants in vaccine preparations. They include plant saponins, cytokines, bacterial cell wall products, particulates, viral-like particles, and nucleic acid motifs. Some function by stimulating antigen-presenting cells (APCs) through interaction with pathogen-associated molecular patterns (PAMPs) or Toll-like receptors. Others function to stabilize the antigen for targeted presentation by APCs to T lymphocytes.

PASSIVE IMMUNIZATION

An example of passive immunization was mentioned above when describing how the newborn is protected with maternal IgG. As a therapeutic class, passive immunization is an extremely useful clinical tool. Immunoglobulins are routinely given to patients to prevent or treat disease, and they are especially potent in immune-deficient individuals. Pooled antibodies from immune donors can be given intravenously. These **intravenous immunoglobulins** (**IVIG**) used to treat primary immune deficiencies can restore regular immune function, although continued administration is required every 3–4 weeks due to the half-life of the IgG isotype given.

Historically, antibodies raised in animals could also be administered to humans to treat infections. For example, serum raised in horses could be given to neutralize tetanus toxoid. Unfortunately, the heterologous antisera, being nonhuman in nature, were recognized as foreign when given more than once. This led to hypersensitive responses. Fortunately, science has evolved the technology to produce monoclonal antibodies in the laboratory that are homologous in physical amino acid structure (antibody of the same species) which limits

cross-reactivity to heterologous epitopes. The FDA now has a panel of approved antibody-based products for passive immunization and targeted immunotherapy, many of which have been "humanized" to include human constant regions while retaining the antigenic specificity targeted by the original immunoglobulin.

THERAPEUTIC USES OF IMMUNOGLOBULINS

The ability to utilize passive antibodies as therapeutic agents has exploded in the last decade. In addition to IVIG, monoclonal antibodies are successfully used to treat multiple autoimmune disorders. Specifically, antibodies which either neutralize inhibit binding of tumor necrosis factor (TNF) to its receptor are important therapeutic tools to fight manifestation of pathology during autoimmune responses. Likewise, monoclonals can serve a targeted role in the fight against cancers of both hematologic and solid tumor form. These directly target B-cell malignancies, breast cancer, CML (chronic myelogenous leukemia), and CLL (chronic lymphocytic leukemia), to name a few.

Another example for antibody use as a therapeutic agent is of clinical importance in a specific complication of red blood cell (RBC) surface antigen incompatibility between mother and fetus. Rh antigens, also called *Rhesus antigens*, are transmembrane proteins expressed at the surface of erythrocytes. They appear to be used for the transport of CO_2 and/or ammonia across the plasma membrane. RBCs that are Rh positive express a specific antigen designated the D type (RhD antigen). About 15% of the population have no RhD antigens and thus are "Rh negative." A Rh-negative mother who carries a Rh-positive fetus runs the risk of producing immune antibodies to the Rh antigens on the fetal RBC. The exposure during primary pregnancy is minimized. However, the mother may generate Rh antibodies after birth if the mother comes into contact with fetal blood cells during placenta rupture. Some fetal RBCs enter the mother's blood stream, thus allowing production of maternal-derived anti-Rh antibodies. Upon subsequent pregnancies, the next Rh-positive fetus will be at risk since the mother will retain a low level of circulating antibodies against the Rh antigen. Destruction of fetal erythrocytes will ensue by passive immune transfer of maternal antibodies to fetus, resulting in erythroblastosis fetalis (hemolytic disease of the newborn). It is of great clinical importance to identify Rh-mismatched mother and fetus. If there is a mismatch,

the mother is clinically treated with anti-Rh antibodies (Rh immune globulin (RhIG) or Rhogam) which react with the fetal RBC. Ensuing antibody–antigen complexes are removed prior to maternal recognition of foreign Rh antigen.

OTHER WAYS OF MODIFYING IMMUNITY

Finally, it should be noted that removal of pathogenic antibodies and other immune factors is an important immunotherapeutic option. An example again relates to a child born to a mother suffering specific autoimmunity. Maternal antibodies are passed to the child, and the child exhibits disease symptoms mimicking those in the mother. A specific case can be seen with a mother who has active Graves' disease; maternal antibodies passed to the newborn initiate relatively high activation of the thyroid gland, leading to clinical symptoms of hyperthyroidism. Successful treatment can be accomplished by plasmapheresis whereby reactive maternal antibodies are filtered from the serum of the newborn; elimination of reactive antibodies eliminates clinical presentation in the child.

SUMMARY

- Vaccines are a safe and proven mechanism to induce strong immunity. Immunization has had tremendous impact on human life quality and longevity by eliminating devastating pathogens.
- Vaccines vary to accommodate targeted immune recognition of pathogens, in advance of encounter with the infectious agent. Strategies incorporate the physical basis and nature of the antigen with stabilizing delivery vehicles. Adjuvants can be added to promote directed immune function.
- The nature of the antigen used in immunization is critical for elicitation of subsequent response. Technological advances in molecular production allow a broad range of antigens for use in vaccination.
- Passive administration of immunoglobulins or immune factors allows short-term protection in the absence of preexisting immune response.

CHAPTER 10

Cancer Immunology

Chapter Focus: To investigate natural (effective) responses to tumor development and formulate how immune components function to eliminate potentially dangerous precancerous events. This will be followed by a discussion of challenges faced when protective responses fail and tumors develop. Categories of tumor antigens will be described, related to impact on self recognition. A review of effector mechanisms in tumor immunity will be given. A section will also contain information on cancers of the immune system when the protective cells themselves become the cause of tumorigenic activity. Finally, aspects of immunodiagnosis and immunoprophylaxis will be addressed.

UNDERSTANDING IMMUNE DEFENSES AGAINST CANCERS

The field of cancer immunology investigates interactions between the immune system and tumors or malignancies. At the heart of this is the specific recognition of cancerous cells and the antigens they express. In essence, this is the concept of natural **immunosurveillance**, proposed in 1957 by Burnet and Thomas. They suggested that lymphocytes act as sentinels to recognize and eliminate continuously arising, nascent transformed cells. The stepwise immunosurveillance process ensues beginning with recognition and elimination of dysregulated cells; both innate and adaptive immune cells recognize and kill tumors at early stages during development. A balanced state of equilibrium can be assumed, representing control of cellular and tissue growth. In time, there is potential for cellular escape from naturally protective responses, resulting in tumor pathogenesis.

TUMOR ANTIGENS

At the heart of immunosurveillance is the concept that cancerous cells express tumor antigens that can be recognized by different immune cell phenotypes. Tumor antigens fall into general categories, which basically include many normal gene products that are turned on at

Introductory Immunology. DOI: http://dx.doi.org/10.1016/B978-0-12-420030-2.00010-X

inappropriate times or appear on cell types that normally do not express those particular proteins. Examples include a group of antigens produced in adult tissue that are normally only present during developmental stages. These are the **oncogenes**, or **oncofetal antigens**, that are of embryonic origin. Additionally, gene products may be expressed that function to inhibit growth suppression, allowing uncontrolled expansion of that particular cell phenotype.

Mechanisms for which mutant cellular gene products arise are varied. A somatic mutation or point mutation may occur, or a genetic rearrangement may happen. In either case, a change in protein function takes place, usually leading to alterations in normal cell function or protein structure. This can happen during cell cycle and replication, through improper DNA repair mechanisms or by action of carcinogens. However, the end result is typically unrestricted growth in a non-regulated manner.

Viral gene products may also trigger **oncogenesis**. Indeed, retroviral encoded oncogenes may subvert normal cellular control mechanisms, turning the cell host into a vehicle for continued viral expansion. Along the way, the infected cells become cancerous. Viral products can also directly influence cellular expansion, directly via introduction of point mutations or targeted host gene amplification, or by chromosomal translocation events which generate new gene products with untold consequences.

For simplicity, any overexpressed, mutated, dysregulated, or rearranged gene product expressed by a cancerous cell is considered a tumor antigen. Sometimes these are referred to as **tumor-specific transplantation antigens**. These tumor antigens play a role in differentiating tumors as being immunologically different from healthy tissue and allow the immune system to recognize cancerous cells as "non-self." A **proto-oncogene** is a normal gene that undergoes mutation to allow increased expression. It may regulate other genes, through functional activation or suppression. Activation of cellular proto-oncogenes in human cancers most often function in a way that affects cell growth. What is critical to keep in mind is that the protein is primarily a "self" protein, albeit one that is expressed with a mutation or minor change in antigenic structure. Thus, these antigens are typically very difficult for detection by typical lymphocytic activation processes that deal with foreign antigenic recognition.

A special class of tumor antigens includes those proteins that are carcinogen-induced. **Carcinogens** can induce mutations in normal genes that were previously silent, giving rise to an array of different gene products. By definition, the oncogenic and viral-induced mutations are similar between individuals, however, the carcinogen-induced mutations appear antigenically unique. As such, there is very little or no cross-reactivity in these tumor antigens between individuals due to the random mutations induced by the chemical or physical carcinogenic substance.

EFFECTOR MECHANISMS IN TUMOR IMMUNITY

Both innate and adaptive immunity play defined roles in the fight to control tumor expansion (Table 10.1). The nature of the cancer is a significant factor in how the immune system functions to combat these rapidly expanding dysregulated populations. Just as it was shown for the fight against infectious agents, the location of the pathogen was critical for understanding immune mechanisms available to mount, direct, and complete a functional immune response. The same is true for cancerous cells. Specifically, dispersed cells are far easier to target compared to solid tissue masses with little to no direct blood flow or common lymphatic drainage. But even solid tumors can be infiltrated by a broad range of immune cell types, in a productive manner to limit tumorigenesis.

Natural Killer Cells and Innate Response to Tumor Cells

Innate immune cells play a critical role in the antitumorigenic process. While natural killer (NK), natural killer T cells (NKT cells), and γδ T cells all have tumoricidal functions, the NK cells are most import for surveillance functions. NK cells are large granular lymphocytes that

Table 10.1 Immune Effector Mechanisms to Fight Tumor Cells

Effector Mechanism	Activity
NK cells	• Cytolysis, apoptosis, ADCC • Lytic activity on tumors expressing low MHC molecules
CD8 + cytotoxic T cells	• Cytolysis, apoptosis • Reject viral or chemical-induced tumors
CD4 + helper T cells	• Help to CTLs • Cytokine support for other effectors
B cells	• Antibodies and complement-mediated lysis • Antibodies for contribution to ADCC by NK cells
Macrophages/neutrophils	• Presentation of altered self-antigens • Cytokine production to support adaptive effector cells

nonspecifically kill tumor cells. NK cells express CD16 and CD56 and share many surface molecules with T lymphocytes, but do not express specific antigen receptors. Essentially, they are non-T and non-B lymphocytes that lack surface CD3, CD4, CD8, and CD19. NK cells mediate lysis of target cells by release of lytic granules and perforin-induced pore formation. In this manner, they function like CD8 + CTLs, but the NK cells are able to kill "self" in the absence of antigenic recognition. A likely method involves "recognizing" the absence of histocompatability molecules on the target cell, a common occurrence in cancerous cells. NK cells may also kill target cells using Fas/Fas-ligand (FasL)-mediated apoptotic pathways. Of note, killing by NK cells is enhanced by cytokines present in an inflammatory environment, such as IFN-α, IFN-β, and IL-12. Further activation can occur in the presence of activated T cells. Activated NK cells produce IL-2, IFN-α, IFN-γ, and TNF-α.

Another mechanism for NK cell activation utilizes a receptor for the constant portion of IgG; antibodies which recognize tumor targets are captured by the NK Fc receptor (CD16), which triggers killing of target cells using an **antibody-dependent cellular cytotoxic** (ADCC) mechanism (Figure 10.1).

