

Waldenstrom's Macroglobulinemia

Basic Immunology



SUPPORT • EDUCATION • RESEARCH

IWMF

International Waldenstrom's
Macroglobulinemia Foundation



Waldenstrom's Macroglobulinemia

Basic Immunology

by Guy Sherwood, M.D.

The IWMF Vision Statement

Support everyone affected by Waldenstrom's macroglobulinemia while advancing the search for a cure.

The IWMF Mission Statement

To offer mutual support and encouragement to the Waldenstrom's macroglobulinemia community and others with an interest in the disease.

To provide information and educational programs that address patients' concerns.

To promote and support research leading to better treatments and ultimately, a cure.

Published by the International Waldenstrom's Macroglobulinemia Foundation (IWMF)

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Revised 2014, 2018

FOREWORD

This is an updated comprehensive review of immunology as it relates to Waldenstrom's macroglobulinemia (WM). An understanding of the immune system is important from the standpoint of this disease.

This booklet begins with an overview of the immune system and then concentrates on the cells involved. Cell growth and death is briefly addressed. There is an extensive section on cytokines and an excellent review of the immunoglobulins, which are so important. Structure of the immunoglobulin genes as well as a brief and lucid discussion of the genetics of immunology are included. Adoptive cell therapy, a new approach for Waldenstrom's macroglobulinemia and related disorders, is briefly discussed.

This is an exciting time for WM. An understanding of the immune system is critical to take advantage of the new scientific advances.

Robert A. Kyle, MD The Mayo Clinic

2014, 2018

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PREFACE

This is a revised and expanded 2018 edition of a booklet originally conceived in early 2007 as an extension for a brief article I wrote in 2001 entitled “Immunology 101.” I had just been diagnosed with Waldenstrom’s macroglobulinemia (WM) and was beginning my search of the available medical literature for additional sources of information about this mysterious disease.

What I found was disappointing insofar as basic reference sources for this rare type of immune system cancer. The majority of medical journals that carried the occasional and brief article on WM required frequent forays back into my old and already outdated immunology textbooks from the 1980s. Fortunately, together with my trusted copy of *Dorland’s Illustrated Medical Dictionary* and more up-to-date editions of standard medical school immunology textbooks that I borrowed for extended periods of time from the local hospital library, I began to slowly revisit the fascinating world of human immunology.

By far the most important source of information I “discovered” was the International Waldenstrom’s Macroglobulinemia Foundation (IWWMF) and its fabulous Internet discussion list, IWWMF-Talk, now known as IWWMF-Connect. On IWWMF-Connect, a wealth of practical information is offered and discussed by actual patients, be it treatment-related issues, important emotional support, or strategies for dealing with this bizarre and incurable disease. It soon became evident that many patients found great comfort in educating themselves about their illness.

The rapid pace of medical research and the wonderful support the IWWMF has given to dedicated WM researchers has resulted in significant and continuing advances in the understanding of this illness and treatment options for it – a major reason why this revision was needed. As more and more new treatments are being studied in clinical trials around the world, WM patients need now, more than ever, to educate themselves about their disease and the critical role their immune system plays in the genesis of WM as well as their treatment response.

I have made a conscientious effort to write this booklet in layman’s terms as much as possible, and a Glossary is provided for selected terms that appear in bold type when they are first mentioned. You will also see outlined text boxes that discuss genetics and immunology as they pertain to recent discoveries in the field of WM research. I have endeavored to present the most accurate information available; however, I am sure that some of the information will need to be updated as new discoveries are made. Of course, I invite any corrections that can be made to improve this booklet. I encourage WM patients to seek additional sources of information and maintain a continuous thirst for knowledge in the wonderful and truly fascinating world of human immunology.

For the past few years there has been increasing interest and corresponding research into the molecular biology and genetics of the immune system and of WM. The IWWMF Board of Trustees therefore encouraged me to revise this booklet and add some information on basic cell biology, as well as an expanded section on basic genetics. It is my hope that this modest booklet will assist WM patients in their quest for a greater understanding of this disease and thus help them mobilize resources in their successful struggle with WM and cancer survivorship.

Guy Sherwood, MD

January, 2018

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Revised 2014, Revised 2018

INTRODUCTION TO THE IMMUNE SYSTEM

We live in an environment where we are continuously challenged by a huge variety of disease-causing organisms: bacteria which leave us miserable with sinusitis, viruses that cause horrible shingles pain, fungal organisms that discolor our toenails, complex organisms like malaria that kill millions every year, and bizarre protein particles called prions implicated in mad cow disease. Fortunately, as humans we have evolved an immune system which can protect us from many organisms, rendering most infections short-lived and leaving little permanent damage.

Immunity, Antigens, and Immunogens

Immunity is the mechanism used by the body to protect itself against these environmental agents that are foreign to the body. A foreign molecule on the surface of an infectious agent (e.g. bacteria, virus, or other pathogen) is called an **antigen**. An **immunogen** is an antigen capable of inducing an immune response. Immunogenic compounds are usually characterized as being foreign to the individual, having a high molecular weight (large size), and being chemically complex. Thus, bacteria and proteins such as pollens can cause immune responses, whereas smaller molecules such as most simple drugs (unless they are attached to a “carrier” molecule) in general do not evoke an immune response. In essence, all immunogens are antigens, but not all antigens are immunogens.

The Human Immune System

There are two types of immunity: **innate immunity** and **acquired immunity**.

Innate immunity (also known as non-adaptive immunity) consists of all those elements with which an individual is born and that are always present and available at very short notice to protect the individual from infection. Examples of innate immunity are the protective barrier of the skin, the mucous membranes of the upper respiratory system, the cough reflex, the acidic pH of the stomach, and enzymes such as **lysozyme** that are present in tears. Internal elements also play a role in innate immunity, including fever, specialized proteins found in the blood, chemicals such as **interferon** released by immune cells, and certain immune cells that act as non-specific security guards to any foreign invader.

Of greater interest to us, however, is acquired immunity (also called adaptive immunity). This type of immunity is considerably more specialized and complex. In fact, acquired immunity is a relatively new evolutionary manifestation, present only in vertebrates. The main difference between innate and acquired immunity is that an acquired immune response is highly specific for a particular antigen; thus, an individual needs to have an initial contact with the foreign antigen, which in turn triggers a chain of events leading to this form of immunity. The acquired immune response not only improves with each successive exposure to the particular antigen, it in effect “remembers” the antigenic properties of a particular infectious agent and can prevent it from causing disease at a later date.

The immune system’s initial exposure to a foreign agent or pathogen is called **immunization**. This immune response triggers a number of events, such as activation of certain cells referred to as **leukocytes (white blood cells)** and subsequent production of antibodies.

BASIC CELL BIOLOGY

Cells are the basic structural and functional biological units of all living things. The human body is composed of trillions of cells that provide structure for the body, take in nutrients from food, produce energy, and carry out numerous specialized tasks. Cells also contain the body’s hereditary material and are the smallest unit of life that can reproduce independently.

Cells have many specialized parts called organelles, each with a different function. In the interest of brevity, we will focus principally on five parts of the cell: plasma membrane, cytoplasm, mitochondria, nucleus, and two of the cell’s many small structures: **ribosomes** and **proteasomes** (Figure 1).

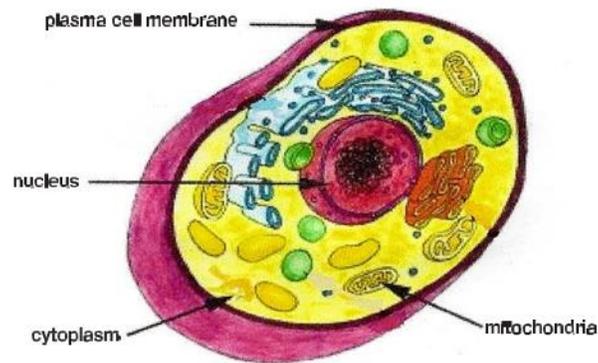


Figure 1 A typical cell and some associated structures.

The plasma membrane is the outer lining of the cell. It separates the cell from its environment, is important in the cell's communication with its environment, and allows materials to enter and leave the cell. Within the plasma membrane is a variety of embedded protein molecules that act as channels and pumps to move different molecules into and out of the cell. Cell surface membranes also contain receptor proteins that allow cells to detect external signaling molecules (e.g. the CD20 receptor that communicates with the commonly used immunotherapy agent rituximab) (Figure 2).

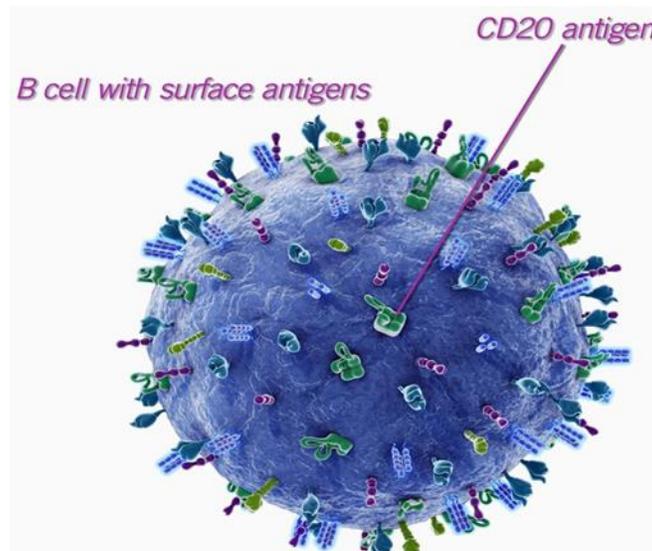


Figure 2 A B-cell with its surface antigens, including CD20.

Within the cell, the cytoplasm (or protoplasm) contains many molecules such as proteins and nucleic acids and organelles such as the mitochondria and the nucleus, all enclosed within the plasma membrane. Many complex biochemical reactions are executed within the cytoplasm, often initiated by signals from the receptors on the plasma membrane, and eventually in turn end up influencing **DNA** (or **deoxyribonucleic acid**) replication in the nucleus.

Mitochondria are complex organelles that convert food into energy for the cell. They have their own genetic material and can make copies of themselves.

The nucleus serves as the cell's command center, sending directions to the cell to grow, mature, divide, or die. It also houses the cell's hereditary material (**DNA**). The nucleus is surrounded by a membrane called the nuclear envelope, which protects the DNA and separates the nucleus from the rest of the cell (Figure 3).

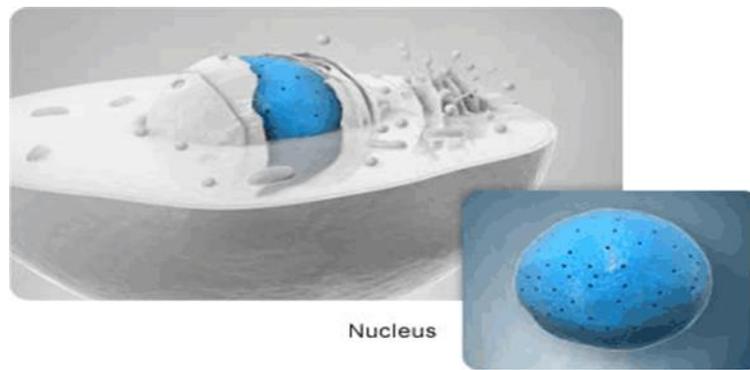


Figure 3 A cell nucleus.

There are two structures that deserve special mention. Ribosomes are the cell's manufacturing plant for proteins. Using the code from the cell's genetic material, they are able to create multiple types of proteins. Conversely, proteasomes are structures located in the cell nucleus and cytoplasm whose main function is the degradation and recycling of proteins. Cells can regulate the concentration of particular proteins with the use of proteasomes. The proteins are degraded into smaller bits of protein that can be reused to synthesize new proteins.

Bortezomib (or Velcade as it is more commonly known) inhibits the normal function of proteasomes. This is believed to result in a rapid and marked increase in the level of un-degraded proteins in the cell, leading to cell death. Essentially, the cell becomes engorged with "garbage" proteins as the garbage truck has stopped doing its rounds. Cells that are very active in protein metabolism (i.e. WM cells making copious amounts of IgM) are particularly susceptible to proteasome inhibition.

CELL GROWTH AND DEATH

Many complex processes are involved in maintaining appropriate cell growth. Cell growth and division is such an important undertaking that many checks and balances are in place to ensure tight control over all the processes involved. Despite safeguards such as DNA repair, failures in internal and external cell communications (or signaling) can result in uncontrolled cell growth. Cancer can occur in many ways, but it invariably is always dependent on multiple signaling errors. Cancer begins when a cell gains the ability to grow and divide regardless of the usual signals or even in the absence of such signals. When the cell loses the ability to respond to death signals, it divides out of control, eventually forming a tumor. In a properly functioning cell this unregulated growth triggers a signal for self-destruction, called **apoptosis**. Similarly, if a cell is beyond repair, it initiates apoptosis (Figure 4).