Adaptive Response to Tumor Cells

Adaptive immunity plays a strong role in elimination of tumor targets, especially when innate surveillance mechanisms are delayed. These mechanisms employ the specific antigen receptors on T cells, of both the CD4 + helper subset and the CD8 + cytotoxic variety. Current thinking suggests that a T helper type 1 effector response is required for effective antitumor activity. CD4 + T cells recognize antigen presented by MHC class II molecules on an APC (antigen-presenting cell; dendritic cell, or macrophage), following which they provide help through cytokines such as IL-2 and IFN-γ to activate CD8 + T cells. It is likely that macrophages play a critical role in detecting cancer cell debris, phagocytosing cellular membranes followed by localized draining via lymphatics to regional lymph nodes. Helper T cells mature and provide productive assistance to CD8 + cells, which in turn recognize altered tumor transplantation antigens presented on target cells in the context of MHC class I histocompatability molecules. Cytotoxic lymphocytes function to kill their targets using perforin, granzymes, and destructive cytokines (TNF-β, IFN-γ). Recent evidence also suggests a

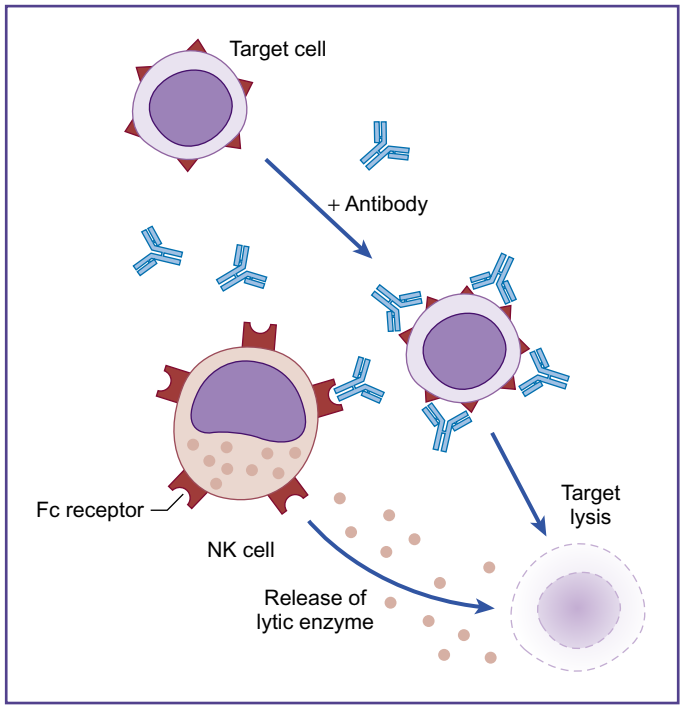


Figure 10.1 ***ADCC.*** *ADCC is a phenomenon in which target cells coated with antibody are destroyed by specialized killer cells, such as NK cells. The killing cells express receptors for the Fc portion of antibodies that recognize tumor antigens resulting in release of lytic enzymes at the site of contact. Target cell killing may also involve perforin-mediated membrane damage.*

role for Fas and FasL in the specific lymphocytic process to control tumor expansion.

Antibodies play an adaptive role toward tumors. The cell-dependent mechanism involving antibodies and NK cells was mentioned above. However, it is critical to also know that antibody and complement are another powerful tool to limit tumor cell expansion. As with all manners of antibody recognition, this is dependent upon the altered surface antigenic structures present on cancer cells; the greater the antigenic modification, the more likely that antibody-based mechanisms will be successful at limiting tumorigenesis.

ESCAPE MECHANISMS OF TUMOR ELIMINATION

The survival pressure for the expanding tumor cell population is high, and tumor-related "escape" from immune surveillance is common.

Multiple mechanisms are the result of changes to the tumor itself, while other escape methods rely on alterations in host immune function. Regarding the tumor population, there are many instances where cancer cells demonstrate modulation of the expression of key target molecules, thus contributing to immune avoidance. This includes reduction of MHC molecule expression, change to antigenic nature of the tumor-associated surface antigens, and shedding of tumor antigens so that the cell is no longer recognized as an "altered self" cell.

Often a deficiency in the host immune response allows escape of tumor cells and resistance to attack. It is quite common for the tumor microenvironment to become infiltrated by immune suppressive cells, including T regulatory cells that downregulate functional responses. Additional cell phenotypes can be involved in the escape phenomenon. Myeloid-derived suppressor cells (MDSCs) represent a heterogeneous population that comprises myeloid progenitor cells and immature macrophages, immature granulocytes, and immature dendritic cells. Some of these phenotypes are quite adept at modulating tumor-associated macrophages, skewing their ability to present tumor antigens to incoming helper and cytotoxic T lymphocytes. Finally, it should be noted that influences that affect systemic immunity in the host, such as infection or general immunodeficiency, may also contribute to impairment of natural immunosurveillance function.

TUMORS OF THE IMMUNE SYSTEM

Problems also arise when the protective cells themselves become the cause of tumorigenic activity. Indeed, immune system cells are not "immune" from the dysregulated responses discussed above. Although varied, the cancers generally fall into two classes: myeloid-based or lymphoid-based **leukemias**, representing cancers of the white blood cell leukocytes. A partial list of common leukemias is discussed below.

Multiple myeloma is a cancer of plasma cells, a type of activated B-cell phenotypic for producing antibodies. In multiple myeloma, abnormal plasma cells will characteristically accumulate in the bone marrow, interfere with the production of normal blood cells, and damage parenchymal tissue beds. Most cases feature a diagnostic overproduction of a single type of immunoglobulin protein readily found in circulation (called M-protein). **Burkitt's lymphoma** is another example of a B-cell

cancer in which tumor cells from the patient possess identical immunoglobulin heavy chain gene rearrangements. **B-cell chronic lymphocytic leukemia**, also known as **chronic lymphoid leukemia** (or CLL), is another common type of small lymphocytic lymphoma which can be distinguished based on maturity of the immunoglobulin variable-region heavy chain (IgVH) gene mutation status.

Hodgkin's lymphoma is a type of lymphoma also originating from white blood cell leukocytes. Hodgkin's lymphoma is characterized by spread from one lymph node group to another. Microscopic examination reveals characteristic histology with the presence of multinucleated Reed–Sternberg cells. Different pathologic subtypes are possible, based on cell morphology and composition of infiltrate seen within lymph nodes. The Hodkin's lymphomas may be characterized as distinct from the **non-Hodgkin lymphomas**, NHL, which represent a diverse group of blood cancers that expand the definition of cancerous lymphomas. Types of NHL vary significantly dependent upon the underlying cause (e.g., viral, genetic, iatrogenic). T-cell lymphomas generally fall into this category, usually associated with viral etiology.

Chronic myelogenous leukemia (CML) represent another type of leukocyte cancer characterized by unregulated growth of predominantly myeloid cells in the bone marrow and subsequent accumulation of these cells in the blood. CML may be considered a clonal bone marrow stem cell disorder. This cancer represents a myeloproliferative disease with expansion of mature granulocytes of neutrophil, eosinophil, and/or basophil origin. CML is associated with a characteristic chromosomal translocation called the Philadelphia chromosome. A chromosomal fusion event occurs between the Abelson (Abl) tyrosine kinase gene at chromosome 9 and break point cluster (Bcr) gene at chromosome 22. A chimeric oncogene results (called Bcr-Abl) with a resulting product implicated in disease pathogenesis. **Chronic neutrophilic leukemia** is another chronic myeloproliferative neoplasm, albeit much less common. This leukemia represents myeloid hyperplasia in bone marrow, although there is an absence of the chromosome rearrangement found in the CML.

IMMUNODIAGNOSIS AND IMMUNOTHERAPY

The use of antibodies targeted toward specific antigens is a powerful tool to histologically detect and identify tumors within tissue.

Immunohistochemistry is a laboratory technique where tissue sections can be screened for presence of tumor antigens, allowing for diagnostic identification of cancer cell phenotype on surgical specimens. The ability to accurately detect and classify the type of cancer has great potential for subsequent therapeutic intervention. Likewise, detection of specific tumor antigens on phenotypic cells allows accurate prediction of tumor aggressiveness, as well as its potential for responsiveness to therapeutic intervention.

Significant advances in our understanding of tumor immunology have led to a multitude of therapies based on manipulation of the immune system (Figure 10.2). Specifically, understanding of which arm of the immune response is responsible for rejection and destruction of tumors has led to the development of incredibly powerful clinical tools. For example, BCG immunotherapy is commonly used for treatment of early stage bladder cancer; stimulation of innate responses allows local environments to respond in a positive manner to limit tumor growth. Likewise, Imiquimod, a topical therapeutic which

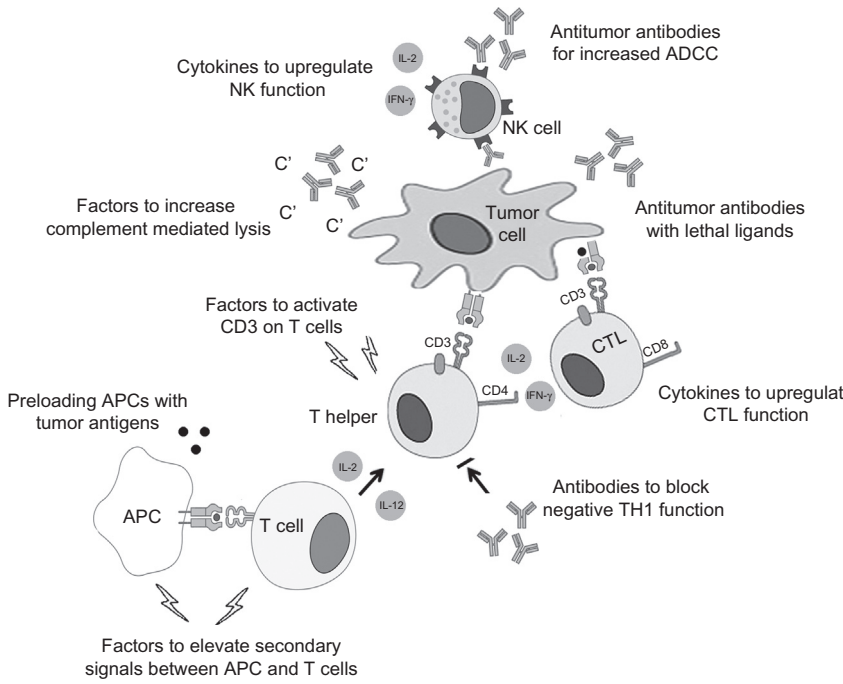


Figure 10.2 ***Therapeutic interventions to boost anticancer immune function.***

supplements local production of IFN-γ, can be advantageous when accompanying radiation therapy or chemotherapy. Future therapeutics will stimulate innate responses, as exemplified in animal studies where addition of nucleotide analogs stimulates Toll-like receptors (TLR7) to augment cellular responses. Another example is the use of monoclonal antibodies, which are quite effective to target and destroy tumors.

SUMMARY

- Tumor cells differ from normal counterparts by indefinite proliferation and changes in growth regulation.
- Normal cells can be transformed by chemical and physical carcinogens, or by transforming viruses. They typically express tumor-specific antigens on their cell surface.
- Innate immune responses to tumors include NK cell killing, ADCC, and macrophage-mediated cell killing. Adaptive immunity requires activation using specific receptors of both CD4+ helper and CD8+ cytotoxic lymphocytes. The CTL-mediated cell lysis is a particularly powerful and specific mechanism to control tumor growth.
- Some tumors cells utilize immune response evading mechanisms.
- Cancer immune therapy includes monoclonal antibodies, antibodies coupled with toxins, chemotherapeutic agents, or radioactive elements.

CHAPTER 11

Transplantation Immunology

Chapter Focus: To examine immune regulation of transfer, or grafting, of tissues from one person to another. Transplanted organs have the potential to be rejected by the host's immune system unless the recipient is either tolerant or immunosuppressed. Concepts associated with mechanisms underlying the immunobiology of transplantation will be discussed. The goals are to present genetic relationships between individuals that are critical for transplantation and to categorize immune-mediated events between donor and host post-transplant. Mechanisms will be defined, with details on the contributing cells and factors involved in transplant acceptance vs. rejection. Rejection topics will be discussed, including graft-versus-host disease (GVHD). Finally, classes of immunosuppressive agents will be presented to assess therapeutic intervention as a way to control immune features that affect graft acceptance and rejection.

TRANSPLANTATION DEFINED

The concept of organ replacement has become an important part of modern medical therapy. It has been known experimentally that skin can be transferred to different sites on the same person, with great success. This is referred to as an **autograft**; all molecules are identical within the individual and the syngeneic tissue is recognized as "self." However, tissues transferred between nonrelated individuals (**allograft**) are not readily tolerated; their cellular components are recognized as foreign antigens. Immune responses are initiated within the recipient to eliminate the foreign tissue. Likewise, tissues from nonrelated species (**xenograft** or **heterograft**) share a similar fate and are rapidly rejected unless a high degree of immunosuppression is present. The rules that govern graft acceptance and rejection, and the immunological basis of successful graft acceptance, are well defined (Figure 11.1).

Introductory Immunology. DOI: http://dx.doi.org/10.1016/B978-0-12-420030-2.00011-1

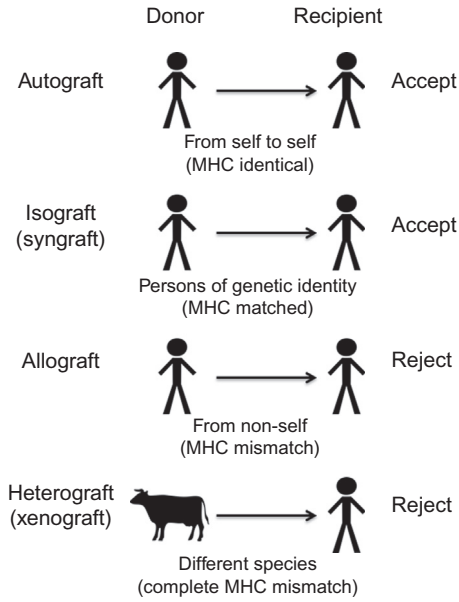


Figure 11.1 ***Transplantation acceptance as function of recipient and donor genetic similarity.*** *Tissue transplantation is governed by immunological rules that allow graft acceptance according to degree of genetic relatedness between recipient and donor.*

TISSUE HISTOCOMPATIBILITY

The basic architecture of tissues between individuals is quite similar. Indeed, a kidney is a kidney; the liver functions in a similar manner from one person to another. Unfortunately, significant differences in molecules present on the surface of cells exist between genetically different individuals. These discrepancies must be taken into consideration during transfer of blood, cells, and tissue. Specifically, serum must be matched to limit interactions with naturally occurring reactive antibodies, blood cells must be matched for carbohydrate markers on their surface, and solid tissues must be matched for overall genetic histocompatibility.

Natural Isohemagglutinins

A subpopulation of IgM isotype antibodies includes the **natural isohemagglutinins**, which are reactive with the red blood cell molecules of the ABO series. The **ABO blood group** epitopes are carbohydrates in nature; antibodies elicited by environmental (bacterial) carbohydrate motifs cross-react with human A or B blood group antigens on red blood cells. It is therefore critical to match the ABO blood types when

Table 11.1 Natural Isohemagglutinins		
Blood Type	**Antigen Present on RBCs**	**Isohemagglutinin Reactivity**
A	A	Anti-B
B	B	Anti-A
AB	A and B	None
O	None	Anti-A and Anti-B

giving whole blood or serum. Table 11.1 gives the reactivity of isohemagglutinin antibodies normally found in patients with the various blood groups.

In addition to the ABO antigenic category, an antigen called the Rh factor (rhesus factor) is also present on blood cells. The Rh factors must be matched; Rh negative blood is only given to Rh negative patients. A Rh negative individual will make antibodies to the Rh factor if Rh positive blood is given. If antibodies are present to the Rh factor, they will cause agglutination of the donated blood cell. Rh positive blood or Rh negative blood may be given to Rh positive patients, as those individuals do not make antibodies to molecules they already possess on their own cells.

Human Leukocyte Antigens

The major histocompatibility complex (MHC) antigens are the strongest indicator for inducing allograft rejections. These are the **human leukocyte antigens** (HLA) discussed previously that allow T cells to recognize presented antigen as a first step in activation events. Relative to immune function, the class I HLA molecules (HLA-A, HLA-B, HLA-C) are found on all nucleated cells and mediate recognition of endogenous antigen by CD8+ cytotoxic T cells. The class II HLA molecules (HLA-DR, HLA-DP, HLA-DR) are on the surface of professional antigen-presenting cells (APCs), and show exogenous antigen to CD4+ T helper subsets. Subsets of these molecules are inherited from both parents, allowing unique patterns to be expressed in their offspring. The nature of these molecules includes a high degree of polymorphism, which essentially creates high differences between individuals. As a group, these sets of HLA surface molecules are referred to as **alloantigens**. During transplantation, the histocompatibility alloantigens expressed on donor tissue are recognized by both CD4+ T helper and CD8+ cytotoxic lymphocytes present in the

recipient host. The greater disparity between the host and the donor, the greater the lymphocytic reactivity and chance for subsequent tissue rejection. **Minor histocompatibility antigens**, as well as tissue specific differences between the host and the donor, may also contribute to graft rejection.

Tissues transplanted to **immunoprivileged** sites do not typically require MHC matching. For example, corneal transplants do not routinely require HLA matching. The fetus is another example of "tolerated" nonmatched tissue. Although there are common antigens between mother and child, there are also numerous paternal-derived moieties. Factors that allow tolerance include downregulation of MHC on the developing fetus and change in environmental cytokines or factors produced by both the mother and the fetus.

ALLOGRAFT REJECTION

Allograft rejection involves a series of humoral and cellular responses (Figure 11.2). The immune response involved in allograft rejection spans a wide variety of defined mechanisms. Preformed antibodies can bind to donor tissue, establishing a nidus for direct killing via complement deposition. Antibodies can also function in concert with natural killer cells in an antibody-dependent cell cytotoxic manner to lyse nonmatched target tissue. CD4+ cells recognize class II MHC molecules on the donor tissue (HLA-DP, HLA-DQ, HLA-DR), and are induced to secrete IL-2, IFN-γ, and TNF-α. These in turn activate CD8+ cells, natural killer cells, and incoming macrophages. The Th1 CD4+ cells also give signals to activate the Th2 CD4+ group to secrete cytokines IL-4, IL-5, and IL-10, which can induce B cells to undergo activation and immunoglobulin production, as well as isotype class switching.

The mechanisms involved permit the establishment of rejection categories. These include characterization of rejection as being hyperacute, accelerated, acute, and chronic.

Hyperacute Rejection

Hyperacute rejection occurs within minutes of transplantation in individuals who are MHC mismatched, or in individuals preexposed to the donor's MHC types by prior grafting or blood transfusion. The end

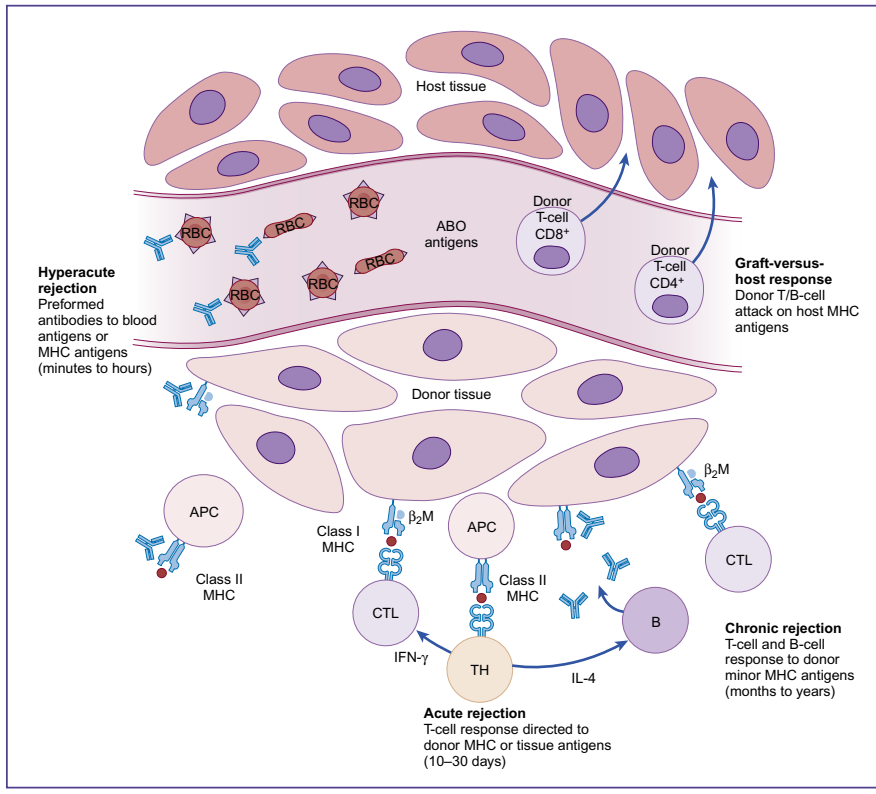


Figure 11.2 ***Allograft rejection.*** *Immune-mediated tissue rejection is characterized by the response speed toward donor tissue, which is directly related to immune mechanisms involved in the rejection process.*

result is graft tissue loss in a nonreversible manner. The basis relates to preexisting antibodies reactive with the mismatch. Natural IgM antibodies are present to cross-reactive epitopes on pathogens which mimic the carbohydrates within components on nonmatched blood cells. These antibodies immediately recognize the foreign tissue and activate complement, which in turn releases factors attracting and activating neutrophils.

Accelerated Rejection

Accelerated rejection, also called "second set" rejection, is relatively rare but occurs with multiple transplants from genetically related donors. Recipients that have rejected a previous allograft tend to reject a second allograft from the same donor significantly faster.

Acute Rejection

Acute rejection, also called "first set" rejection, occurs between 10 and 30 days' post-grafting in untreated recipients. This is the expected time for reactive T-cell populations to expand and react. Both T helper and T cytotoxic cells are usually required, although the direct cytotoxic event is delivered by CD8+ CTLs. Reactions usually occur later in immunosuppressed recipients, depending on level of immunosuppression success.

Chronic Rejection

Chronic rejection occurs over months to years post-transplant. It is a complex reaction involving maturation of both T and B lymphocyte responses. Antibodies are directed at the foreign (non-self) antigens within the graft. Subsequent deposition of antibody–antigen complexes leads to targeted destruction of graft tissue and indirect damage to vascular beds. Chronic rejection leads to permanent damage that is difficult (if impossible) to reverse with immumosuppressants.

The molecular mediators involved in graft rejection are depicted in Figure 11.3. It is relatively straightforward to envision the acute and chronic mechanisms discussed as being a direct recognition by the host T cell to a combination of foreign MHC and foreign antigens. However, keep in mind that it is also possible for alloantigens to be presented by host APCs. When host cells pick up pieces of the donor tissue and present donor-derived peptides, they can also be targeted for destruction. Indirect recognition of host lymphocytes may therefore contribute to destruction of self-tissue that is physically near the grafted organ.

GRAFT-VERSUS-HOST DISEASE

GVHD occurs when immunocompetent lymphocytes from the donor tissue are inadvertently delivered to the host during the transplantation process. The recipient host, who is immunosuppressed at the time of transplantation, does not reject the alloreactive cells. Over time, these infiltrating donor cells expand, culminating in a pool of donor cells reactive to host tissue. GVHD can occur when there is a mismatch of HLA (class I or class II), or if there are a significant number of differences in minor histocompatibility antigens, such as that seen in closely matched siblings. A common GVHD occurrence is post-bone marrow

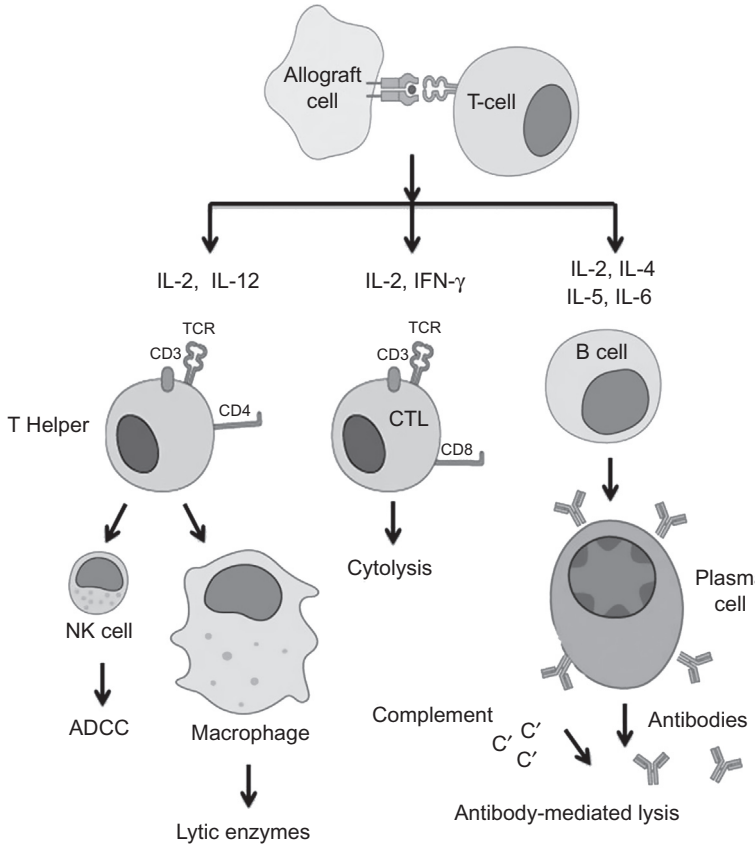


Figure 11.3 ***Allograft rejection is mediated by cellular and molecular mediators.*** *The specific molecular mediators of allograft graft rejection are a function of the responding immune cell phenotypes involved in tissue recognition.*

transplantation, where alloreactive hematopoetic stem cells are delivered as a form of gene therapy. These pluripotent stem cells give rise to all phenotypes of white blood cells; however, the pool of transplanted cells often contains mature lymphocytes that are capable of recognizing differences in HLA between the donor and host. Stringency of T-cell depletion prior to transplantation reduces this occurrence.

PRE-TRANSPLANTATION HISTOCOMPATIBILITY EVALUATION

Multiple laboratory methods are used to evaluate tissue histocompatibility between donor and recipient. Methods allow matching of tissues between individuals, with a higher degree of graft survival directly

related to level of similarity. Any donor-recipient HLA incompatibility can result in an immune response, rejection, and possible graft loss. And while immunosuppressants may obviate the impact of HLA matching for both short- and long-term graft outcome, it is preferred to limit mismatch prior to transplantation.