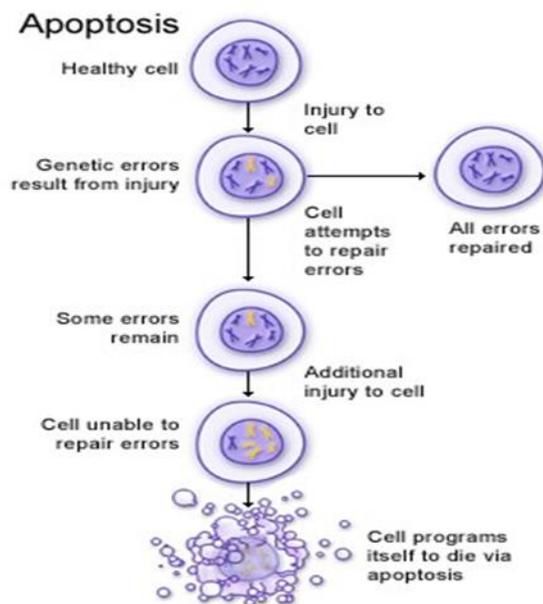


Figure 4 The process of apoptosis (programmed cell death).

CELLS OF THE IMMUNE SYSTEM

HEMATOPOIESIS

Hematopoiesis is the process by which blood cells grow, divide, and differentiate in the **bone marrow**. The **hematopoietic stem cells (HSCs)**, which reside in the bone marrow, are the common ancestors to virtually all the functional cells found in the blood, lymph, and organs of the immune system (Figure 5). The HSCs are self-renewing; when they divide, some of their daughter cells remain as HSCs. In this fashion, the pool of stem cells never becomes depleted. Although the HSCs represent less than 0.01% of the cells found in the adult bone marrow, they give rise to a larger, intermediately differentiated population of daughter cells, or **progenitor cells**, which in turn divide several times and differentiate into mature cells. By the time a cell completes its last programmed cell division and reaches its designated final stage, it loses all ability to proliferate or to alter its functional status and is therefore said to be terminally differentiated.

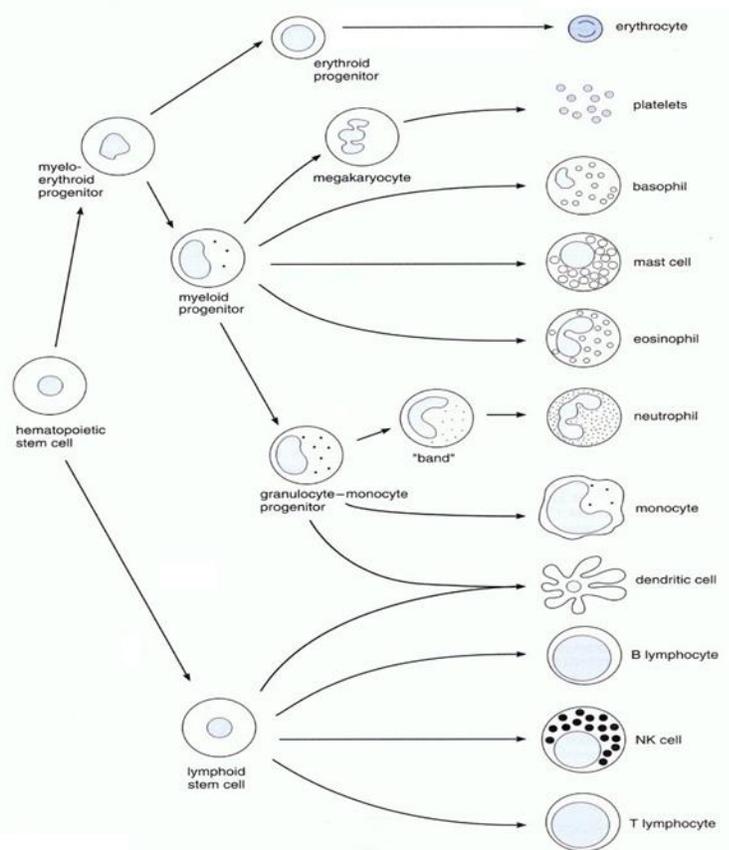


Figure 5 Schematic overview of hematopoiesis, emphasizing the erythroid, myeloid, and lymphoid pathways. This highly simplified depiction omits many recognized intermediate cell types in each pathway. [Adapted from *Medical Immunology 10th edition*, Parslow, T.G., et al., 2001]

Human HSCs express a characteristic surface protein **CD34** (also found on cells that line blood vessels) that is useful in recognizing and isolating HSCs.

Incredible amounts of mature blood cells are produced by the bone marrow every day, and the rate of production of each cell type is precisely controlled at several levels in order to: (1) maintain an available pool of HSCs for self-renewal, (2) regulate the proliferation and differentiation of the functional cells at all stages, and (3) adjust the activity of each hematopoietic pathway in response to the body's physiologic demands.

The three general types of cells produced in the bone marrow by HSCs are: (1) **erythrocytes (red blood cells)** which are primarily responsible for oxygen transport to the body's tissues, (2) **platelets**, responsible for control of bleeding; (3) and leukocytes (white blood cells), the vast majority of which are involved in the body's defense from foreign invaders.

Cells of the Acquired Immune System

The acquired or adaptive immune response is produced by a variety of cells and by the biologically active molecules which they secrete (Figure 6). Although the leukocytes play the predominant role in most immune responses, other cells in the circulatory system and tissues also participate in specific ways to the immune response. Communication between cells and the activation of certain target cells of the immune system are carried out by molecular messengers called **cytokines**. Three types of cells are recognized as major players in acquired immunity.

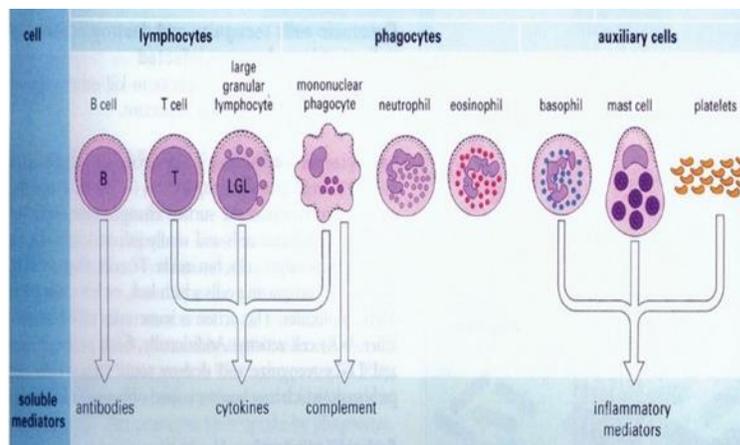


Figure 6 Leukocytes and associated soluble components of the immune system. [Adapted from *Immunology Sixth Edition* by Roitt, I, et al., 2001]

Phagocytes

The **phagocytes** are the white blood cells responsible for essentially disposing of foreign invaders. **Macrophages** are able to “ingest and digest” antigens and “process” them for presentation to and subsequent activation of the T-cells of the acquired immune system (Figure 7). These long-lived **antigen-processing cells** or phagocytes play a necessary and very effective role at activating specific T-cells and are strategically placed throughout the body where they can best intercept and capture antigens. As these phagocytic cells migrate to different tissues, they become Kupffer cells in the liver, microglial cells in the brain, “A” cells in synovial joints, alveolar macrophages in the lungs, mesangial phagocytes in the kidneys, the macrophages in the **lymph nodes** and **spleen**, and finally the **monocytes** that circulate freely in the blood.

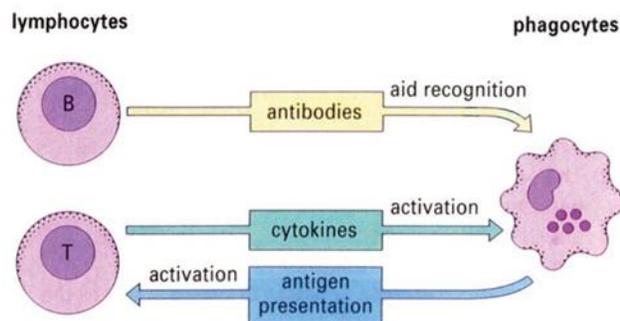


Figure 7 Interaction between lymphocytes and phagocytes. [Adapted from *Immunology Sixth Edition*, Roitt, I., et al., 2001]

Polymorphonuclear neutrophils, or **neutrophils** for short, are another important group of phagocytes. They are short-lived, however, and constitute the majority of the leukocytes found in the blood. They are very reactive, multiply very quickly (hence the high “white count” in an infection for example), and are able to migrate into tissues as well, usually in response to an inflammatory insult.

Lymphocytes

The human body contains more than a trillion lymphocytes, which consist of two major types known as **B- (bone marrow-derived) cells** and **T- (thymus-derived) cells**. In the blood, 75% of lymphocytes are T-cells and 10% are B-cells (the remaining 15% are **natural killer (NK) cells** and **dendritic cells** (see below)). The hematopoietic stem cell in the bone marrow gives rise to a progenitor cell called a **lymphoid stem cell**, which in turn serves as a common precursor for both T- and B-cells, as well as NK cells and dendritic cells. Human B-cell development takes place entirely within the bone marrow, whereas T-cells leave the bone marrow as immature precursors and travel through the bloodstream to the **thymus** where they proliferate and differentiate into mature T-cells.

B-cells or B-lymphocytes are genetically programmed to encode on their exteriors a surface receptor molecule specific for a particular antigen. Once stimulated by this specific antigen, B-cells multiply and subsequently differentiate into **plasma cells**.

These plasma cells, which are now no longer able to multiply, secrete large amounts of antibodies of the same specificity for a particular antigen as that of the receptors on their precursor cells' membranes. At the same time, a proportion of daughter cells turn into resting mature B-cells that are capable of being activated for a subsequent and even more rapid response. These latter B-cells become committed **memory cells** (Figure 8). Antibodies, also known as **immunoglobulins (Igs)**, are virtually identical to the original receptor molecule on the B-cell, making them very specific for the antigen that initially activated the B-cell.

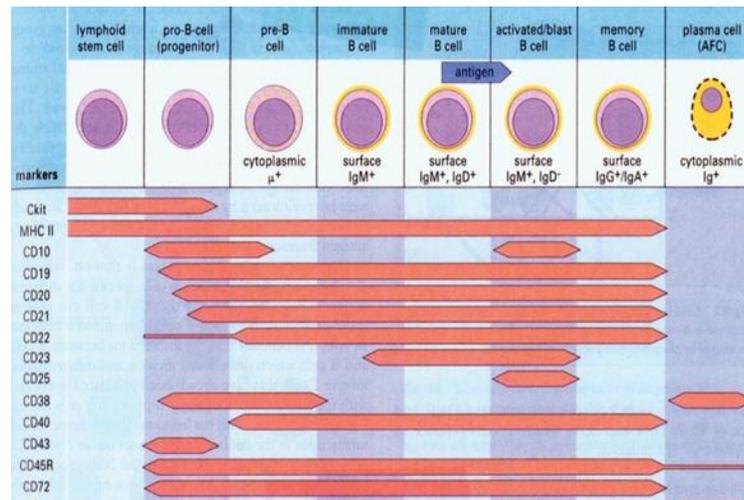


Figure 8 B-cells differentiate from lymphoid stem cells into virgin B-cells and may then be driven by antigens to become memory cells or plasma cells. [Adapted from *Immunology Sixth Edition*, Roitt, I., et al., 2001]

As these cells mature, they express different surface molecules called **CD markers** for example, a B-cell expresses CD20 throughout most of its development but loses this expression and acquires CD38 when it becomes a plasma cell. This change in surface molecule expression provides a convenient way to identify the various stages of B-cell development and the types of cancers that can arise from them.

T-cells or **T-lymphocytes**, of which there are several different varieties, also demonstrate specificity to antigens via surface receptors (**T-cell receptors**), and they also proliferate and differentiate when stimulated by antigen-presenting cells. The T-cell receptors share many properties with the immunoglobulin receptors of the B-cells. T-cell receptors are generally of greater number and complexity, participating in a tremendous amount of immune system functions. The activated T-cells release substances in the circulation called **lymphokines**, which play important biochemical roles in the immune response. T-cells are made up of distinct subpopulations that have very different immunologic functions and express their own distinctive surface markers. T-cells do not produce antibodies, but they do have a variety of other very interesting functions.