Any potential donor must undergo extensive screening prior to transplantation. This begins with a test for host antibody reactivity to donor target cells. In essence, this simple test can identify reactive anti-donor antibodies by examining the level of cytotoxicity and lymphocytotoxicity of these antibodies to lyse cells in the presence of complement components. The purpose of the cross-match is to detect clinically relevant IgG anti-donor antibodies to prevent hyperacute, accelerated, or chronic rejection. The next level of tests examines cellular reactivity, accomplished using host and donor T cells in a **mixed lymphocyte reaction** to assess direct reactivity to allogeneic MHC between individuals. Basically, if T cells are reactive to allogeneic molecules, they will undergo rapid replication and produce diagnostic secretion of cytokine subsets.

Recent technological advances now permit identification of haplotype distinctions between individuals without cell culture methods. These methods are especially useful when comparing relationships of parents and siblings to the recipient. One particular method, called HLA-DNA (also called PCR typing), uses polymerase chain reaction (PCR) and sequence-specific oligonucleotide probes to rapidly identify DNA-genomic subtypes. DNA primers can be used which are specific for individual or similar groups of HLA alleles, allowing amplification of relevant genomic DNA. This type of screening is especially useful when donor tissue is of cadaveric (deceased) origin.

IMMUNOSUPPRESSIVE DRUGS TO PREVENT ALLOGRAFT REJECTION

At the present time, there is no successful clinical protocol to induce complete tolerance to allografts. All patients require daily, lifelong treatment with immunosuppressive agents to inhibit graft rejection. All immunosuppressive agents used in clinical practice have drawbacks relating to toxicity and side effects, or to failure to provide sufficient levels of downregulated lymphocytic response. On one hand,

inadequate immunosuppression allows the recipient to mount an immune response, causing allograft rejection. On the other hand, excessive immunosuppression can lead to development of opportunistic infections and neoplasia.

Immunosuppressive Therapy

Immunosuppressive agents are often used to control reactions prior to graft rejection. These agents fall into categories dependent on targeted function and immune modification desired (Table 11.2). Prior to transplantation, agents are given to the recipient at a relatively high level to quiet immune reactivity, allowing for greater success and acceptance of tissue immediately post-transplantation. After the tissue has been placed within the host, the major concern revolves around targeting the immune system in a manner which prevents reactivity. Maintenance therapy is usually given at a low level to keep the immune system operational but quiet, without completely shutting down reactivity to opportunistic infections. It is only when clinical symptoms arise indicating initiation of active rejection that specific and aggressive immune suppressants are used. In this case, targeted therapeutics are administered to support mechanisms which disrupt immune events and even kill rapidly expanding lymphocytes that demonstrate reactivity to the donor organ.

Table 11.2 Immunosuppressant Drugs and Therapeutics

Class	Mechanism of Action	Example
Corticosteroids	Blocks multiple cytokine expression	• Prednisone • Prednisolone • Methylprednisolone
Cytotoxic agents	Blocks DNA synthesis or replication in proliferating cells; can suppress APC processing	• Azathioprine (AZA) • Cyclophosphamide • Hydroxychloroquine
Immunophilin ligand	Blocks T-cell activation and gene transcription	• Cyclosporine (CsA) • Tacrolimus (FK506)
Proliferation signal inhibitors	Blocks intracellular kinases	• Sirolimus (SRL) • Mycophenolate mofetil (MM)
Immunosuppressive antibodies	Specific targeting of adaptive cellular functions	• Anti-lymphocyte globulin • Anti-thymocyte globulin • Anti-CD3 MAb (OKT3) • Rh(D) immune globulin • Etanercept (anti-TNFα/β) • Daclizumab (anti-IL2R)

Immunosuppressive therapy has had a significant impact on both the prevention and treatment of rejection. Yet, suppressing the immune response has consequences, such as increased risk of infections and certain types of malignancies. While steroids remain an important immunosuppressive clinical tool, recent advances in protocol development limit their use to minimize known side effects. Other therapeutics such as Tacrolimolus are effective; however, a major concern remains surrounding nephrotoxicity. Targeted agents, including polyclonal and monoclonal antibodies, are becoming increasingly useful in the arsenal against rejection of transplanted tissue. However, they often leave the recipient highly susceptible to infection, which remains a major cause of mortality post-transplantation.

SUMMARY

- The immunological rules for transplant acceptance or rejection are governed by recipient responses to histocompatibility molecules on donor cells.
- Allograft reactivity, and the speed of rejection, is governed by cell phenotypes and molecules involved in reactivity to donor histocompatibility antigens.
- GVHD represents a state where immune competent cells from the donor tissue escape initial destruction and lead to subsequent reactivity against recipient tissues.
- Modern laboratory techniques can use genetic sequences to identify potential histocompatibility mismatches. Therapeutics have evolved to specifically target immune responses detrimental to graft acceptance.

CHAPTER 12

Assessment of Immune Parameters and Immunodiagnostics

Chapter Focus: To provide an overview of *in vitro* antibody binding to antigen that allows for the development of successful immunoassays. Information presented will compare and contrast past and current methods of immune detection. There will also be a discussion of commonly used assays to determine cell function in the clinical setting and an introduction to concepts behind large-scale data collection and analyses used in the laboratory.

ANTIBODY–ANTIGEN REACTIONS

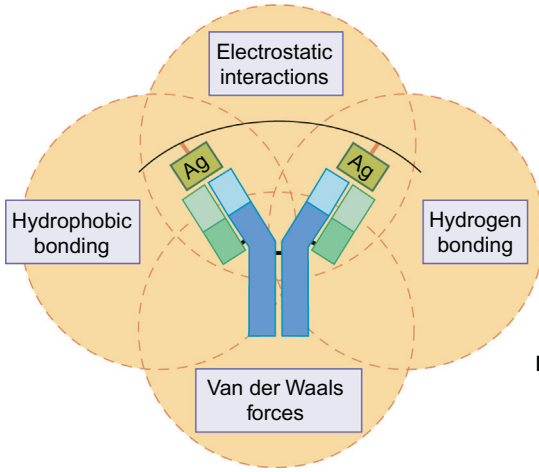
The unique structural characteristics of antibodies (or immunoglobulins) can be exploited for use in the laboratory. Advances in molecular design related to monoclonal antibody engineering, protein biochemistry, and recombinant DNA technology have allowed continued refinement of application for use in clinical practice and in cutting-edge experimental research. The requirements for antibody–antigen reactions are discussed below.

Affinity

The reaction of antigen with its homologous antibody, and the subsequent physical manifestation of that binding, is a two-stage phenomenon. The initial or primary binding reaction between the complementarity-determining region (CDR) domains on the Fab portion of the **antibody** (**Ab**) and the **antigen** (**Ag**) occurs fairly rapidly and invisibly.

The interaction of the CDR of the antibody with its cognate antigen is not a covalent interaction. Instead, binding is mediated by van der Waals forces, electrostatic interactions, and hydrophobic interactions (Figure 12.1). Hence, Ab–Ag binding interactions are analogous to those observed in enzyme–substrate reactions and can be defined similarly through physical laws of mass action. The rate at which the antibody forms the Ab–Ag complex is referred to as its **affinity**, or strength of binding, while the dissociation constant represents the

Introductory Immunology. DOI: http://dx.doi.org/10.1016/B978-0-12-420030-2.00019-6



Major antigen (Ag) classes

Carbohydrates
Polysaccharides
Proteins
Glycoproteins
Nucleic acids
Lipids

$$K = \frac{k_{association}}{k_{dissociation}} = \frac{[Ab–Ag]}{[Ab]–[Ag]}$$

Requirements for immunogenicity

Physiochemical complexity
Molecular weight > 6 kDa
Foreignness (non-self)
Degradability

Figure 12.1 ***Forces contributing to antibody–antigen interactions.*** *The interactions between antibody and antigen follow the laws of mass action and can be described in a representative equation.* K *represents the equilibrium constant;* $k_{association}$ *is the association constant,* $k_{dissociation}$ *is the dissociation constant, [Ab] represents the free antibody concentration, [Ag] represents the free antigen concentration, and [Ab–Ag] represents complexed antibody with antigen.*

stability of the Ag–Ab complex. Both effective binding and stability contribute to the strength of an Ag–Ab interaction. Hence, an antibody with a low affinity but a higher dissociation constant is more effective at binding antigen than an antibody with a very high affinity constant but a smaller dissociation constant. Strength of binding is also influenced by antibody isotype. IgG antibodies typically have a higher affinity for antigen than IgM antibodies. However, the pentameric form of IgM allows it to bind multiple antigens (5–10 paratopes in IgM vs. 2 paratopes in IgG). Thus, IgM has a higher **functional affinity** (**avidity**) than IgG (Figure 12.2).

SECONDARY MANIFESTATIONS OF ANTIBODY–ANTIGEN BINDING

Cross-linking of Ag and Ab is dependent on several factors, including the isotype and specificity of the antibody, and the size and epitopes (unique conformations) contained within the antigen. This reaction is affected by the number of binding sites available for each Ab and the maximum number of binding sites on an Ag or particle. This concept is defined as the **valence** of the antigen or antibody; the valence of Ab and Ag has to be ≥2 for precipitation to occur. It is important to note

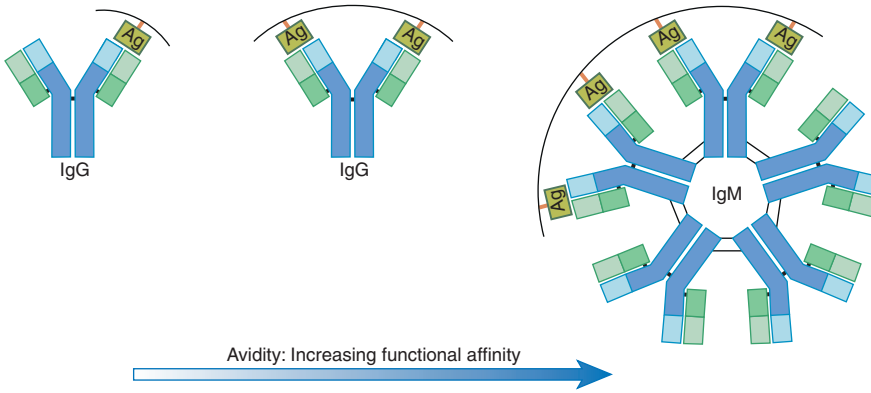


Figure 12.2 ***Multivalent interactions of antibody–antigen binding.*** *Avidity represents functional antibody binding to the antigen, increasing as more epitopes are bound.*

that steric considerations limit the number of distinct Ab molecules that can bind to a single antigen at any one time.

The isotype of antibodies, determined by the expression of specific heavy chains (**discussed in Chapter 3**) plays an important role in cross-linking complex antigens. The flexible hinge region of IgG, for example, allows this antibody to simultaneously bind two identical antigens. The hinge region of IgM is not as flexible as IgG, yet its pentameric form allows it to bind multiple antigens at once. The number of antibody binding sites, or epitopes, on an antigen affects cross-linking as well, as does the physical properties of the antibody contact points (called the **paratope**) that interact with the epitope. As shown in Figure 12.3, the presence of multiple epitopes recognized by the same antibody (**unideterminant**) or multiple epitopes recognized by different antibodies (**multi-determinant**) on an antigen increases the possibility of cross-linking and precipitate the particle. In contrast, antibody binding to very small antigens, called **haptens**, cannot cross-link. Finally, Ag–Ab cross-linking is more likely to occur if the antibody in solution is **polyclonal**, i.e., it is comprised of multiple immunoglobulins with many different antigen specificities. Of note, normal human serum samples contain polyclonal antibodies with many different specificities; therefore, agglutination and precipitation reactions occur *in vivo* naturally.

In both agglutination and precipitation reactions, various amounts of soluble antigen are added to a fixed amount of serum containing

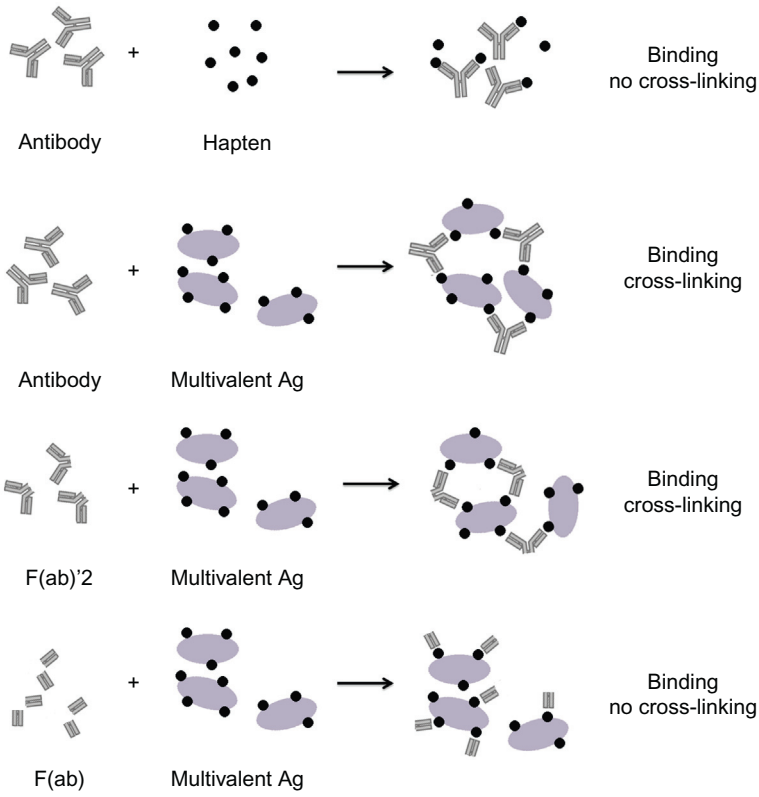


Figure 12.3 ***The Coombs' reaction.*** *The Coombs' reaction dictates the agglutination process. Multivalent antigens are easily precipitated with polyclonal antibodies, but not when monoclonals are used. As each antibody binding complexity is reduced, the ability to form precipitates depends on the complexity of the antigenic structure.*

antibody. As illustrated in Figure 12.4, when small amounts of Ag are added, Ab−Ag complexes are formed with excess Ab, and each molecule of Ag is bound by Ab and cross-linked to other Ab molecules. No cross-linking can occur due to this **"prozone" effect**. When enough Ag is added, all of the Ab and Ag complexes fall out as precipitate (**zone of equivalence**). When an excess of Ag is added, only small Ag−Ab complexes form (no cross-linking) and the precipitate is reduced. The highest dilution of serum that induces agglutination but beyond which no agglutination occurs is called the **titer**; this measurement is often used to compare the relative amount of Ab binding Ag in clinical samples.

Agglutination reactions occur when polyclonal antibody binds to multi-determinant particulate (insoluble) antigens. A very good example

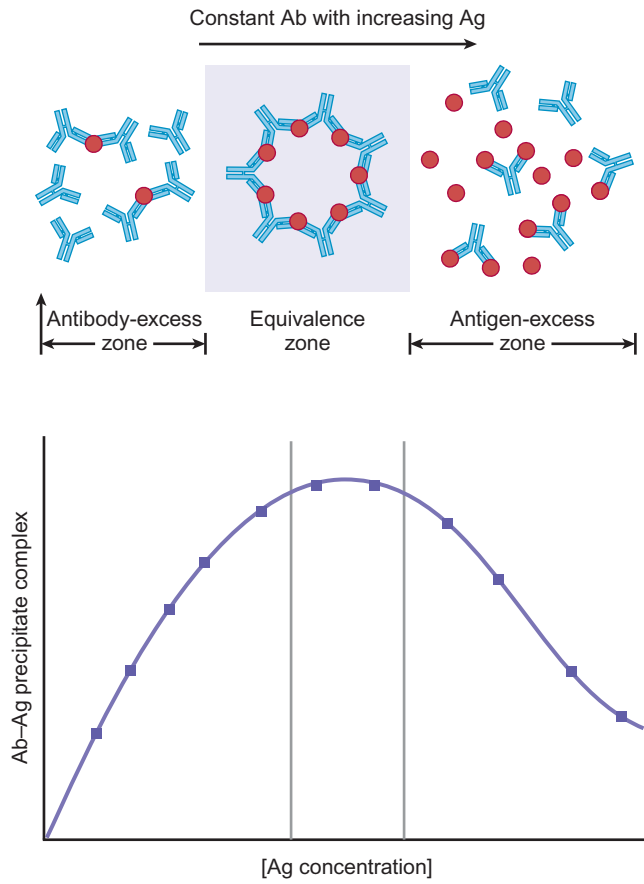


Figure 12.4 ***Physical interactions dictate antibody–antigen interactions.*** *The amount of precipitate formed between antibody and antigen is a result of relative concentrations of excess vs. equivalence.*

of a commonly used agglutination assay is red blood cell typing. Red blood cells express ABO antigens, which can be recognized by IgM antibodies that are very efficient at cross-linking. Therefore, serum from a person who expresses the O phenotype will contain antibodies specific for A and B blood group antigens. Incubation of either type A or type B blood with this serum will result in binding of the endogenous IgM antibodies to the red blood cells and agglutination, or clumping due to Ab–Ag cross-linking will occur. Conversely, type O blood cells will not be recognized by serum from type A or type B donors. Hence, type O blood is a "universal donor" phenotype, while patients with AB phenotype are "universal acceptors."

Due to the presence of sialic acid on the membrane, RBC can possess a net negative charge. In solution, this charge, called the **zeta potential**, can prevent cross-linking. This is best illustrated by the inability of IgG to cross-link RBC, while the larger structure and multiple binding sites of the IgM antibody can overcome this potential. In some cases, however, it is necessary to detect IgG binding to RBC. The **Coombs' reaction** is a widely used agglutination reaction, useful for detection of maternal IgG antibodies directed against Rh+ antigens found on the fetal RBC.

In the **precipitation reaction**, Ab binds to soluble Ag. Cross-linking of multivalent Ag by divalent Ab forms a lattice structure linking the smaller Ag complexes together. When the lattice grows to a large enough size, it loses solubility and precipitates out of the solution. Precipitation reactions are governed by the same rules as agglutination reactions; lattice formation will only occur in the zone of equivalence and will be inhibited either by Ab excess or Ag excess. Soluble precipitation reactions are performed by adding various concentrations of Ab to a constant concentration of Ag (or the reverse) and performing serial dilutions to determine the titer. Hence, these assays may be considered semiquantitative, as they can determine the relative quantity of Ab in one sample vs. another.

Nephelometry is a widely used method for accurately measuring precipitated quantities of immunoglobulin classes in serum. In this assay, proteins in the sample react with specific antibody to form particulates. As light passes through the aggregated suspension, a portion of the light is scattered and measured for comparison against stored standards. Thus, this is a quantitative method using liquid-phase precipitation principles.

SOLID-PHASE PRECIPITATION ASSAYS

To generate a simple yes/no answer to determine if specific Ag or Ab is present in a sample, it can be helpful to slow down the rate of diffusion in a gel matrix and immobilize precipitates for subsequent visualization either directly or with the aid of various staining methods. Several qualitative and quantitative methods are in wide use in medicine today for analysis of numerous hormones, enzymes, toxins, and for analysis of the products of the immune system itself.

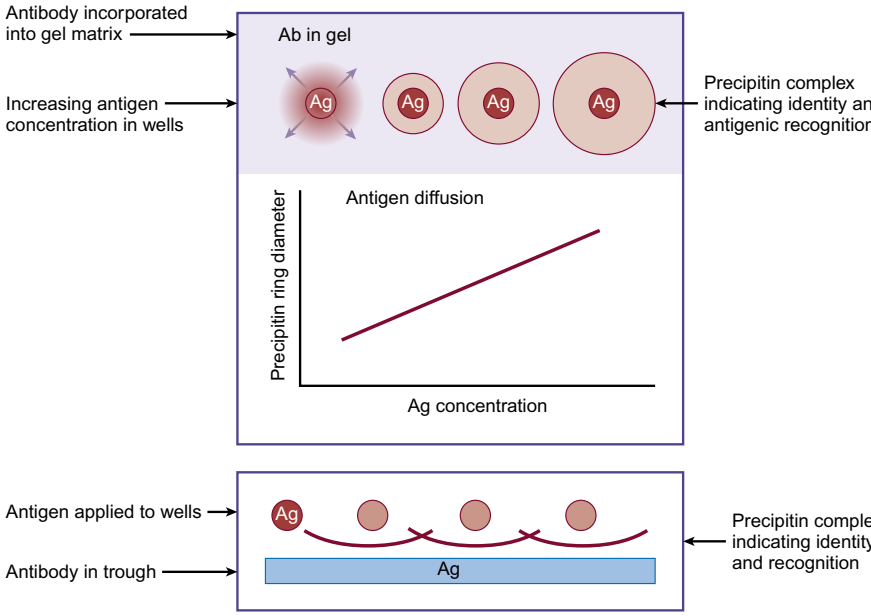


Figure 12.5 ***Diffusion assays.*** *The radial diffusion assay (top) is characterized by precipitin reactions occurring as applied antigen diffuses through a gel matrix containing reactive antibodies. The diameter of the precipitin ring formed can be quantitated by comparison to known standard antigen concentrations. A variant on the radial diffusion assay (bottom) allows qualitative determination as antibody and antigen diffuse toward each other through the assay matrix.*

Radial Immunodiffusion

In this reaction, a known antibody or an antigen is infused into the gel matrix. A test sample is placed in the center of the gel. As the unknown sample diffuses into the surrounding agar, a precipitation reaction will occur if there is a positive Ab–Ag interaction (Figure 12.5). Since precipitation happens only at the zone of equivalence, a ring will form some distance away from the high concentration of antigen at the center. The radial immunodiffusion can be a quantitative assay if the diameter of rings formed from various known quantities of antigen is used to generate a standard comparison curve.

Ouchterlony Double Diffusion Assay

The Ouchterlony Assay, developed by Orjan Ouchterlony in the 1950s, allows comparison to determine the relatedness of two antigens. The assay is called a **double diffusion** assay because both the antigen and antibodies are diffusing. It is a qualitative assay—a reaction occurs, or it doesn't. Antibodies and antigens are placed in separate, but close wells.

The molecules then diffuse slowly into the agar in a radial fashion, toward each other. A positive thin opaque line will form in the agar at right angles to a line connecting the centers of the two wells if precipitation occurs.

Immunoelectrophoresis

Immunoelectrophoresis is a variation of the Ouchterlony double diffusion in gel technique, designed to analyze complex protein mixtures containing different antigens. Electrophoresis first separates proteins according to their size and mobility in the electric field within a gel matrix. A mix of antibodies specific for the proteins is then added to a trough cut in the agar. The individual proteins and their specific antibodies will diffuse toward one another, and lines of precipitate form representing interaction. The medical diagnostic use is of value where certain proteins are suspected of being absent (e.g., hypogammaglobulinemia) or overproduced (e.g., multiple myeloma).

Western Blot

The mechanisms that underlie immunoelectrophoresis form the basis for the more commonly used western blot (also called immunoblot). A mixture of antigens is first separated by size via electrophoresis on a gel after which the proteins are transferred onto a solid matrix such as nitrocellulose that tightly binds proteins. Patient serum containing antibodies suspected of binding to the antigen can then be added, followed by antihuman detection antibodies that have an enzyme covalently attached. Substrate for the enzyme is added, turns colors when enzyme is present, and the colored line determines detection of antigen if it is present (Figure 12.6). Importantly, since western blotting separates proteins by size before their detection by antibody, it is possible to identify several specific antigens in one sample. A good example of this is the test used to detect reactivity to HIV—serum from patients who are infected with the virus usually contains antibodies that bind to multiple proteins (GAG, POL, and ENV) resulting in the visualization of multiple reactive lines.

IMMUNOASSAYS

The methods discussed thus far in this chapter rely on the physical manifestation of binding of polyclonal Abs to multivalent, multideterminant Ags. As a general rule, immunoassays utilize methods

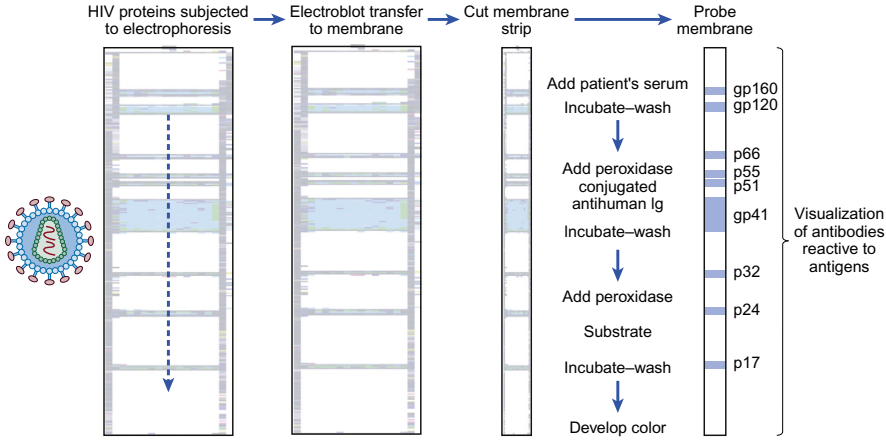


Figure 12.6 ***Western blot (immunoblotting).*** *Reactivity to antigens assessed by western blot analysis using serum antibodies is pictured. HIV antigens separated by gel electrophoresis are transferred to a solid matrix (nitrocellulose), then probed with patient serum. Reactive antibodies against HIV antigens are visualized, thus determining patient exposure to the viral agent.*

with great sensitivity and specificity to detect antibody binding to antigen, with direct or indirect labeling of the antibody constant region. Many immunoassays rely heavily on the use of monoclonal antibody preparations to bind to known antigenic epitopes.