Over 75% of mature T-cells express the surface marker **CD4**, and these **CD4 T-helper cells (Th cells)** are further subdivided into **T-helper-1 cells (Th1 cells)** and **T-helper-2 cells (Th2 cells)** based predominantly on the type of cytokines they produce. Th1 cells are particularly effective in enhancing immune responses that involve macrophages and other phagocytes. They interact with the **class II major histocompatibility complex molecules (class II MHC)** presented to them by the antigen-presenting cells (macrophages, dendritic cells, etc.). This leads to the release of cytokines such as IL-2 from the T-cells and subsequent activation of B-cells to help them divide and make antibodies, as well as to the activation of macrophages and other phagocytic cells that will neutralize or destroy the antigen in question. Th2 cells have a predominant role in activation of antibody producing B-cells (Figure 9) and also with allergic disorders, interacting with **mast cells** and **eosinophils**.

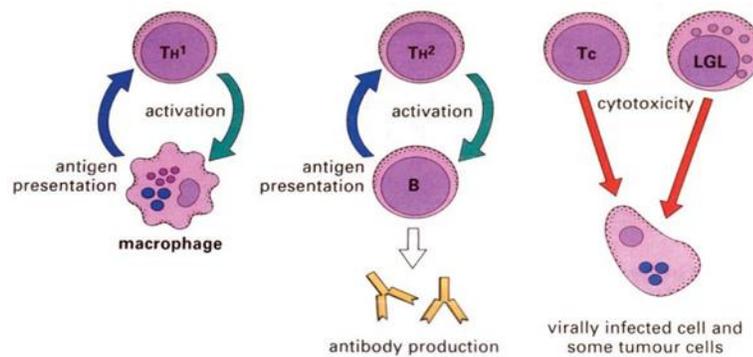


Figure 9 Functions of lymphocytes. [Adapted from *Immunology Sixth Edition*, Roitt, I., et al., 2001]

CD8 T-cytotoxic cells (Tc cells) express the surface marker CD8. They interact predominantly with the class I **major histocompatibility complex molecules (MHC I)** and have the ability to recognize and destroy cells which have become infected by a virus or some other intracellular pathogen. Tc cells are important in donor transplant (allograft) rejection and may play a role in immune surveillance against malignancy.

Natural killer cells (NK cells) or large granular lymphocytes are able to recognize tumor cells and virus-infected cells that tend to display changes on their cell surfaces. NK cells and Tc cells work closely together in this regard. NK cells are also able to destroy cells which have become coated with specific antibodies (e.g., rituximab on B-cells).

Exciting new research called “adoptive T-cell therapy or CAR-T” seeks to harvest and genetically alter a patient’s T-cells to attack cancerous cells. T-cells travel through the body and scan for antigens on the surface of foreign cells. If an antigen matches a T-cell receptor, the T-cell activates and launches an attack. A T-cell receptor has been identified that recognizes the abnormal form of a protein called MYD88 (the “antigen”), which is found in the majority of WM patients. A new project will engineer T-cells from WM patients to recognize this antigen and re-infuse them in large numbers into individual WM patients. The T-cells are expected to seek out and destroy WM cells throughout the body. Hopefully, such engineered T-cells will lead to improved disease control and possibly a cure.

Other Types of Leukocytes and Immune System Cells

Eosinophils have the ability to recognize and participate in the destruction of large parasites such as worms. When stimulated, they release toxic chemicals called lysozymes from granules inside the cells.

Mast cells arise from the same common bone marrow precursor cell as **basophils**. Tissue mast cells possess surface membrane receptors for IgE and, as a result of this interaction, release numerous chemicals associated with the typical allergic reaction. Recently the interaction between mast cells and the WM B-cell in the cellular microenvironment of the bone marrow has been the subject of intense research. The presence of mast cells in the bone marrow is helpful in raising the possibility of a diagnosis of WM.

Basophils are similar to mast cells in that they release biological molecules that produce inflammation in the tissues. Basophils, unlike mast cells, are mobile and have the ability to circulate.

Dendritic cells are bone marrow-derived cells that migrate to nearly all tissues and play a major role in antigen presentation and T-cell activation.

Platelets, although not of white blood cell lineage and known primarily for their role in blood clotting, nonetheless participate in the immune response primarily through their role in inflammation. Following their aggregation at the site of an injury to a blood vessel, they release substances which in turn attract leukocytes.

BIOLOGICAL MOLECULES OF THE IMMUNE SYSTEM

Many critical interactions between cells of the immune system are controlled by protein molecules found in the blood, lymph nodes and tissues, and bone marrow. The serum concentration of a number of these molecules increases rapidly during an immune response and thus they are called acute phase proteins, or **acute phase reactants** (C-reactive protein or CRP is one example). Cytokines, antibodies (immunoglobulins), and complement proteins are the three major biological molecules involved in the immune system.

Cytokines

Cytokines are a diverse group of biological molecules that are involved in the communication occurring between cells and that influence the growth, mobility, differentiation, and function of the target cells in question. Together they are involved in immune and inflammatory responses, wound healing, hematopoiesis, **angiogenesis** (growth of new blood vessels), and many other biological processes. Cytokines exert their actions by binding to specific surface receptors on the target cells. Some cytokines such as **erythropoietin** (Procrit) and **G-CSF** (Neupogen) can influence distant cells; most cytokines act locally on adjacent cells, such as the interaction between mast cells and WM cells in the bone marrow (called **paracrine action**), or act on the producing cell itself (**autocrine action**). Cytokines produced by lymphocytes are called lymphokines, whereas cytokines produced by monocytes or macrophages are called **monokines**.

Interleukins (ILs) are produced mainly by T-cells and are involved primarily in the division and differentiation of other cells.

Interferons (IFNs) are produced in response to viral infections: some are produced by the virally-infected cell itself, whereas others are produced by certain activated T-cells.

Colony-stimulating factors (CSFs) are primarily involved in the division and differentiation of bone marrow stem cells and with the precursors of white and red blood cells. Some CSFs can also exert their actions outside of the bone marrow.

Chemokines are involved primarily in the movement of cells around the body, from the peripheral blood to the appropriate tissues.

Many other cytokines exist, and of these, the tumor necrosis factor (TNF) family and transforming growth factor (TGF) family are the subjects of active research in molecular biology.

Research supported by the IWMMF seeks to understand the mechanisms that result in the increased serum IgM levels seen in WM patients and to determine which factors in the bone marrow support WM cell growth. Proteins called cytokines play an important role in promoting IgM production and secretion. Cytokines with perplexing names such as BlyS/BAFF, IL-6 and IL-21 play key roles in supporting WM cell growth and promoting IgM production. These cytokines use signaling pathways to increase IgM secretion, and blocking these very complex pathways significantly decreases IgM production.

Complement Proteins

Complement proteins consist of about 20 proteins found in the blood that act together in a specific orderly sequence to facilitate the inflammatory reaction. The complement proteins can also interact with other components of the immune system such as phagocytes and antibodies to destroy pathogens. The complement system will be discussed in further detail later.

Antibodies

Antibodies (Abs), also known interchangeably as immunoglobulins (Igs), are a group of immune system molecules produced by B-cells. These will be discussed in greater detail in a separate section entitled ANTIBODIES/IMMUNOGLOBULINS.

ORGANS AND TISSUES OF THE IMMUNE SYSTEM

Proliferation, differentiation, and maturation of lymphocytes take place in the organs and tissues of the immune system, collectively known as the lymphoid organs.

The maturation of T- and B-cells into antigen-recognizing lymphocytes takes place in the **primary lymphoid organs** or **central lymphoid organs**. Once lymphocytes are generated in primary lymphoid organs, they migrate to the **secondary lymphoid organs and tissues** where they are stimulated by antigens to undergo further division and differentiation (Figure 10).

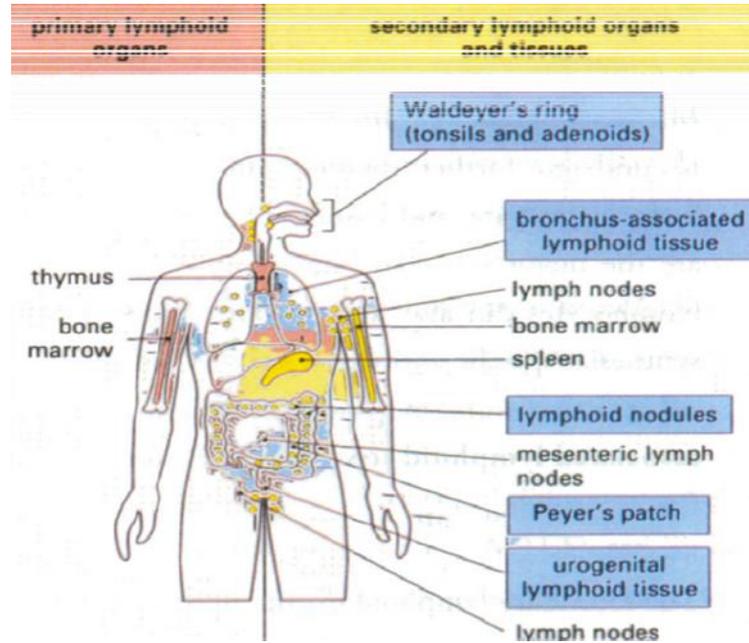


Figure 10 Major lymphoid organs and tissues. Thymus and bone marrow are primary lymphoid organs and are the sites for maturation of T- and B-cells, respectively. Immune responses occur in the secondary lymphoid organs and tissues. [Adapted from *Immunology Sixth Edition*, Roitt, I., et al., 2001]

Primary Lymphoid Organs

The bone marrow (and the liver in the fetus) is the site of development and maturation of B-cells. Erythrocytes (red blood cells), granulocytes (neutrophils, eosinophils, and basophils), monocytes, and platelets are also produced through hematopoiesis in the bone marrow.

The thymus is an organ located in the thoracic cavity, overlying the heart and major blood vessels. This organ consists of two lobes and is the site of T-cell development and maturation. Progenitor T-cells from the bone marrow migrate to the thymus where, through further differentiation, they become committed to respond to a specific antigen and develop cell surface proteins called T-cell receptors (TCRs).

Secondary Lymphoid Organs and Tissues

Mature lymphocytes are stimulated by antigens to undergo further division and differentiation in secondary lymphoid organs and tissues, the major ones being the spleen and the lymph nodes. Mature lymphocytes can also interact with antigens and differentiate to synthesize specific antibodies in other areas of the body. Lymphoid tissue that is found in association with mucosal surfaces (**mucosa-associated lymphoid tissue** or **MALT**), tonsils, and the appendix are a few examples.

The secondary lymphoid organs and tissues serve two major roles in the immune system: they are very effective at trapping and concentrating antigenic foreign substances, and they are the main sites for the production of antibodies and the generation of antigen-specific T-cells.

The spleen lies in the upper left abdomen behind the stomach. It is the largest secondary lymphoid organ and is very efficient at filtering and concentrating foreign substances in the blood. B-cells make up approximately 50% of the population of the spleen, with T-cells accounting for 30-40%. Macrophages in the spleen are able to recognize aged or damaged platelets and red blood cells and dispose of them by **phagocytosis**.

The lymph nodes and lymphatic system form an intricate network of canals (lymphatic vessels) and filtering stations (lymph nodes) strategically placed throughout the body, both in deep areas of the body near organs as well as in superficial areas under the skin. Like the spleen, the lymphatic system is very good at trapping antigens present in the lymphatic circulation where macrophages, T-cells, and B-cells can interact and mount an immune response. Upon antigenic stimulation, structures in the lymph nodes form germinal centers that contain dense populations of lymphocytes, mostly B-cells, which are actively dividing and differentiating. Lymphocytes are in constant movement throughout the body, allowing for the strategic placement of immune system cells that will permit the highest likelihood of interaction with a foreign organism.

ANTIBODIES/IMMUNOGLOBULINS

Antibodies or immunoglobulins (Igs) are one of nature's most brilliant solutions to the problem of antigens or "foreign" material. Acquired immunity, which as we now know is relatively new in evolutionary terms, is characterized by the ability of the immune system to produce an antibody in response to a specific antigen. The initial contact by the antigen that leads to this antibody production is called immunization. The subsequent activation of lymphocytes and the production of immunoglobulins ultimately lead to the neutralization of the foreign agent.

The immunoglobulins are part of a large family of related, but non- identical, biologically active molecules that are the critical ingredient at every stage of acquired immunity. Each immunoglobulin molecule is **bifunctional**. One region, or fragment, of the molecule, called the **Fab** fragment, is concerned with antigen binding, while another fragment of the Ig molecule, the **Fc** fragment, facilitates the so-called **effector functions** and can bind to **effector cells**. Effector functions include binding of the Ig to host tissues and to various cells of the immune system and to activation of the complement system. Immune system cells such as macrophages, neutrophils, natural killer (NK) cells, eosinophils, and mast cells have surface receptors for immunoglobulins. These cells interact with the Fc region of the immunoglobulin to initiate functions such as phagocytosis, tumor cell killing, and release of biologically active molecules.