Solid-phase immunoassays are a group of assays in which the antigen or the antibody is coated on the surface of a plastic microplate and sensitive indicators (radioactivity, enzymatic action, or fluorescence) are used to detect the presence of Ag or of Ab. These assays may be further characterized according to the types of antigens being analyzed: soluble or cellular.

Enzyme-Linked Immunoadsorbent Assay

The enzyme-linked immunoadsorbent assay (**ELISA**) is solid-phase assay where a detection antibody molecule is coupled to an enzyme that converts added substrates to a colored product for spectrophotometric detection (Figure 12.7). A very common enzymatic label is horseradish peroxidase (HRP), which, when incubated with a peroxidase substrate such as tetramethylbenzadine (TMB), results in a blue colored solution detected at 650 nm. In general, two types of ELISA assays are used in the clinic and in biomedical research. The first, a direct binding ELISA, uses an antigen bound to a plastic plate. Sample antibody is incubated and allowed to bind, and detection

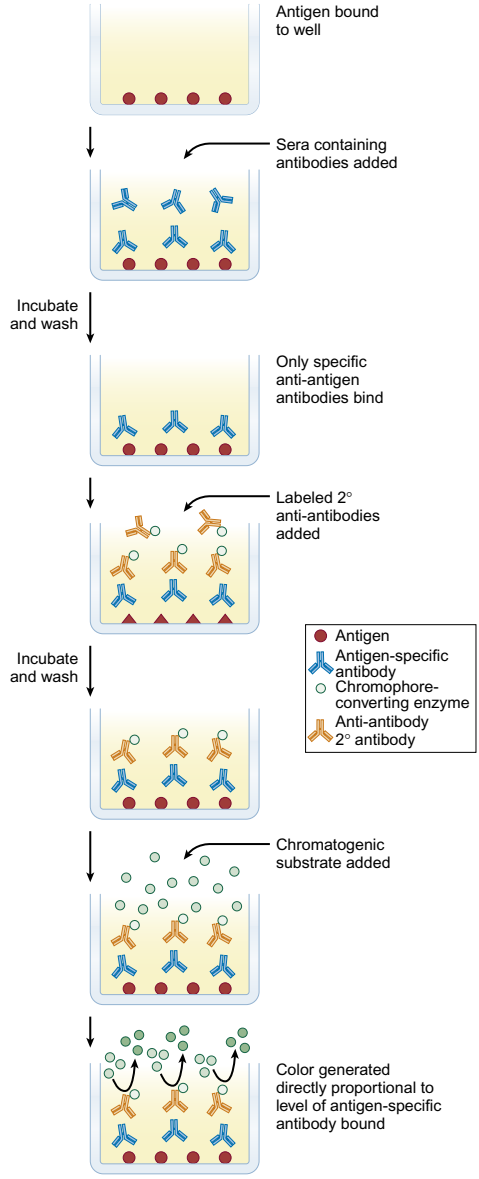


Figure 12.7 ***ELISA.*** *Serum added to wells coated with antigen is probed with antibodies. Enzyme-conjugated secondary antibodies detect the primary complex. Catalytic conversion of chromogenic substrate allows quantitation against known standards.*

occurs via a secondary-labeled antibody. A second type of ELISA, the "sandwich" or "capture" ELISA, uses a pair of specific sets of unique antibodies to bind antigen. The primary antibody is coated on the plate, then sample containing antigen is added. Antigen binds to

the coated antibody, then a labeled secondary antibody that binds to a different epitope on the same antigen is added to create a tiered complex. Color change indicates binding and thus the presence of specific antigen in the sample.

A variation on the ELISA method is the **ELISPOT assay**. In this assay, a nitrocellulose membrane is substituted for the solid plastic surface of the well. Primary antibodies or antigens are bound to this membrane, then incubated with living cells in a solution. Cells secreting a product (e.g., an antibody or cytokine) which is captured on the membrane. Labeled secondary antibody is added, and reactivity determined through use of a precipitable substrate that forms detectable spots on the membrane. The number of cells secreting the protein of interest may then be enumerated.

DETECTION OF CELLULAR ANTIGENS

It can be of great diagnostic value to determine if a particular antigen is found on or within cells of a particular tissue. Assays can be performed directly on biopsies of tissue and visualized using a microscope. The immunofluorescence method utilizes covalent attachment of fluorescent organic compounds to specific antibodies that then can be used to detect antigen in the tissue sample. The fluorescent compounds excite at different wavelengths and thus can be detected using special microscope lighting. This is a highly sensitive and specific assay, and individual cells can be stained with up to 12 different excitable compounds. Visualization is accomplished by **direct immunofluorescence**, where an antibody specific for the antigen in question is directly labeled with the fluorophore and used to identify the antigen. Or it may be accomplished by **indirect immunofluorescence**, where a two-step method in which the unlabeled antibody specific for the antigen in question is reacted first and then the slide is flooded with a fluorescent molecule to detect the bound antibody. Immunofluorescence is used in clinical laboratories to screen for anti-DNA antibodies in suspected cases of autoimmune systemic lupus erythematosus.

Immunohistochemistry

Immunohistochemistry is a powerful technique to localize antigens on tissues embedded in paraffin and placed on glass slides. Incubation with specific enzymatically labeled antibodies allows detection of molecules within the tissues; detection is enhanced using enzymes, such as HRP or alkaline

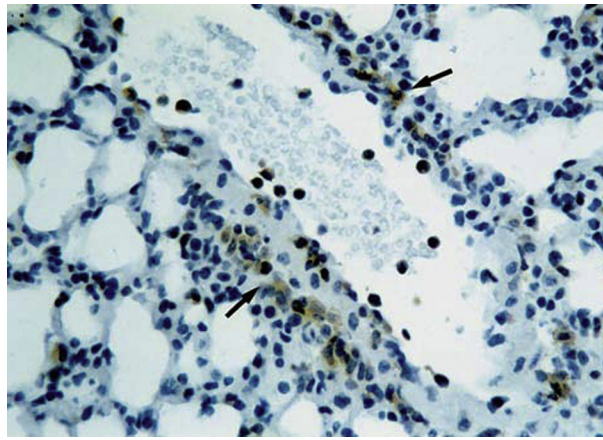


Figure 12.8 ***Immunohistochemical method.*** *Antibodies can identify cell structure and phenotype within tissue. Pictured are lymphocytes cuffing pulmonary vascular regions (arrows). Enzyme (HRP)-conjugated antibodies directed against surface $CD3^+$ were incubated with the tissue section. Conversion and deposition of chromogenic substrate (diaminobenzidine) enables visualization of T lymphocytes (brown stain).*

phosphatase, for which addition of substrate then colorizes the membranes of the cells expressing the antigen of interest (Figure 12.8). While this method is usually less sensitive than immunofluorescence, it is often used in clinical pathology laboratories to permit determination of antigenic placement within overall general tissue morphology.

Fluorescence-Activated Cell Sorting Analysis

Fluorescence-activated cell sorting (FACS) analysis is used to identify, and sometimes purify, one cell subset from a mixture of cells (Figure 12.9). This is an extremely effective tool to identify and/or isolate specific cell subsets, as it allows for rapid identification as well as quantification of cells expressing specific surface molecules. Antibodies directed against surface molecules (such as CD surface proteins) may be directly tagged with fluorescent compounds or indirectly identified with secondary tagging (indirect immunofluorescence). Labeled cells are passed single-file through a laser beam by continuous flow in a fine suspension stream. Each cell scatters some of the laser light. Detection of scattered light in a parallel manner (forward scatter) is indicative of cell size, whereas detection of scattered light at a perpendicular angle (side scatter) is indicative of cellular granularity. These physical parameters alone can subdivide granular polymorphonuclear cells (PMNs) mononuclear cells, and RBCs. Flow cytometers are equipped with multiple lasers to generate different excitation wavelengths as well as a

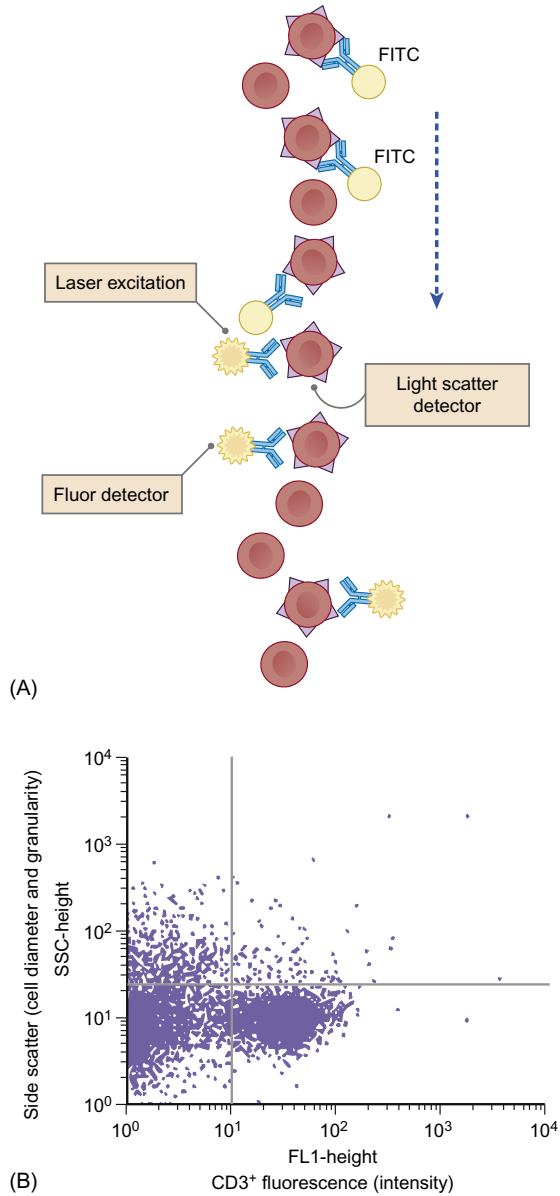


Figure 12.9 ***Fluorescence-activated cell sorting.*** *Flow cytometric analysis allows direct identification of cell phenotypes using fluorescently tagged antibody molecules. Modern cytometers can measure multiple parameters simultaneously, including low-angle forward scatter (proportional to cell diameter), orthogonal scatter intensity (measuring cellular granularity), as well as fluorescence intensity at several wavelengths (A). Pictured is a scattergram of splenocytes screened for reactivity with an FITC-labeled anti-CD3 antibody to quantitate T lymphocytes (B). A shift to the right on the* x*-axis indicates the presence of positively stained cells with increasing levels of CD3 surface molecule. FITC, fluorescein isothiocyanate; SSC, side scatter.*

series of optical detectors that capture the unique emission wavelengths generated by the various fluorescent labels. Thus, specific monoclonal antibodies labeled with unique fluorescent compounds may be used to detect up to 20 unique antigens on a single cell. Newer methodologies also allow for detection of intracellular cytokines to match surface molecules found on individual cell phenotypes. The instrument can identify the cells of interest based on size or fluorescent label. Downstream of the laser beam, an electrostatic deflection system within the instrument, can sort the cells of interest into batches. Sorting of cells can also be accomplished using antibodies coupled to magnetic beads. The cells are then placed over a magnetized column, and labeled cells can be isolated from the nonlabeled population.

Multiplex Bead Arrays

A relatively new technology combines aspects of an ELISA with the sensitivity of flow cytometry. The **multiplex bead arrays** rely on engineering of microspheres internally "coded" with two fluorescent dyes. Combinations of the dyes can be used to generate up to 100 individual "bead sets," each of which can be coated with a specific antibody. Different bead sets may be incubated with samples (plasma or cell culture supernatant) in a single tube allowing for the measurement of multiple parameters at once. The antibody-coated beads bind to their specific antigen target, then biotin-labeled secondary detection antibodies are used to "sandwich" the antigens bound on the beads. A reporter molecule, such as streptavidin–phycoerythrin, then indicates each complex. In a method similar to that used in flow cytometry, the beads are passed single-file through a laser beam; complexes are identified by fluorescence with internal fluorochromes unique to each bead, emitting specific signals detected by digital processors. In this manner, multiple analytes can be detected in a single sample, making this a powerful tool for screening patient responses in different disease conditions.

ASSAYS TO DETERMINE IMMUNE FUNCTION

Assessment of immune function is a useful clinical tool to establish competency as well as defects present in the patient. The tests below allow indication of immune disorders with identification of response for excess or deficiency.