The immunoglobulins have many common structural features, but they differ from one another in the portion of the Ig that binds specifically to the respective antigen. Essentially, each immunoglobulin molecule consists of two identical **light chains**, and two identical **heavy chains**, linked together by disulfide chemical bonds. The typical immunoglobulin molecule can be represented schematically like a "Y" (Figure 11). The heavy chains (twice the size of the light chains) form the central portion of the "Y" configuration, with a light chain on either side of the molecule. The two antigen binding sites (Fab) are generally located at both arms of the "Y" whereas the effector site (Fc) is located at the base of the "Y" antibody structure.

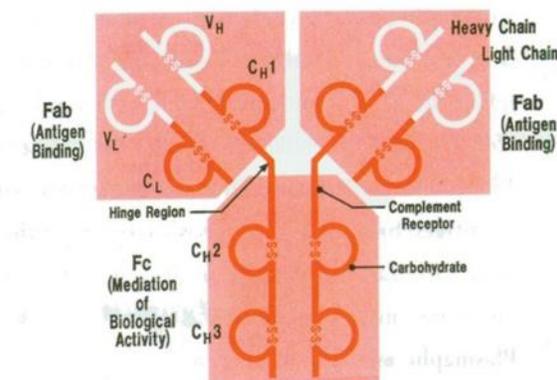


Figure 11 Basic schematic representation of an immunoglobulin molecule. The areas in white on the two arms of the "Y" represent the antigen binding sites, while the base binds with effector cells. [Adapted from *Immunology – A Short Course*, Benjamini, E.,

There are two distinct types of light chains, **kappa light chains** and **lambda light chains**. No known functional differences exist between these two types, and each type can associate with any of the various classes of the heavy chains.

Humans have five different classes (or **isotypes**) of heavy chains, which differ considerably in their physical and biological properties. Both of the heavy chains in any given Ig are identical. The heavy chain determines the class of the immunoglobulin, either IgG, IgM, IgA, IgD, or IgE.

IgG Immunoglobulins

IgG is thought of as the “typical” antibody and is the major immunoglobulin in normal human blood, accounting for 70-80% of the total immunoglobulin pool. It is the predominant immunoglobulin of internal components such as the blood, cerebrospinal fluid, and peritoneal fluid (fluid present in the abdominal cavity). IgG is the only class of immunoglobulin that crosses the placenta, conferring the mother’s immunity to the fetus (the fetus and early newborn are only able to synthesize IgM). IgG is the smallest immunoglobulin, with a molecular weight of 150,000 Daltons (a molecule of water has a molecular weight of 18 Daltons). It is divided into four subclasses, IgG₁, IgG₂, IgG₃, and IgG₄. The synthesis of IgG is largely governed by antigen stimulation, so that in germ-free animals, IgG levels are very low but rise rapidly when the animal is exposed to a normal environment. IgG is a relatively short-lived antibody, with a **half-life** of IgG₃ at 8 days, and IgG₁, IgG₂, IgG₄ at 21 days. It can readily diffuse out of the body’s circulation into the tissues – only 45% of the body’s IgG is in the bloodstream. For this reason, **plasmapheresis** (PP) can only theoretically remove some IgG.

The **monoclonal** antibody rituximab, which is frequently used in the treatment of WM, is an IgG immunoglobulin; in fact, many if not all of the monoclonal antibodies in use or in clinical research are of the IgG class.

The IgG molecule plays an important role in **antibody-dependent cell-mediated cytotoxicity (ADCC)** (see next section). IgG greatly facilitates the destruction of pathogenic organisms by phagocytic cells (macrophages, neutrophils, and natural killer cells) by attaching itself to the pathogen with its Fab portion and attaching its Fc portion to the **Fc receptors** on the phagocytes. IgG can activate the complement system in **complement-dependent cytotoxicity (CDC)** (see next section). IgG molecules can cause the **agglutination** or clumping together of **antibody-antigen complexes** which can then be absorbed and destroyed by phagocytes. IgG can also neutralize viruses by attaching to the virus surface receptors, in turn preventing the viruses from attaching themselves to the target cell and thus preventing infection.

An abnormally large amount of monoclonal IgG is often a hallmark of the malignancy multiple myeloma. IgG has been associated with such diseases as multiple sclerosis, as well as a number of other autoimmune disorders.

IgM Immunoglobulins

IgM is often referred to as the macroglobulin (hence the term Waldenstrom’s macroglobulinemia) because of its size. The IgM is secreted by the cell as a pentamer consisting of five units of the basic immunoglobulin structure joined together by a “J” chain. It is the largest immunoglobulin, weighing in at 900,000 Daltons. Because of its size, 80% of the IgM is in the bloodstream; plasmapheresis can thus remove it quite readily. IgM makes up about 6-10% of the total immunoglobulins in normal individuals, and its half-life is approximately 7 to 8 days. IgM antibody predominates in the early primary immune response to most antigens, although it tends to become less abundant subsequently. An elevated IgM in normal individuals usually indicates a recent infection or recent exposure to antigen (or recent clinical immunization). The fetus only synthesizes IgM, beginning at approximately five months of gestation; the IgM molecule does not cross the placenta, and an elevated level of IgM in the newborn (or fetus) is indicative of a congenital or perinatal infection. IgM, often accompanied by IgD, is the most common immunoglobulin expressed on the surface of immature B-cells. WM cells express both IgM and IgD on their cell surface. Mature plasma cells, on the other hand, typically do not express IgM and IgD.

The IgM antibody is a very powerful antibody. It is the most efficient initiator of complement-dependent cytotoxicity (CDC). IgM is a very efficient agglutinating antibody, mainly because of its large pentameric form (Figure 12).

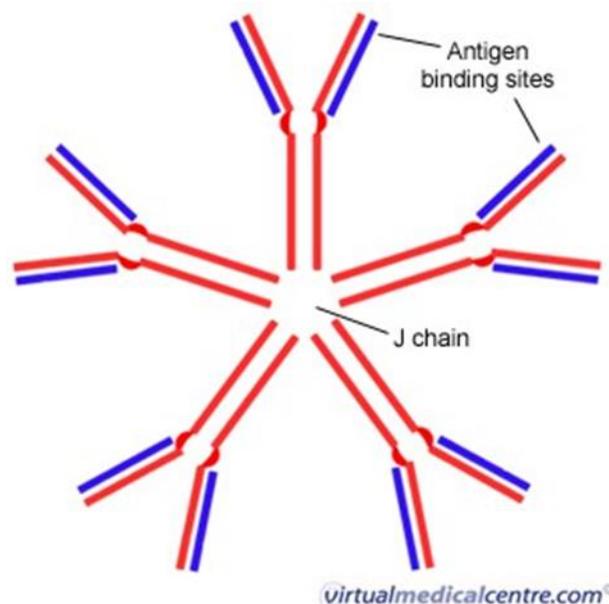


Figure 12 Schematic representation of the pentameric form of IgM with the “J” joining chain.

The IgM antibodies include the **isohemagglutinins** – the naturally occurring IgM antibodies against the red blood cell antigens of the ABO blood group. People with type A blood have IgM isohemagglutinins to the B antigens; those with type B blood have antibodies to the A antigens; and an individual with AB blood has neither anti-A nor anti-B antibodies. Interestingly, people with type O blood group can have IgM and IgG isohemagglutinins to the A and B antigens. Transfusion reactions arise as a result of ABO incompatibility, in which the recipient’s isohemagglutinins react with the donor’s red blood cells.

Autoimmune hemolytic anemia (destruction of red blood cells) can be caused by IgM **autoantibodies** as well as IgG autoantibodies. Autoimmune hemolytic anemias can occur from one of several causes: idiopathic hemolytic anemia, in which there is no evidence of underlying disease; as the result of an abnormal drug reaction; in malignant lymphomas such as WM and chronic lymphocytic leukemia (CLL) and infrequently in multiple myeloma; and as seen in autoimmune diseases like systemic lupus erythematosus. Large doses of corticosteroids and splenectomy are often required to control symptoms. **Cold agglutinin disease**, caused primarily by either infections or cancers such as lymphoma, is usually characterized by high levels of agglutinating IgM antibodies capable of causing complement-dependent immune hemolysis (destruction) of the red blood cells. These autoantibodies are very temperature sensitive and characteristically react optimally in the presence of a cold stimulus. **Cryoglobulinemia**-Cryoglobulins are proteins that precipitate when cooled and dissolve when heated. They may be classified as Type I (monoclonal: IgG, IgM or IgA). Only IgM is associated with Waldenstrom’s Macroglobulinemia. They are rarely of clinical consequence because they form at 0 degrees C and therefore are asymptomatic and of no clinical consequence. They occur in approximately 10% of patients with WM. Mixed **cryoglobulinemia** (Type II) is caused by monoclonal IgM proteins that have activity very similar to rheumatoid factor (RF) – an autoantibody frequently noted in many autoimmune diseases, rheumatoid arthritis for example. The IgM reacts with the Fc portion of **polyclonal IgG cryoglobulins**, resulting in an IgM- IgG **cryoprecipitable immunocomplex** that has limited solubility in the blood (particularly when exposed to the cold) and causes a host of clinical manifestations. The most commonly affected tissues include those of the skin and kidney. A strong association between mixed cryoglobulinemia and the hepatitis C virus (HCV) has been reported. Type III cryoglobulins are polyclonal and no monoclonal protein is present. Blood samples of patients with cryoglobulins should be collected and processed at body temperature to avoid inaccurate laboratory results for some of their blood tests.

Peripheral neuropathy may affect up to 30% of patients with WM. To date, five distinct antigenic targets on peripheral nerves for the IgM immunoglobulin have been identified. Treatment consists either of symptom relief or definitive reduction of the offending IgM molecule by chemotherapy, immunotherapy, or plasmapheresis.

A large amount of IgM in the blood, as in WM, can result in increased **serum viscosity (SV)**. Symptoms of hyperviscosity are often first recognized by the patient and include chronic bleeding from the nose, gums, and less commonly from the gastrointestinal tract; headache; ringing in the ears (tinnitus); dizziness (vertigo); impaired hearing; blurring or loss of vision;

distended, sausage-shaped veins in the retina; and swelling of the optic disc at the back of the eye (papilledema). These symptoms will often warrant immediate therapy in the form of plasmapheresis, frequently followed by additional therapy to control the underlying disease.

IgA Immunoglobulins

The IgA immunoglobulin is 160,000 Daltons in size. IgA represents approximately 10-15% of serum Igs. The half-life of IgA is estimated to be 5.5 days. On B-cell surfaces or in the blood, IgA exists as a monomer comprised of only one four-chain unit. Most IgA is present not in the blood, however, but in secretions of saliva, tears, sweat, milk, the genito-urinary and gastrointestinal systems and the tracheo-bronchial tree. Secretory IgA frequently combines to form primarily two unit dimers and rarely up to three to five unit polymers (which can also cause hyperviscosity in the blood).

IgE Immunoglobulins

Although it normally represents only a small fraction of all serum antibodies (0.004%), IgE is extremely important from a clinical standpoint because of its involvement in allergic disorders. The IgE molecule weights about 200,000 Daltons and has a half-life of 2 days. The mast cell and basophil cell have a unique, very reactive Fc receptor that is specific for IgE antibodies, and thus the IgE molecules are found predominantly attached to these cells. When IgE comes into contact with an antigen (also known in this case as an **allergen**), the mast cell or basophil releases inflammatory molecules that trigger many of the acute manifestations of an allergic reaction. Elevated IgE serum levels can also be seen in infections caused by multicellular parasites such as intestinal worms.

IgD Immunoglobulins

IgD is the least well known and characterized immunoglobulin. It is present in very small amounts, less than 1% of the total plasma immunoglobulins. The molecular weight of IgD is approximately

185,000 Daltons, it is a very fragile molecule, and its half-life is 2 -3 days. IgD is not secreted by plasma cells and has no known function in the serum. It is a major component of the surface membrane of many B-cells. Its presence on B-cells serves as a marker of B-cell differentiation and may control lymphocyte activation and suppression. There is much interest in cancer research in further understanding the complex role of the IgD immunoglobulin as it relates to the abnormal differentiation of the B-cell into a malignant cell. As was noted earlier, WM cells express IgM and IgD molecules on their surface membranes.

Interactions of Immunoglobulins and Antigens

Immunoglobulins form multiple bonds with antigens at the Fab sites on the Ig. Although these bonds are weak compared to the typical covalent bonds found in biochemistry, the large number of interactions results in a large total binding energy (**avidity**). The strength of these bonds is dependent on the distance between the interacting groups. A "good fit" is essential between the antigen and the Fab binding site of the antibody. The strength of the bond between an antigen and an immunoglobulin is known as the antibody **affinity**. Immunoglobulins are capable of expressing remarkable specificity to an antigen and are able to distinguish between small differences in the chemical composition of the antigen. The electrical charges of the antigen, the amino acid sequence of protein antigens, as well as the three dimensional shape of the antigen, are crucial determinants of antigen-antibody specificity, avidity, and affinity.

ANTIBODY-DEPENDENT CELL- MEDIATED CYTOTOXICITY (ADCC) AND COMPLEMENT- DEPENDENT CYTOTOXICITY (CDC)

One of the major classes of immunoglobulins, the IgG antibody, plays an important role in antibody-dependent cell-mediated cytotoxicity (ADCC). In this form of cytotoxicity, the Fab portion of the specific IgG binds with the target cell, be it a microorganism or a tumor cell (bound by rituximab, for example), and the Fc portion of the IgG antibody binds with specific Fc receptors found on lymphocytes called natural killer (NK) cells and certain other cell types. Thus the NK cells are able to come in contact with the antigen-bearing target cell, which might be bacteria, multicellular parasites, or even tumor cells, and destroy the target by releasing various substances called cytotoxins. The antibodies can thus be said to “arm” the NK cells to perform ADCC. This is the major mechanism whereby rituximab appears to exert its cytotoxic effects on the WM cancer cell. Rituximab attaches its Fab sites to the CD20 molecule (target antigen) found on WM B-cells and its Fc site to the Fc receptor on effector cells (natural killer cells and macrophages). Rituximab thus recruits the body’s own immune system to destroy the malignant WM B-cell.

Higher circulating NK cell levels and responses to rituximab in WM patients appear to be influenced by polymorphisms (natural genetic variations) present on the Fc receptor of natural killer cells called FcγRIIIA (CD16). A simple difference in the sequence of amino acids from phenylalanine to valine at position 158 of the FcγRIIIA receptor can result in significantly better rituximab/NK cell binding, subsequently more potent ADCC, and a better response to rituximab therapy. Some researchers have therefore suggested that rituximab administration can be adjusted according to the FcγRIIIA genetic makeup of an individual.

Certain types of antibodies can activate the complement pathway when bound to an antigen. IgM and IgG antibodies are principally involved in complement-dependent cytotoxicity (CDC). The activation of complement results in the release of several important biologically active molecules and leads to the destruction, or lysis, of the target cell membrane if the antigen is on the surface of the cell in question. Some of the components of complement bind to the target antigen and cause phagocytes, which carry receptors specific to the complement protein, to destroy the target antigen. The activation of the complement pathway can result in the production of chemotactic molecules, which serve to attract phagocytic cells. The release of histamine and other inflammatory molecules by mast cells and basophils can also be facilitated by components of complement. Briefly, the activation of complement by an IgG antibody such as rituximab has profound effects on the host and on the target antigen if it is a live cell, such as a malignant WM B-cell.

FUNDAMENTALS OF GENETICS

In the past few years there has been an explosion of research and discoveries in cancer genetics of special interest to WM patients. This revised section, which has been requested by many intellectually curious WM patients and caregivers, will introduce the interested reader to the fundamentals of genetics and illustrate how several of these principles can be applied to the study of WM-related genetic anomalies.

Genetic Material

The cell has genetic material contained in the cell nucleus and in the mitochondria. There are two different kinds of genetic material in the cell: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

DNA basically contains the instructions for making the proteins essential for life. DNA carries all of the information the cell or organism needs for its physical characteristics, determining as well which cells should grow and when and which cells should die and when. Essentially the information contained in DNA ensures that the cell or organism functions correctly.

The information in DNA needed to make proteins is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T). The sequence of these bases determines the blueprint for building proteins and ensuring the proper function of a cell (and by extension an organism). This blueprint is referred to as the “genetic code.” DNA bases pair up with each other, A with T and C with G, to form units called base pairs. Each base is also attached to a sugar molecule (deoxyribose) and a phosphate molecule and forms a molecule called a **nucleotide**. Nucleotides are arranged in two long strands that form a spiral called a double helix. The structure of the double helix resembles a ladder: the base pairs form the ladder’s rungs and the sugar and phosphate molecules form the vertical sidepieces of the ladder (Figure 13). Each strand of DNA in the double helix can serve as a pattern for replicating itself, an important property of DNA.

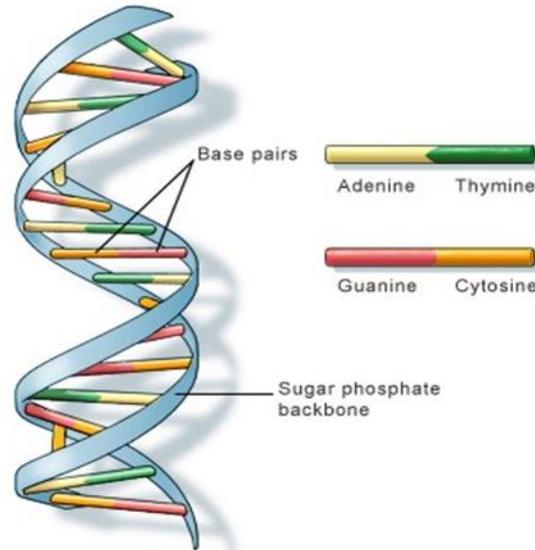


Figure 13 The double helix structure of DNA.

RNA (ribonucleic acid) is a nucleic acid molecule similar to DNA but containing the sugar ribose rather than deoxyribose. RNA is used for information transport (messenger RNA or mRNA), enzymatic functions (ribosomal RNA), and to aid in the building of proteins (transfer RNA or tRNA).

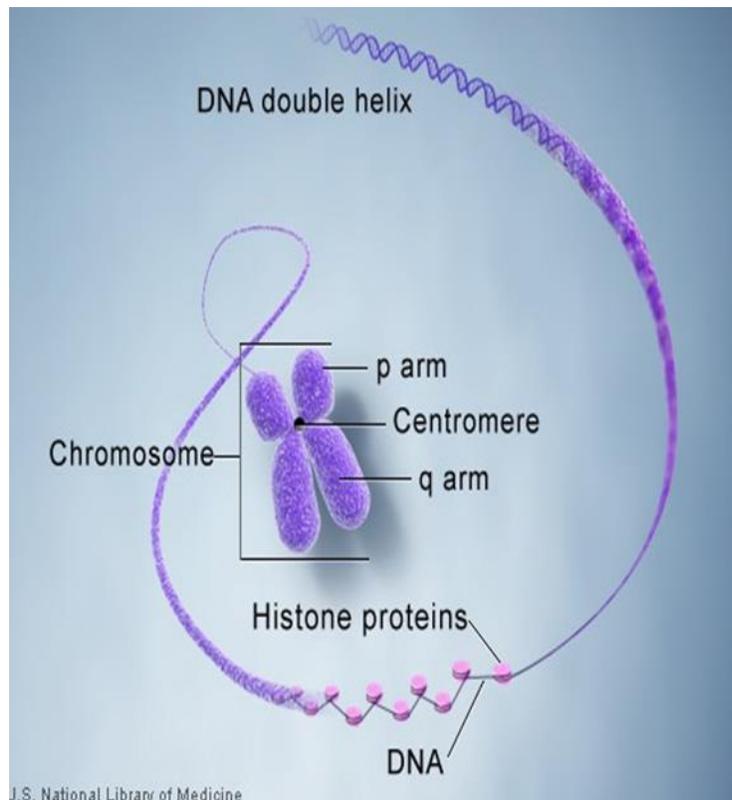
Genes

Genes are the basic physical and functional unit of heredity. The order of the DNA nucleotides within a gene specifies the blueprint needed to make a specific protein. It is estimated that humans have over 20,000 genes that can vary in size from a few hundred DNA bases to more than 2 million bases. Only a small number of genes (less than 1% of the total) are slightly different between people. Small differences in the sequence of DNA bases within a gene contribute to each person’s unique physical features and are called **alleles**. Alleles are thus simply variants of a gene.

Chromosomes

The DNA molecules which make up the genes are packaged into linear structures called **chromosomes**, which are tightly coiled around specialized structural proteins called histones. Each chromosome is divided into two sections (or arms) by a constriction point called the **centromere**. The short arm of the chromosome is called the “p” arm, and the long arm of the chromosome is called the “q” arm. The exact location of the centromere gives the chromosome its characteristic shape and can be used to help describe the location of specific genes (Figure 14).

One of the major obstacles to WM research in the past has been the lack of large-scale genetic studies performed in collaboration with multiple research centers. The design of targeted therapies in WM has been hindered by the lack of adequate genetic and molecular characterization of WM cells. In order to address this issue, the IWMF has funded the development of an integral tissue bank (bone marrow samples, blood samples, and saliva samples) that is linked to clinical characteristics from patients with different stages of WM. The tissue bank will make these samples available to other researchers and permit the screening of WM patients for common cancer-causing mutations that have been described in other tumors.



J.S. National Library of Medicine

Figure 14 DNA is tightly coiled around a histone into a chromosome with a short “p” arm and a long “q” arm.

We may resemble our parents, but we are never exactly like them. In humans the genome is divided into 46 chromosomes, including 22 corresponding chromosome pairs and a pair of sex chromosomes, X and Y (Figure 15). About half our DNA comes from our mother, and the other half comes from our father. The distribution of chromosomes we get from our parents is virtually random, and each child may receive a different subset of the parents’ DNA (unless he or she is an identical twin). Women carry two X sex chromosomes, men carry one X and one Y; fathers therefore “determine” the sex of their offspring by donating either an X (daughter) or a Y (son) sex chromosome to the mother’s X chromosome.

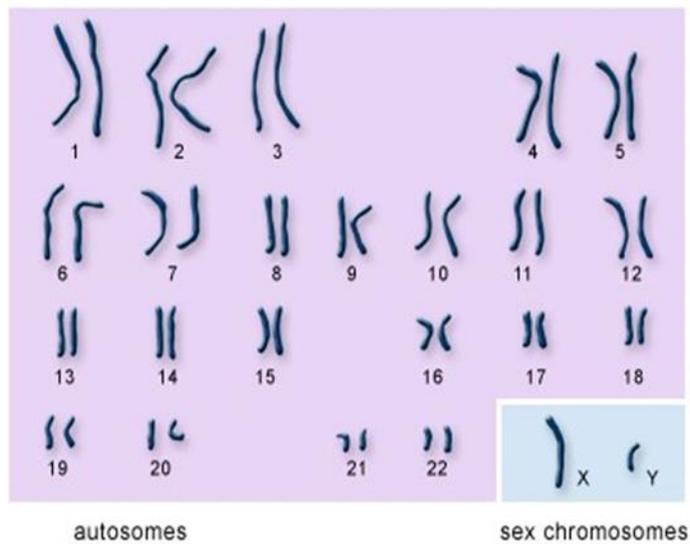


Figure 15 The 23 pairs of chromosomes typically found in humans.

Proteins

Proteins are large, complex molecules that are essential for the structure, function, and regulation of cells and, by extension, the body's tissues and organs. Proteins are made up of smaller units called **amino acids** and can occur in chains (or polypeptides) of hundreds or thousands of amino acids. There are 20 different types of amino acids that can be combined to make a protein. The amino acid sequence in a protein is defined by the sequence of DNA bases within a gene (more on this later). The protein's unique three-dimensional structure defines its specific function.

Common examples of proteins important in immunology include:

- Immunoglobulins (antibodies) that bind to specific foreign particles and help protect the body from viruses and bacteria.
- Enzymes which are important proteins that allow the cell to carry out almost all of the thousands of chemical reactions in cells in a very quick and efficient manner.
- Messenger proteins that transmit signals between different cells, tissues, and organs and help coordinate biological processes.
- Transport/binding proteins that bind and carry atoms and small molecules within cells and throughout the body.

Gene Expression

Proteins have their own unique amino acid sequence and are assembled using information encoded in genes. The genetic code is a set of three-nucleotide sets called **codons**, and these codons specify which of the 20 amino acids is included in the protein; each three-nucleotide combination designates an amino acid (Figure 16). As there are a possible 64 codons and only 20 amino acids, some repetition in the genetic code exists. The order of codons in the gene specifies the order of amino acids in the protein. A **"start" codon** designates the beginning, and a **"stop" codon** designates the end of the gene.