Complement Fixation Test

The complement fixation test is a blood test that can determine the presence of antigen-specific antibodies by incubating patient serum with antigen and complement. This assay takes advantage of the requirement for complement to be activated by the combination of antigen–antibody complexes (Figure 12.10). Should specific complexes form, the complement cascade will be activated. A target for this

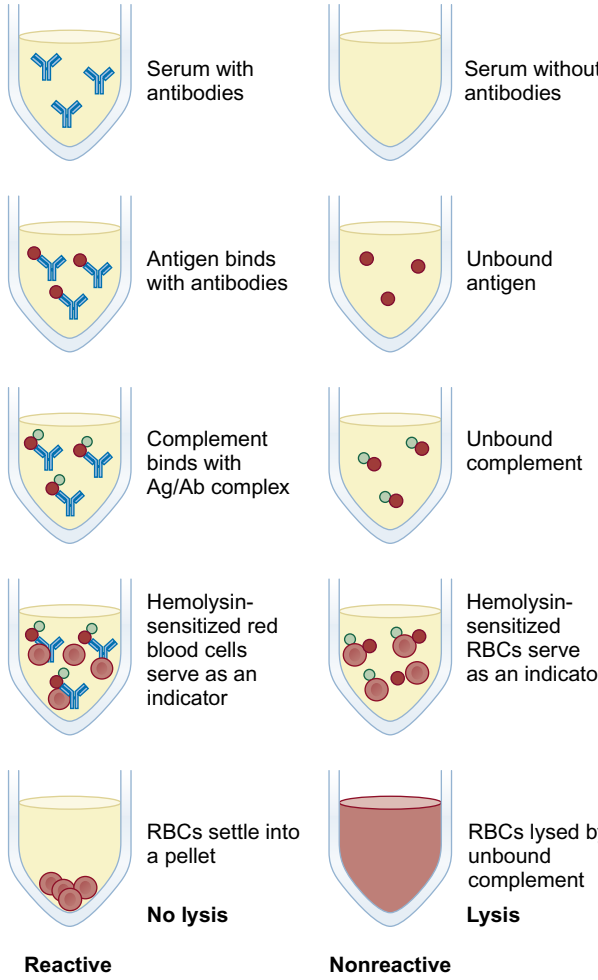


Figure 12.10 ***Complement fixation assay.*** *In the complement fixation assay, complement components bind to antibody–antigen complexes, thereby making complement unavailable for hemolysis of indicator red blood cells. In the absence of specific antibody–antigen interactions, complement assembly results in cell lysis.*

activation is thus required to determine reactivity. Sheep red blood cells (sRBC) bound to anti-sRBC antibodies are often used as an activation target. In a positive test, the complement is bound to an antigen–antibody complex and thus is sequestered away from interaction with target sRBC–Ab complexes. The RBCs remain unlysed and settle to the bottom of a concave shaped well. In a negative or non-reactive test, the complement remains free to interact with the sRBC–Ab complexes causing them to lyse. The complement test is a powerful tool to confirm presence of antibodies following exposure to a specific microorganism. For example, the Wasserman reaction is a diagnostic complement fixation test to detect antibodies to the syphilis causing organism *Treponema*; a positive reaction indicates the presence of antibodies and therefore syphilis infection.

Lymphocyte Function Assays

Lymphocyte function can be compromised in certain diseases or can occur as a result of a genetic abnormality. A diagnosis can be confirmed in many cases if it is known whether or not the B or T cells are normal, if the existing B cells can make antibodies, or if the T cells can produce the correct cytokines. For example, cytotoxicity assays measure the ability of cytotoxic T cells or NK cells to kill radioactive target cells that express a specific antigen for which the cytotoxic T cells may be sensitive.

Blast transformation assays are used to measure cell reactivity of unique phenotypic populations within peripheral blood lymphocytes. Cells are incubated in the presence of specific antigen or mitogen. A **mitogen** is any agent capable of stimulating cellular activation. For example, **lipopolysaccharides** can cause polyclonal stimulation of B cells. Several **lectins**, including **concanavalin A** and **phytohemagglutinin**, are effective T-cell mitogens. And **Pokeweed mitogen** stimulates polyclonal activation of both B and T cells. If reactive, lymphocytes will proliferate. Historically, radioactive nucleotides (^{3}H-thymidine) were added; the amount of radioactivity incorporated into DNA was determined as a quantitative measure of proliferation. The reduction of tetrazolium salts is now recognized as a safer and accurate alternative to radiometric testing; yellow tetrazolium salt (MTT) is reduced in metabolically active cells to form insoluble purple formazan crystals, which can then be quantified by spectrophotometric means.

OTHER TOOLS TO MEASURE IMMUNOLOGICAL STATUS

Monoclonal Antibodies

Polyclonal antibody mixtures consist of antibodies specific for a multitude of different epitopes on even simple antigens. Subpopulations of antibodies with different affinities exist even in the subset specific for a single epitope. Due to cross-reactivity of antibodies and the need for more controllable assays, it is sometimes of great advantage to have a homogeneous antibody preparation specific for a single epitope and with high affinity. Kohler and Milstein developed a method for making antibodies that are **monoclonal**, i.e., all antibodies are derived from a single precursor plasma cell so that all antibodies are identical. Genetic engineering methodologies now allow easy and rapid production of monoclonal antibodies, with swapping of human sequences for homologous protein utility.

Microarrays

Levels of expression of thousands of genes can be measured simultaneously using **gene chips** or **microarrays**. Briefly, thousands of short cDNAs representing genes from all parts of the genome are attached to a silicon chip. Samples of mRNA from cells in culture are reverse transcribed into cDNA, labeled with different fluorochromes, and used to measure differential expression of distinct genes. The chips are exposed to laser light and the unique wavelengths emitted by the fluorochromes are measured and compared to control samples. Hence, the relative binding of the different cDNAs to each unique sequence can be determined.

Finally, recent advances in sequencing technology and computing algorithms have allowed the development of methods to rapidly screen and characterize polyclonal immune responses to antigen. Collectively, these methods are referred to as **high-throughput Ig sequencing (Ig-Seq)** technologies, and they take advantage of the unique manner in which Ig genes recombine to specifically amplify heavy and light chain variable sequences isolated from class-switched B cells (or TCR from memory T cells). Genomic DNA isolated from a population of B cells is amplified using primers complementary to rearranged V(D)J sequences. Alternatively, cDNA can be amplified using a primer pool complementary to leader peptides or framework regions of V gene segment combined and specific primers for heavy or light chains.

The samples are then sequenced; bioinformatic approaches are used to generate output data. Results from these analyses can be applied to many experimental and clinical questions to understand the generation of responses and their role in human health and disease.

SUMMARY

- The affinity of the antibody–antigen reaction is defined through physical laws of mass action.
- Understanding the nature of antibody–antigen reactions has allowed the development of specific assays to detect functional immune response.
- The immunoassays have become strong clinical tools to determine immune reactivity and allow assignment of developmental status in individuals with immune disorders.

ACKNOWLEDGMENT

Chapter contributed in collaboration with Keri C. Smith, PhD.

GLOSSARY

Accessory Molecule Cell surface molecules participating in cellular interactions to modulate strength and direction of specific immune response.

Acquired Immunodeficiency Syndrome (AIDS) An infectious disease caused by the human immunodeficiency virus (HIV) characterized by loss of CD4+ T helper lymphocytes.

Acute Phase Proteins Any of the nonantibody proteins found increased in serum during active and immediate innate responses; includes complement factors, C-reactive protein and fibrinogen.

Acquired Immunity/Adaptive Immunity Network of antigen specific specialized lymphocytes that function to eliminate or prevent systemic infection. Responses take days or weeks to develop and results in immune readiness (memory) that may be sustained for long periods.

Adjuvant Excipient added to an immunogen to direct immune response during vaccination.

Affinity Binding strength of antibody for its cognate antigen.

Affinity Maturation Process by which B lymphocytes mature to increase specificity of antibody for its cognate antigen.

Allelic Exclusion Expression of only one gene while the alternate copy (allele) remains silent.

Allergy Misdirected hypersensitive immune reaction to normally harmless foreign substances. An "allergen" is any antigen that elicits Type I hypersensitivity (allergic reactions)

Allogenic Genetically different from a similar species member.

Allograft Tissue graft from a non-self donor of the same species.

Anaphylactic Shock Systemic allergic reaction to circulating antigen, resulting from interaction with IgE antibodies on connective tissue mast cells, followed by release of inflammatory mediators which confer "shock."

Anaphylotoxin Complement system enzymatic fragments (C3a, C4a, C5a) that mediate host defense functions, including chemotaxis and activation of cells bearing fragment receptors. Causes enhanced vascular permeability and mast cell histamine release.

Antibody A surface protein on B lymphocytes that can be secreted in large amounts in response to an antigen. Five subclasses exist, each which unique function to confer protection against infectious assault. Also see Immunoglobulin.

Antibody-Dependent Cell Cytotoxicity (ADCC) Cytolytic process directed toward an antibody-coated target cell via mechanisms whereby effector cells (mostly natural killer cells) with Fc receptors recognize the constant region of target bound immunoglobulin.

Antigen Foreign substance capable of eliciting an immune response. May be of protein, carbohydrate, lipid, or nucleic acid in nature.

Antigen Presentation Organized display of processed antigenic fragments bound to presenting cell surface histocompatibility molecules to allow targeted recognition by the T-cell receptor.

Antigen-Presenting Cell (APC) Specialized bone-marrow-derived cell, bearing cell-surface class II major histocompatibility complex molecules to function in antigen processing and presentation to T cells.

Antigen Receptor Specific antigen-binding molecule on T or B lymphocytes; comprised of amino acids produced from genetic sequences with physical rearrangements of V, D, and J gene subsets.

Antigenic Substance capable of recognition by an immunoglobulin or an antigen receptor. The "antigenic determinant" is the site or epitope on a complex molecule recognized by an antigen receptor (antibody or T-cell receptor). The antigen-binding site "paratope" represents the physical location on the receptor that contacts the molecules.

Apoptosis Process of programmed cell death.

Autograft Tissue grafted from one person to that same individual, with complete match of histocompatibility molecules.

Autoimmune Disorder Pathological condition where the body's own immune system is directed toward self-antigens. Autoreactivity describes immune cells mounting a response against self.

Avidity Combined strength of antibody–antigen interaction, taking into account multiple binding sites between molecules.

B Cell/B Lymphocyte Type of lymphoid cell produced in the bone marrow from lymphoid progenitor stem cells that possesses specific antibody cell-surface antigen receptors; cell capable to produce antibodies when activated.

Basophil Polymorphonuclear granulocytic cell involved in allergic reactions during Type I hypersensitivity.

Blood Group Antigens Red blood cell surface molecules detectable with antibodies produced by sensitization to environmental substances. Major blood group antigens include ABO and Rh (Rhesus) markers used in routine blood screening to designate blood type.

CD3 Complex Set of signal transduction molecules assisting in T cell activation once the antigen receptor has been engaged.

Cell-Mediated Immunity/Cellular Immunity Adaptive immune responses initiated by antigen-specific T cells.

Chemokines Family of related small polypeptide cytokines involved in directed migration and activation of leukocytes. "Chemotaxis" is targeted movement in response to a chemical stimulus.

Class Switch Change in production of antibody isotype due to maturation of the B lymphocyte response to a particular antigen.

Cyclosporine A Powerful immunosuppressive agent.

Cluster of Differentiation (CD designation) Commonly used designation for specific cell surface molecules. Useful in discriminating between different cell phenotypes and for assessment of functional cellular activity.

Combinatorial Joining Physical joining of nucleic acid sequences during development to generate novel proteins involved in antigen binding receptors on B and T lymphocytes.

Complement System of serum proteins involved in inflammation and immunity; mediates activities which include activation of phagocytes, direct cytolysis of target cells, and coating (opsonization) of microorganisms for uptake by cells expressing complement receptors.

Concanavalin A (con A) Mitogenic lectin derived from the jack bean that stimulates T lymphocytes to undergo mitosis and proliferation.

Cross-Reactivity Binding of antibody to a epitope or molecule similar in structure to the antigen used to elicit antibody response.

Cytokine Class of small molecule immune mediator secreted by leukocytes as a mechanism of immune regulation and cross-talk. Cytokines produced by lymphocytes are called "lymphokines" or "interleukins."

Cytotoxic T cell T lymphocytes bearing CD8 cell surface molecules that respond to antigenic stimulation through elicitation of toxic mediators. Critical for antiviral and antitumor responses.

Defensins Natural molecules able to limit growth of microorganisms.

Degranulation Process by which myeloid leukocytes release digestive proteins stored in cytoplasmic vesicles.

Delayed-Type Hypersensitivity (DTH) See Hypersensitivity, Type IV.

Dendritic Cell Primary phagocytic antigen-presenting cell capable of initiating immune response and lymphocytic activation, accomplished by cytokine secretion.

Enzyme-Linked Immunoadsorbent Assay (ELISA) Assay used to detect antigens bound to solid wells in a plate format. Labeled reagents are used for quantitation, by linking enzymes to an antibody to allow substrate color change for recognition of antigenic detection.

Endocytosis Mechanism utilizing receptors or pinocytosis whereby materials are uptaken from solution into plasma membrane vesicles by cells.