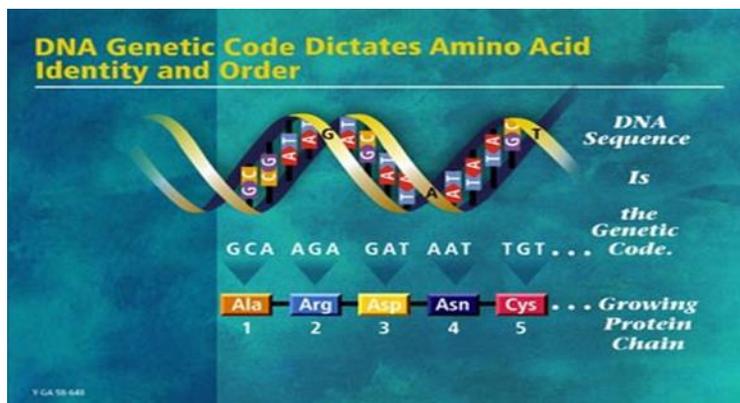


Figure 16 Assembling an amino acid chain from codons.

The process by which genes make proteins is called gene expression (Figure 17). Each strand of DNA in the double helix can serve as a pattern for duplicating the sequence of bases. There are two principal steps in gene expression: **transcription** and **translation**.

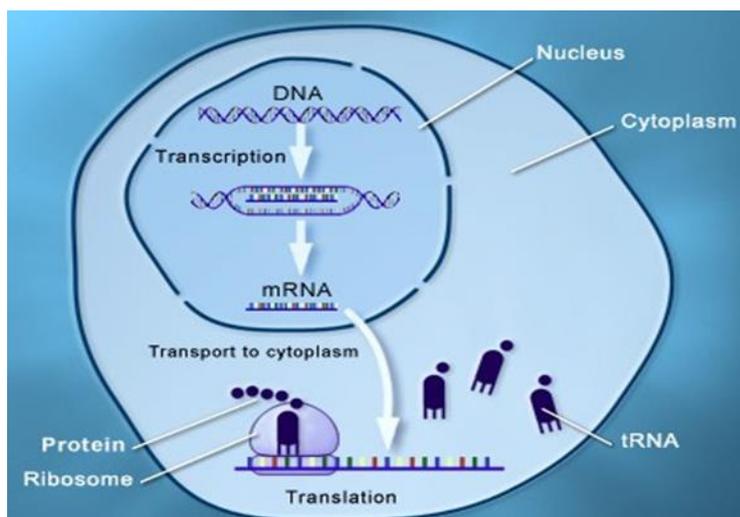


Figure 17 The process of gene expression – assembling a protein by transcription and translation.

During transcription, the gene's DNA sequence is copied to a single- stranded messenger RNA molecule (mRNA) whose nucleotide sequence is complementary to the DNA from which it was transcribed. The transcription is performed by an enzyme called RNA polymerase. The ensuing information, now coded in the mRNA, is carried out of the nucleus into the cytoplasm.

Translation is the process of synthesizing a protein from a mRNA molecule. Translation is carried out by specialized cellular organelles called ribosomes. The mRNA is loaded onto the ribosome which matches each codon to the corresponding amino acid and adds the new amino acid(s) to the growing protein molecule. The new protein must fold to its active three-dimensional structure before it can carry out its cellular function.

Gene Regulation

Cells have the capability to turn on or off only a fraction of their many genes. This is called gene regulation. Gene regulation occurs most commonly during transcription (see above) but can occur at any point during gene expression. Gene regulation enables embryonic stem cells to develop into either red blood cells or white blood cells (or any type of cell) and to react quickly to changes in their environments. Transcription factors are specialized proteins that bind to regulatory regions of a gene and increase or decrease the level of transcription, thereby determining the amount of a certain protein made from a gene. Tight regulation of cell growth and division ensures that a dividing cell's DNA is copied properly.

MicroRNAs (also called miRNAs) are small non-coding RNA molecules which function as transcriptional and post-transcriptional regulators of gene expression. They can bind to messenger RNA, and when they do, the messenger RNA is silenced and can no longer be translated into proteins by the ribosomes. Abnormal expression of microRNAs has been implicated in

numerous disease states, including cancer, and is currently being investigated in WM. Transcription factors are specialized proteins that bind to regulatory regions of a gene and increase or decrease the level of transcription, thereby determining the amount of a certain protein made from a gene.

Epigenetics

Although the human genome still retains its status as the blueprint for the cell, the **epigenome**, the way the DNA and genes are marked and packaged inside the cell nucleus with the addition of chemical compounds, directs the cell which instructions in the blueprint to follow and which to ignore. Gene activity can be affected by modifications (called epigenetic changes), even if these modifications do not change the actual DNA sequence. Epigenetic modifications explain why a white blood cell does not look or act like a brain cell, even though they both carry the same DNA. Epigenetic modifications can influence the production of proteins in certain cells, ensuring that only necessary proteins are produced, or not, and is often the reason why a perfectly normal cell goes bad and becomes a cancer cell.

A number of chemical compounds, added to single genes, can regulate a gene's activity. Two main epigenetic processes have been identified thus far that accomplish this (Figure 18). Small molecules called methyl groups can be added to a particular gene, resulting in that gene being turned off or silenced so that no protein is produced (DNA methylation). The addition of acetyl groups to the specialized structural proteins called histones (that the DNA molecules are tightly coiled around) can modify histones so that different genes are activated or inactivated by allowing or blocking transcription factors and other proteins access to the DNA. This process is called histone acetylation.

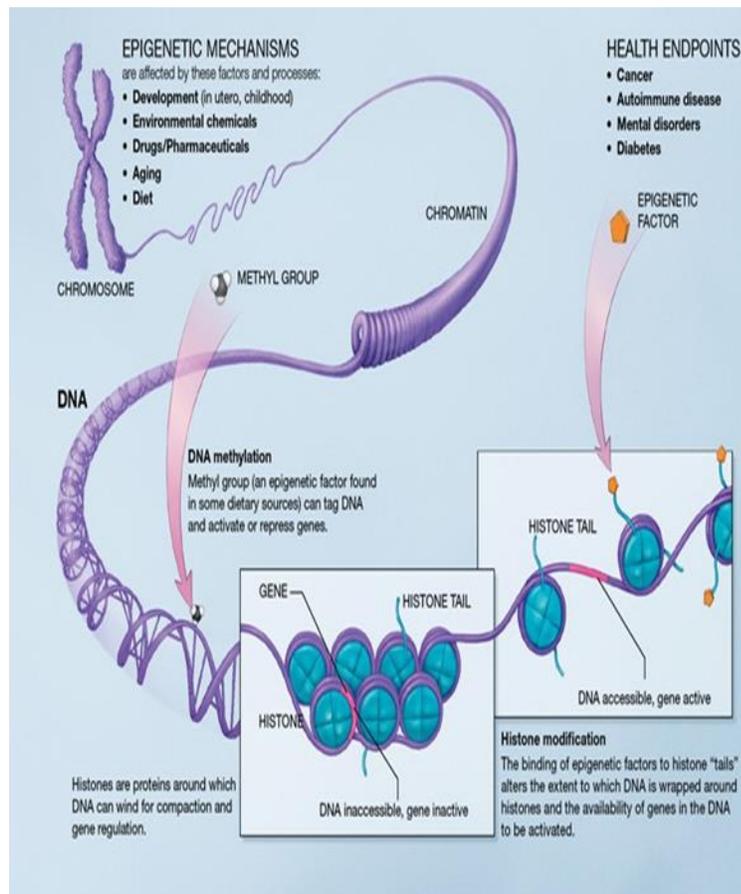


Figure 18 The process of epigenetic modification.

Patterns of epigenetic modification vary among individuals, and even among different cells within an individual. The epigenetic modifications remain as cells divide and in some cases can be inherited.

WM cells are characterized by unbalanced epigenetic activity. A number of new drugs that target histone acetylation and thus induce cell death have been studied in clinical trials. The histone deacetylase inhibitor panobinostat showed activity in patients with relapsed/refractory WM, indicating a potential role for histone deacetylase inhibition in WM.

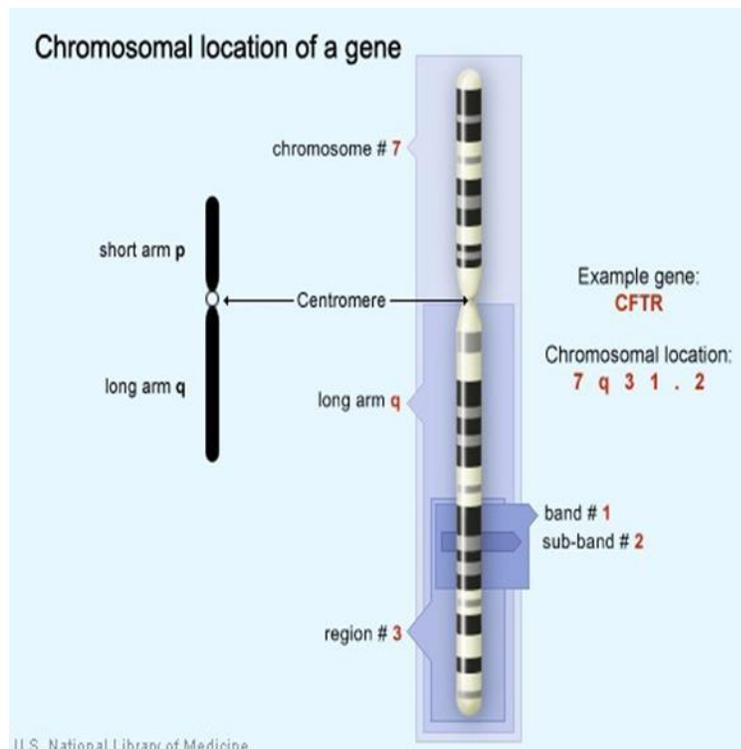
Environmental factors, such as diet and exposure to pollutants, can also lead to epigenetic modifications. Epigenetic errors are known to be related to the development of cancers, metabolic disorders such as diabetes, and degenerative disorders such as ALS (amyotrophic lateral sclerosis).

Identification of Gene Locations

The Human Genome Project, an international research effort completed in 2003, identified the sequence of base pairs for each human chromosome. This has allowed researchers to provide a more specific “address” for the location of many genes. The molecular location describes the precise position of that gene in terms of base pairs, indicates the size of the gene, and also allows researchers to determine exactly how far a gene is from other genes on the same chromosome.

Another type of “map” used by researchers to describe a gene’s position employs its cytogenetic location on the chromosome. The cytogenetic location is based on a distinctive pattern of bands created when chromosomes are stained with certain chemicals.

A combination of numbers and letters provide a gene’s “address” on a chromosome (Figure 19). The address contains several key parts: the number of the chromosome on which the gene can be found (chromosomes 1 through 22 – plus the sex chromosomes designated by X or Y); the arm of the chromosome (the shorter arm is called p, and the longer arm is called q); and the position of the gene on the p or q arm (based on a distinctive pattern of light and dark bands that appear when the chromosome is stained).



U.S. National Library of Medicine
Figure 19 Determining the address of a gene on a chromosome.

The L265P somatic mutation found in the MYD88 gene (myeloid differentiation primary response gene 88) in a majority of patients with WM was identified at the molecular location 38182641 at 3p22.2. Although less prevalent, another group of mutations occurs in the gene CXCR4 resulting in disease progression in patients with WM and a less favorable prognosis. Research is being funded by the IWMF to test an inhibitor of CXCR4 to see if it has potential as treatment for patients with WM who harbor this mutation.

Gene Families

Gene families are sets of several similar genes, usually formed by duplication of a single original gene that provides instructions for making proteins with similar biochemical functions. Gene families can also contain distinct genes that are grouped together based on the fact that proteins produced from these genes participate closely in the same biochemical function(s). Gene families are used by researchers to assist in determining the function of newly identified genes based on their similarity to known genes. One well-studied gene family is the ABO blood group family that determines the A, B, and O blood types.

Gene Mutations

Recall from an earlier section that humans have an estimated 20,000-25,000 genes; new ones are being identified every day. These genes can vary in size from a few hundred DNA bases to more than 2 million bases. Given the inherent complexity of the genome and the rapid turnover of cells in our body, cells sometimes make mistakes during the copying process – like typos – although not as frequently as one may expect. These “mistakes” are referred to as gene **mutations** (Figure 20).

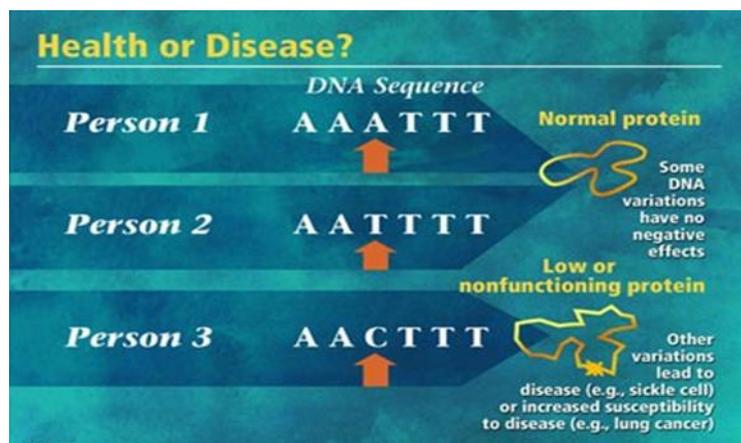


Figure 20 Mutations in the DNA sequence may have no negative effects or may cause disease.

A gene mutation is a permanent change in the DNA sequence of a gene. These mutations may range in size from a single DNA building block (DNA base) to a large segment of a chromosome. Since the genetic code has built-in redundancies, these mistakes may not always have much effect on the protein made by the gene. In some cases, the error might be in the third base of a codon and still specify the same amino acid in the protein. In other cases, it may be elsewhere in the codon and specify a different amino acid. If the changed amino acid is not in a crucial part of the protein, there may be no adverse effect. However, if the changed amino acid is in a crucial part of the protein, the protein may be defective and not work as well, if at all (e.g., the MYD88 mutation identified in WM). This type of change can lead to disease.

There are two ways in which gene mutations can happen: they can be inherited from a parent (hereditary mutations or germline mutations) or they can be acquired throughout one's lifetime (somatic mutations). Hereditary mutations can be passed on to descendants via their parents' reproductive cells and typically remain present throughout a person's life. Acquired (somatic) mutations occur in the DNA of individual cells at some time throughout an individual's lifetime. These types of mutations are not inherited from a parent and are not passed to offspring, unless they occur in an egg or a sperm. Acquired mutations can be caused by environmental factors (pollutants, viruses, radiation) or can occur if a mistake is made during DNA replication.

Natural variations in genes usually have little or no adverse effects on the health of the individual (e.g. blood type, hair color, eye color). Normal genetic variations that produce differing characteristics in individuals in the general population occur with fairly high frequency and are called **polymorphisms**.

A gene mutation that leads to variations in the DNA sequence at a particular location is called a single nucleotide polymorphism, or SNP (“snip”). Most SNPs have no effect on health or development. Some of these genetic differences, however, have proven to be very important in the study of human health. Researchers have found SNPs that may help predict an individual’s response to certain drugs, susceptibility to environmental factors such as toxins, and the risk of developing particular diseases. SNPs can also be used to track the inheritance of disease genes within families.

As noted earlier, not all genetic mutations have bad consequences; some mutations alter a gene’s DNA sequence but do not change the function of the protein made by the gene. Genetic mutations create genetic diversity, which in turn keeps populations healthy. However, some mutations can lead to missing or malformed proteins, and that can lead to disease (e.g. cystic fibrosis, sickle cell anemia). Most inherited genetic diseases are recessive, meaning that an individual must inherit two copies of the mutated gene in question to develop a disease; thus, two genetically similar adults are more likely to give a child two copies of a defective gene this is one reason that marriage between close relatives is discouraged.

Cancer usually results from a series of mutations within a single cell; the mutations can be either hereditary or somatic or both. Often, a faulty, damaged, or missing gene is the principal culprit in cancer. The p53 gene, for example, makes a protein that stops mutated cells from dividing. Without this protein, cells divide unchecked and become tumors.

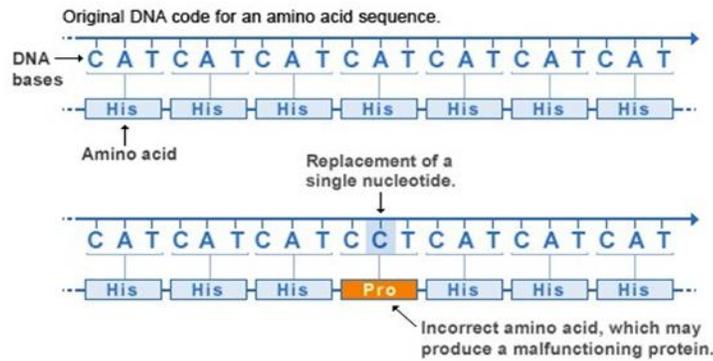
A single mutation in the MYD88 gene sequence causes the substitution of one amino acid (leucine) with another amino acid (proline) at the 265 position (MYD88 L265P). This mutation results in an abnormal signaling cascade (B cell signaling pathway) in the majority of WM patients. MYD88L265P activates an enzyme in this B cell signaling pathway called Bruton’s tyrosine kinase (BTK) which enhances the survival of WM cells by subsequent activation of a protein called NF kappa-B. **Ibrutinib** targets this mutation by inhibiting BTK.

Types of Gene Mutations

As we have previously noted, mutations result from unrepaired damage to DNA (often caused by radiation or chemicals), errors in the process of replication, or from the insertion or deletion of segments of DNA in genes (usually by viruses).

Point mutations, often caused by chemicals or malfunction of DNA replication, exchange a single nucleotide for another within the protein coding region of a gene and can be classified depending upon the resulting error:

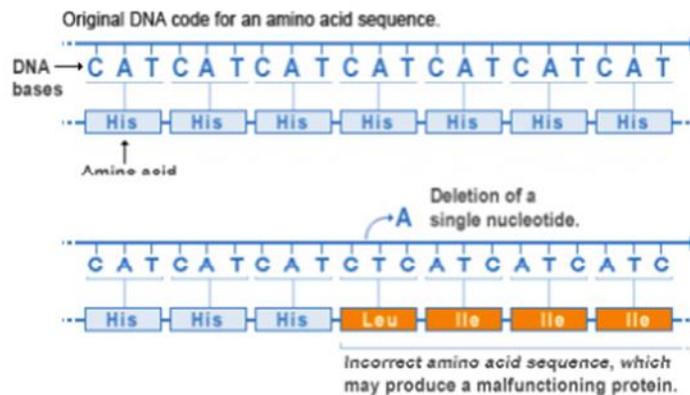
- A silent mutation codes for a change in one DNA base pair that results in the same or similar amino acid and has no effect on protein structure and function.
- A missense mutation codes for a change in one DNA base pair that results in the substitution of one amino acid and may or may not affect the protein structure and function (Figure 21).



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Figure 21 A missense mutation. A change in one nucleotide results in the substitution of one amino acid for another, thereby altering the protein. It may or may not affect the structure and function of the protein.

- A nonsense mutation codes for a change in one DNA base pair that prematurely signals the cell to stop building a protein, resulting in a shortened protein that functions improperly or not at all.
- A frameshift mutation is a genetic mutation that arises when the normal DNA sequence of a gene is disrupted by the insertion or deletion of one or more nucleotides, provided that the number of nucleotides added or removed is not a multiple of three (i.e. a codon). Because of the triplet nature of gene expression by codons, the insertion or deletion can change the reading frame (groups of 3 bases that each code for one particular amino acid), resulting in a completely different, and usually nonfunctioning protein, than the original. Insertions and deletions can be thought of as frameshift mutations.
- Insertions add one or more extra nucleotides into the DNA, in effect changing the sequence of the DNA, and can significantly alter the protein structure and function.
- Deletions remove one or more nucleotides from the DNA and also change the sequence of the DNA, altering the protein structure and function. Small insertions or deletions may add or remove one or a few base pairs within a gene; conversely large insertions or deletions can add or remove an entire gene or several neighboring genes (Figure 22).



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Figure 22 A frameshift mutation. In this case, deletion of a single nucleotide alters the codon reading sequence, resulting in a different protein.

Foreign genetic material (most commonly DNA, but RNA as well) can be artificially introduced into the cell by a process called transfection. The transfection can be transient if the DNA is not integrated permanently into the cell's genome, but the foreign genes can nonetheless be expressed for a limited time (24-96 hours) or can be considered stable if the foreign DNA is actually inserted into the cell's genome. Transfection is a very useful and commonly used research technique.

Chromosomal Abnormalities

Genetic abnormalities resulting from changes in the structure of chromosomes can affect many genes and cause errors in the structure and function of proteins, which in turn may lead to defects in growth, development, and function. These changes can occur at any time: during the formation of reproductive cells, in early fetal development, or well after birth. A chromosome abnormality can result from a missing, an extra, or an irregular portion of chromosomal DNA. Segments of DNA can be rearranged either within one chromosome or can be transferred between two or more chromosomes. The size and location of structural chromosome changes may cause either significant medical problems or may have no impact on an individual's health. Common chromosome structural rearrangements include:

- Translocations – occur when a chromosomal segment breaks off one chromosome and attaches to another. Translocations can be either balanced if no genetic material is gained or lost, or unbalanced if the inverse is the case.
- Deletions – occur when a break in the chromosome results in the loss of genetic material.
- Duplications – occur when a portion of the chromosome is duplicated, resulting in extra genetic material.
- Inversions – occur when a portion of a chromosome breaks off, is turned upside down, and is reattached; genetic material may or may not be lost.

GENETIC PREDISPOSITION

A genetic predisposition is the greater genetic likelihood, or susceptibility, of developing a disease due to the presence of one or more inherited gene mutations. These genetic mutations may contribute to the development of a disease but do not directly cause it. It is therefore important to note that people with genetic predispositions do not always develop the disease to which they may be predisposed. While genes may be a reliable predictor of certain diseases, a person's lifestyle choice, the environment, or other as yet unidentified genes may also be important, perhaps more so, as contributing factors in the development of the disease in question. Essentially, individuals who may be predisposed to an illness by virtue of inherited genes are not always going to express the inherited genes and in turn develop the disease.

One very real concern with evaluating genetic predisposition by genetic testing is that it will be used to discriminate against others. Health insurance companies, life insurance companies, and even employers could demand genetic testing and purposefully reject anyone who has genes that might suggest elevated risk for disease. Countries like the U.S. have signed laws prohibiting discrimination based on genetic factors, but as with any other form of discrimination, it is still possible to break or circumvent these laws.

BASIC GENETICS APPLIED TO IMMUNOLOGY

Immunoglobulin Genetic Structure

Immunoglobulins are an amazingly diverse group of biological molecules. When one considers the millions of different antigenic shapes in the environment and the capacity for immunoglobulins to provide enough different combining sites to recognize them, we can then truly marvel at the complexity of nature and evolutionary mechanisms. It has been suggested that we produce more different forms of immunoglobulins than all of the other proteins of the body put together. We in fact produce more types of immunoglobulin than there are genes in our genome! How can all this diversity be possible? Numerous theories of antibody formation abound, and in this section we will first review the immunoglobulin molecule in more detail and subsequently attempt to provide a simplified explanation of basic immunoglobulin genetics.

In the previous section on IMMUNOGLOBULINS, we were introduced to the basic four-chain "Y" shaped Ig molecule. The two larger heavy (H) chains, roughly twice the size of the smaller light (L) chains, determine the class or isotype of the immunoglobulin (e.g., IgG, IgM, IgA, IgE, or IgD). Any of the light chain types (kappa or lambda) may combine with any of the heavy chain types, but all of the light chains and all of the heavy chains in any single immunoglobulin molecule are

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identical. The chains are held together by strong interchain disulfide bonds to form a bilaterally symmetrical structure (Figure 11). IgA, and in particular IgM, form polymers of multiple Ig molecules (IgM forms a five unit structure called a pentamer, held together by a “J” chain at the center).

The H and L chains are both composed of folded globular shaped **domains**, each of which are 100-110 amino acids long and contain a single intrachain disulfide bond. Light chains always contain two of the domains, whereas heavy chains contain either four or five domains. The domains are typically separated by short unfolded stretches of chains.

When different immunoglobulins are compared, the sequence of the H and L chains can vary widely. This variability is most notable at the Fab portion of the Ig, the tips of the “Y” structure (also called the N-terminal domain). For this reason, this domain is called the **variable region**, abbreviated VH or VL respectively, depending on whether the domain is on the heavy chain or the light chain.

The second and subsequent domains on both chains are much more constant in amino acid sequence and are designated CL or CH1, CH2, CH3, etc., depending on whether the domain is on the light chain or the heavy chain. The biological functions of the immunoglobulin molecule derive from the properties of the **constant region**, which is identical for immunoglobulins within a certain class. This is also where the Fc part of the molecule attaches itself to various cells in the immune system that have Fc receptors. The antigen-binding site (Fab) of the Ig molecule is formed by the VL and VH domains, which are always positioned directly across from each other. Each basic four-chain unit thus contains two separate but identical antigen-binding sites.