Eosinophil Polymorphonuclear granulocytic cell involved in the innate response to parasitic infections.

Epitope Antigenic determinant; portion of antigen capable of interacting with antibody or eliciting a lymphocytic response.

Extravasation Movement across blood endothelial barriers into tissue.

Fab fragment/F(ab)′2 Portion of the antibody heavy and light chains which combine to make up the antigen binding region.

Fas–FasL Cell surface molecule interactions required for activation of apoptosis.

Fc Fragment Portion of the antibody heavy chain that comprises regions able to interact with cellular receptors; confers biological function to the immunoglobulin.

Foreign Non-self.

Germ Line Genetic material in original configuration, representing nonrearranged chromosomes.

Germinal Center Secondary lymphoid tissues sites where lymphocytic populations can proliferate and mature in response to antigen.

Graft-vs.-Host Disease (GVHD) State where donor immune cells develop pathological reactions to recipient post-transplantation.

Granulocyte General term for phagocytic leukocyte containing granular particles.

Granzyme Protein involved in cytotoxic reactions; involved in cell lysis.

Hapten Small low weight molecule that can only elicit immune responsiveness when attached to a larger carrier molecular, thus rendering it immunogenic.

Heavy Chain Larger protein associated with the antibody molecule; confers biological functions, associated with the constant portion of the chain.

Helper T Cell Class of CD4+ T lymphocytes that respond to antigens by secreting cytokine subsets to give help to cells to become effectors of cellular immunity, or to stimulate B cells to make antibodies.

Hematopoietic Stem Cell Precursor cell found in bone marrow. Can give rise to leukocytes.

Herd Immunity Social concept to preventing spread of infection within a community; vaccination of a significant portion of a population provides a measure of protection for individuals who have not developed immunity, due to limitation of infection spread.

Heterograft Graft in which the donor and recipient are of different species. See Xenograft.

Histamine Compound released from neutrophils during immunological and allergic reactions causing vasodilation and smooth muscle contraction.

Histocompatibility Tissue compatibility between individuals based on the presence of polymorphic major histocompatibility molecules present on cellular surfaces.

Human Leukocyte Antigen (HLA) Genetic designation for the human major histocompatibility complex (MHC) molecules. Class I molecules are represented by gene loci HLA-A, HLA-B, and HLA-C. Class II molecules are represented by gene loci HLA-DR, HLA-DP, and HLA-DQ. See MHC.

Human Immunodeficiency Virus (HIV) Lentiviral family member retrovirus with an RNA genome that forms a DNA intermediate incorporated into the host cell genome. Infection leads to loss of CD4+ lymphocytes and an eventual state of acquired immune deficiency.

Humoral Immunity Refers to antibody-mediated immunity.

Hyperacute Graft Rejection Reaction representing immediate recipient antibody reactivity to antigens present in donor tissue during transplantation.

Hypersensitivity Immune reactivity to antigen at levels higher than normal, often leading to clinical states. Reactions are classified by mechanism: Type I (allergic) reactions involve IgE triggering mast cells; Type II (cytotoxic) reactions involve IgG against cell surfaces

resulting in cytolytic events; Type III (immune complex) reactions involve destructive deposition of antibody and antigen complexes; and Type IV (delayed type hypersensitivity; DTH) reactions are T cell mediated.

Hypervariable Regions Portions of the antibody light and heavy chains that represent the most variable amino acid sequences coding for contact with the epitopes on the antigen. Encoded by "complementarity determining regions."

Immunodeficiency Relative decrease in immune responsiveness due to lack of components (innate or adaptive) capable of responding to a foreign influence. Immune deficiency disease is the resultant clinical state when parts of the immune system are missing or defective.

Immunogenic Capable of eliciting an immune response.

Immunoglobulin Any of five classes of antibodies (IgM, IgD, IgG, IgE, or IgA) that function in immune regulation through specific binding of antigens. Also see Antibody.

Immune/Immunity Protection against a specific disease or pathogen resulting from effective innate and adaptive resistance.

Immunization Induction of adaptive immunity by preexposure to antigen or by active infection, thereby generating a memory lymphocytic response.

Inflammation Buildup of fluid and cells that occur in responses to acute injury or trauma.

Innate Immunity Component of the immune system consisting of genetically encoded constitutive factors readily able to respond to pathogens on short notice. Factors involved do not change or adapt during the lifetime of the organism; no associated memory response.

Interferons Specialized subset of cytokines originally discovered as having properties that interfere with viral replication. Mediators of cellular immune function.

Interleukin See Cytokine.

Isograft Tissue graft between individuals of genetic identity. Similar to "syngraft."

Isohemagglutinin Naturally occurring IgM molecules that recognize ABO antigenic determinants on red blood cells.

Isotype Antigenic marker that distinguishes members of an immunoglobulin class. Immunoglobulin isotypes include IgG, IgA, IgE, IgD, and IgM.

Isotype Switching Genetic rearrangement in B lymphocytes to allow change in production of immunoglobulin isotypes.

Lactoferrin Innate iron binding component with bactericidal and bacteriostatic activity, as well as immune modulating properties. Found in neutrophils and secreted onto mucosal surfaces.

Leukocyte Any white blood cell (myeloid or lymphoid) that plays a functional role in either innate or adaptive responses. The myeloid population includes neutrophils, eosinophils, basophils, mast cells, as well as monocytes and macrophages, and dendritic cells. The lymphoid group includes the lymphocyte populations.

Light Chain Smaller protein associated with the antibody molecule; can be either of the kappa (κ) or lambda (λ) variety.

Lipopolysaccharide (LPS) Endotoxin component of gram-negative bacteria cell wall that elicits mitogenic activity.

Lymph Nodes Small, rounded secondary lymphoid organs where mature leukocytes, especially lymphocytes, interact with antigen presenting cells.

Lymphatics Endothelial lined network of vessels permitting flow of lymph to lymph nodes.

Lymphocyte Lymphoid derived leukocyte expressing an antigen specific receptor. There are two broad categories, T cells and B cells. Lymphocytes function as an integral part of the body's adaptive defenses and are critical to distinguish self from foreign antigens.

Lymphoid Tissue responsible for producing lymphocytes and antibodies, including regions in the lymph nodes, thymus, and spleen.

Lymphokine See Cytokine.

M cells Microfold cell found in the follicle-associated epithelium of the Peyer's patch. Function to sample antigen from the small intestine lumen to deliver via transcytosis to presenting cells and lymphocytes located on the basolateral side.

Macrophage Myeloid-derived cell involved in phagocytosis and intracellular killing of microorganisms and antigen presentation to T lymphocytes.

Major Histocompatibility Complex (MHC) Molecule Polymorphic molecules to allow the immune system to distinguish between self and foreign substances. Class I molecules present antigen to CD8+ cytotoxic T lymphocytes, and are on all nucleated cells. Class II molecules present to CD4+ helper T lymphocytes and are found on antigen-presenting cells.

Mast Cell Large myeloid cell found in connective tissues which mediates allergic reactions.

Membrane Attack Complex (MAC) Terminal product of complement cascade, whereby components C5 through C9 self-assemble on a membrane to form a cytolytic pore.

Mitogen Agent capable of stimulating cellular activation and division.

Molecular Mimicry Cross-reactive occurrence during development of autoimmune disorder where a microorganism contains antigenic determinants that resemble those on self-tissues.

Monoclonal Antibodies Antibodies derived from a single B cell specific for a single antigen.

Monocyte Part of the innate leukocyte population; blood precursor to the tissue macrophage.

Mucosa-Associated Lymphoid Tissue (MALT) Diffusion system of concentrated lymphoid tissue found in the gastrointestinal tract, thyroid, breast, lung, salivary glands, and skin. Related to gut-associated lymphoid tissue (GALT) which represents Peyer's patches found in the lining of the small intestines, and the bronchus-associated lymphoid tissue (BALT) representing aggregations of immune cells in the lower respiratory tract.

Myeloid Derived from granulocyte precursor stem cells in bone marrow.

Natural Killer Cell Small granular innate cell derived from lymphoid progenitors. Able to rapidly destroy tumor cell targets by antibody-dependent cell cytotoxicity which permits target destruction in a nonphagocytic manner. They do not express a T-cell receptor.

Natural Killer T Cell Small subpopulation of T cells that express a limited T-cell receptor repertoire; receptors recognize bacterial lipids or glycolipids bound to nonclassical histocompatibility class I–like molecules.

Neutrophil Polymorphonuclear, phagocytic granulocytic cells involved in the acute inflammatory response to pathogens.

Nitric oxide Molecule important in intracellular signaling; free radical and regulator of hydrogen peroxide in phagosomes within phagocytic cells.

Opsonization Process by which a molecule or pathogen is targeted for ingestion and subsequent destruction by phagocytic cells, mediated through complement or antibody interactions. An "opsonin" is a molecule that enhances directed phagocytosis.

Paratope Portion of the antibody that contacts the epitope on the antigen.

Passive Immunity State of immunity acquired through transfer of factors (serum or antibodies), allowing a protective state in the absence of active immunity.

Pathogen-Associated Molecular Patterns (PAMPs) Conserved molecular motifs associated with infectious agents that are able to trigger innate immune function. Cellular receptors on monocytes that recognized conserved molecular motifs associated with infectious agents are called "pattern-recognition receptors (PPRs)."

Perforin Protein involved in cytotoxic reactions; involved in cell lysis.

Phagocyte Any mobile leukocyte that engulfs foreign material. The process of directed uptake is called phagocytosis.

Phagolysosome Internal digestive compartment within phagocytic cells where phagosome and lysosomal enzymes destroy engulfed pathogenic invaders and digest engulfed protein.

Plasma Fluid component of blood containing water, electrolytes, proteins and molecular mediators; plasma does not contain cells.

Plasma Cell Terminally differentiated antibody-secreting B lymphocyte.

Polymorphonuclear Cells (PMNs) Group of white blood cells (neutrophils, basophils, and eosinophils) with multilobed nuclei and cytoplasmic granules.

Primary Immune Response Adaptive immune response representing initial exposure to antigen, predominantly comprised of IgM followed by later presence of other antibody isotypes. "Priming" is the activation of lymphocytic response to antigen for the first time, initiated by antigen-presenting cells.

Primary Lymphoid Tissue Immune organs where lymphocytes develop and mature; organs where antigen-specific receptors are first expressed.

Peyer's Patches Lymphatic nodules located along the small intestine.

Regulatory T Cells (Treg cells) Specialized T lymphocyte subgroup able to regulate immune responses; effective post-thymic development.

Respiratory Burst Phagocytic metabolic activity resulting in the formation of superoxide anion and hydrogen peroxide.

Rheumatoid Factor IgM isotype antibodies reactive with IgG molecules.

Rhesus Antigens (Rh) See Blood Group Antigens.

Secondary Immune Response Immune response induced by repeated antigen exposure, often of higher affinity and with greater speed

than elicited by primary response. Has characteristic maturation of antibody isotype.

Secondary Lymphoid Tissue Immune organs where antigen-driven proliferation of lymphocytes occur in response to antigenic stimulation.

Secretory Component Portion of the dimeric IgA molecule critically involved in release across mucosal barriers.

Seroconversion Indicates when antibody can be first detected against antigen, following infectious challenge or immunization.

Severe Combined Immune Deficiency (SCID) Disease state in which defects in maturation pathways for both B and T lymphocytes result in lack of functional adaptive immunity.

Somatic Hypermutation Change in affinity maturation of the antigen binding site in an antibody following antigenic stimulation.

Superantigen Molecule able to elicit T lymphocyte responses by circumventing normal antigen processing and presentation functions.

Syngeneic Being from individuals that are genetically identical.

T Cell/T Lymphocyte Derived from bone marrow lymphoid progenitor stem cells, possessing specific cell-surface antigen receptors; types include helper T cells of different cytokine secreting subsets as well as cells that confer regulatory and cytotoxic function.

Titer Relative antibody concentration.

Tolerance State of less responsiveness to a substance or a physiological insult; instrumental in prevention of autoimmunity.

Toll-like Receptors Subset of pattern recognition receptors recognizing conserved molecular motifs associated with infectious agents; initiate strong innate immunity when triggered.

Thymocyte Hematopoietic progenitor cell present in the thymus.

Vaccine Immunogenic substance used to stimulate production of protective immunity (antibodies or T cell based) to provide protection against clinical disease.

Vaccination Artificial induction of adaptive immunity by preexposure of antigen or pathogen to generate a memory lymphocytic response.

Variable Domain/Variable Region End portion of the antibody or T-cell receptor which comprises the antigen binding region.

Western Blot Diagnostic antigen identification of a mixture separated by electrophoresis through a gel matrix. Proteins are transferred to a solid matrix (usually nitrocellulose) and probed with specific immune reagents.

Xenograft Tissue graft in which donor and recipient are of different species. Similar to "heterograft."