The antigen specificity of a given Ig molecule is determined by the combined sequences of its VL and VH domains and for this reason can vary widely among immunoglobulins. In effect, three **hypervariable regions** of 9-12 amino acids in length are found within each of the VL and VH domains. Antigen binding primarily involves these hypervariable regions, thus the sequences of these regions are the primary determinants of antigen specificity. In most immunoglobulins, a short segment of amino acids is found between the CH1 and CH2 regions of the H chains. This region permits flexibility between the Fab arms of the Y shaped antibody molecule and is called the **hinge region**. It allows the two Fab arms to open and close to accommodate binding to two antigens. This hinge region can also be cleaved by enzymes such as papain or pepsin, to yield the distinct Fab and Fc fragments of the immunoglobulin (Figure 23).

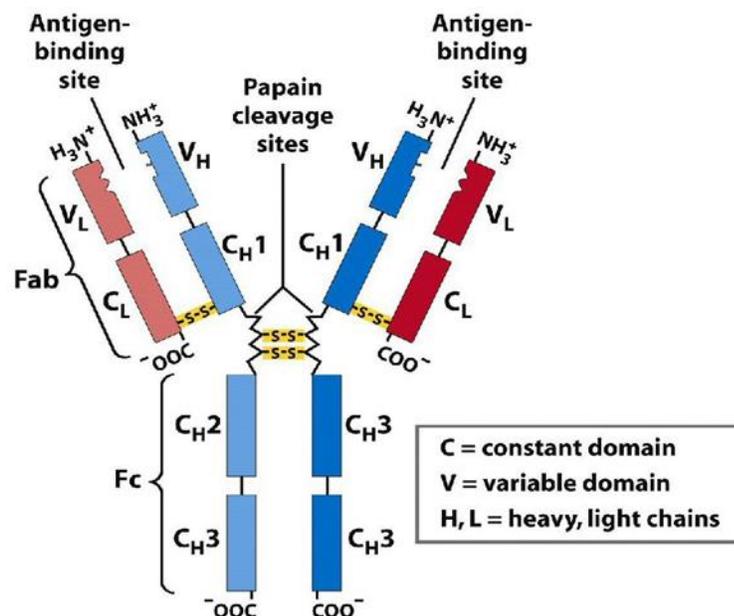


Figure 23 Schematic model of an IgG antibody molecule showing the basic four-chain structure and domains. [Adapted from *Lehninger Principles of Biochemistry Fifth Edition*, W.H. Freeman and Company, 2008]

Immunoglobulin Gene Recombination

Immunoglobulin genes are formed in B-cells by rearrangement of DNA. In order for the immune system to produce the virtually unlimited variety of immunoglobulins of almost infinite specificities to contend with any possible antigen that may be encountered in the environment, the genetic machinery of the B-cell must be able to produce a very large number of variable domain sequences. The sequence of the constant domains, on the other hand, is generally the same for all heavy or light chains of a given immunoglobulin class. Thus, immunoglobulins consist of a relatively small number of different constant domains linked in various combinations to an almost unlimited assortment of variable domain sequences.

Synthesis of Light Chains

In the differentiation of an immature B-cell to an antibody-forming plasma cell, the V and C gene segments of the kappa light chains are joined by a short section of DNA called the joining (J) segment (not to be confused with the J chain of IgM). This joining segment attaches itself first to the V gene segment in rearranged chromosomes and then to the C gene segment (Figure 24). This rearrangement occurs by a complex process called **transposition**. Thus, light chain genes recombine V and J segments to make a gene for the VL domain.

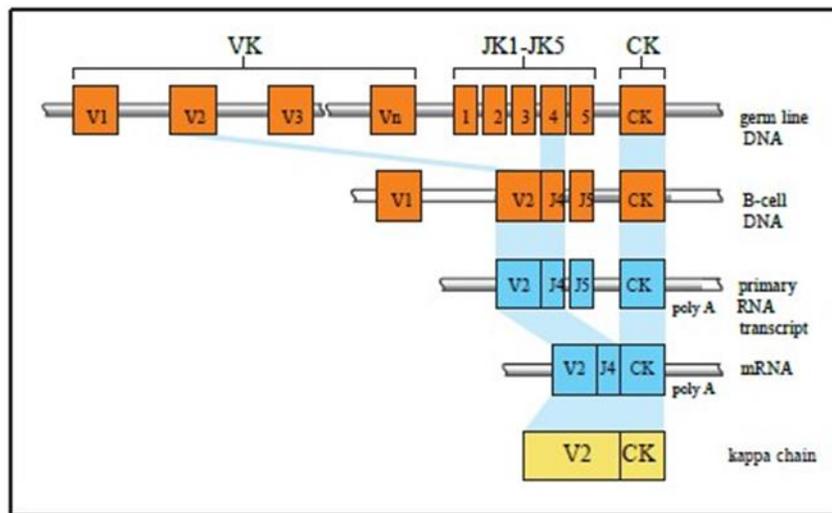


Figure 24 Kappa chain production in humans. [Adapted from *Immunology Sixth Edition*, Roitt, I., et al., 2001]

Following transposition, the entire genetic sequence (the V gene and J gene, together with the single C gene), is transcribed into a large primary RNA transcript in the cell's nucleus. Further modifications or splicing of unnecessary DNA segments is carried out, and the resultant messenger RNA is carried out of the nucleus and translated by ribosomes into the complete light chain. The light chains are then joined with the heavy chains to form the immunoglobulin which is secreted by the cell.

The synthesis of lambda light chains is similar to that of kappa light chains except that in lambda light chain, there are seven C gene sequences, each with its own J segment. The V segment of the lambda light chain combines with one of the J segments and then with its corresponding C segment (Figure 25).

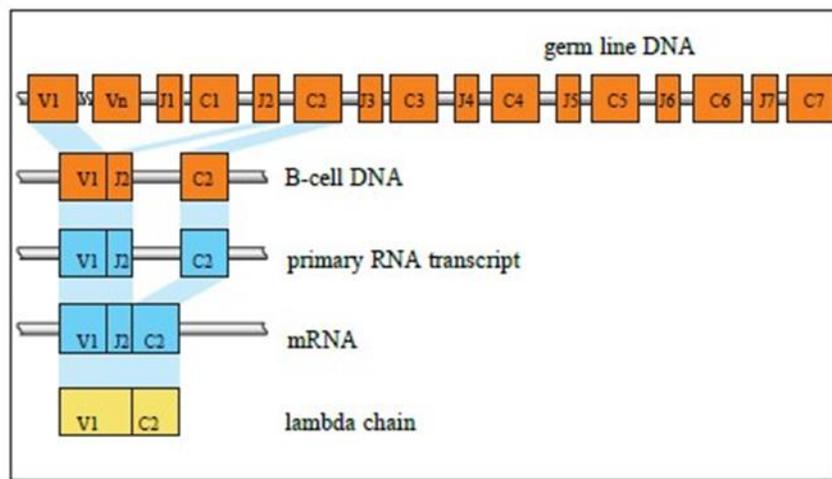


Figure 25 Lambda chain production in humans. [Adapted from *Immunology Sixth Edition*, Roitt, I., et al., 2001]

Synthesis of Heavy Chains

The variable region of the heavy chains is also derived from V and J segment genes. In contrast to the light chain genes, however, a third type of gene segment, called the diversity (DH) segment, is also used in forming a gene for the VH domain (Figure 26). There are an unknown number of DH sequences which lie between the JH and VH segments on chromosome 14. The use of the DH segment permits a further increase in heavy chain diversity. A B-cell must therefore complete two DNA rearrangement events or transpositions. It must first bring together one DH and one JH segment and then attach these to a VH segment. This is called VDJ joining. There can be some imprecision in the VJ and VDJ joining process, such that the site at which one segment fuses with the other can vary slightly. As a result, the DNA coding sequence that remains at the junction of these segments can also vary, resulting in further diversity in the immunoglobulin variable region. This can occur in the synthesis of the light chain VJ sequence and the heavy chain VDJ sequence. The VDJ gene sequence of a heavy chain is then combined with a particular CH gene segment which will in turn determine the class of the immunoglobulin (IgM, IgG, etc.). The remainder of the process leading to a completed heavy chain is similar to that of the light chains.

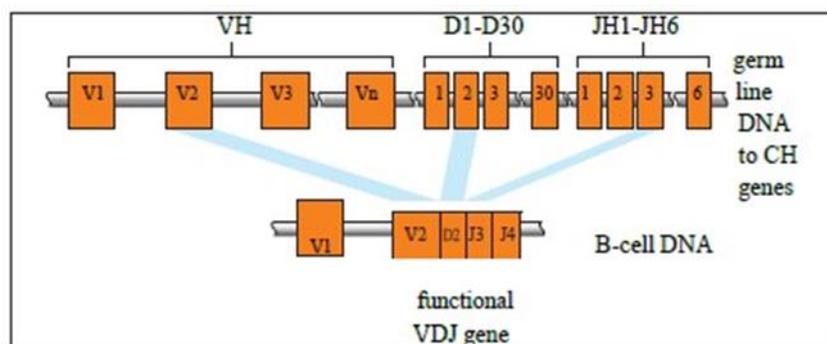


Figure 26 Heavy chain VDJ recombination in humans. [Adapted from *Immunology Sixth Edition* Roitt, I., et al., 2001]

Somatic Hypermutation and Class Switch Recombination

Immunoglobulin heavy and light chain genes can undergo structural changes (somatic hypermutation) after antigen stimulation. It appears that the region of DNA encoding the variable region may be particularly susceptible to mutation. These mutations that occur in the immunoglobulin genes during the lifetime of an individual B-cell can further increase the variety of antibodies produced by the B-cell. Somatic hypermutations occur in the germinal centers of the lymph nodes, and the cells that produce a higher-affinity antibody are selected for survival.

Class switch recombination (CSR) is a mechanism whereby a single B-cell that has been making an immunoglobulin of a single specificity and class can in fact change, from an IgM class to an IgG class for example. The mechanism of this class switch involves rearrangement at the DNA level. This rather complex process, which occurs under the influence of antigen and helper T-cells, involves transposition of the VDJ segment to another of the C region genes. The mechanism of class switching is irreversible (a cell cannot switch to an earlier class) and provides flexibility to the immune response.

PATHOPHYSIOLOGY OF WALDENSTROM'S MACROGLOBULINEMIA

Heavy Chain VDJ Gene Sequences in WM

WM is a cancer of B-cells that is characterized by the presence of a variety of cell morphologies: B-cells, lymphoplasmacytic cells, and plasma cells. This pleomorphism, (or the assumption of various distinct forms) indicates some kind of differentiation among the tumor cell population itself. Since WM is a B-cell malignancy, we can identify the cancer cells by the sequence of the immunoglobulin heavy chain variable, diversity, and joining (VDJ) gene rearrangement.

In humans, there are 6 VH families of variable region genes. In normal individuals, VH3 (55%) and VH4 (26%) are the most commonly found variable segments in B-cell heavy chains. B-cells expressing VH3 appear to be nearly always the target for WM development.

Somatic Hypermutation in WM

It has been noted that the WM IgM VDJ gene sequence present in a majority of WM cells demonstrates evidence of extensive somatic hypermutation, which appears to indicate that the WM cell is in fact derived from an antigen-stimulated B-cell. However, further studies have shown that the somatic hypermutations in question are not typical of antigen-driven selection, and some WM patients show no evidence of somatic hypermutation in the IgM VDJ gene sequence. Normal somatic hypermutations occur most frequently in the germinal centers of secondary lymphoid organs, suggesting that the WM cell may derive from a memory B-cell that bypasses the germinal center.

Class Switch Recombination in WM

WM cells are usually unable to undergo class switch recombination (CSR). Exposure to biological molecules like CD40L and IL-4, which would induce CSR in normal B-cells, has no effect on WM B-cells. The inability to perform the actual transposition required to change immunoglobulin classes may indicate a defective "switch" region in the gene. WM cells are therefore said to be exclusively "pre-switch." This assertion remains controversial as some research has suggested that WM or LPL cells, in certain circumstances, can undergo CSR.

Relevance of Immunoglobulin Gene Recombination to WM

Thus it appears that the latest evidence presented to us from genetic research indicates that WM may derive principally from a IgM- positive memory cell of the VH3 B-cell subset which undergoes somatic hypermutation in the possible absence of antigenic selection, bypasses the germinal center, and has a possible genetic "defect" that prevents class switch recombination, resulting in the persistent production of IgM. As previously stated, these assertions about the origin of WM cells and CSR are continuously challenged in immunological science today.

TOOLS USED IN GENETIC RESEARCH

This section briefly introduces some of the modern tools used by researchers in the field of genetics. Several of these formerly very expensive tests have become simpler and less costly to use, and they are making their way into clinical laboratories for the routine diagnosis and monitoring of many diseases. Indeed, these tools are making possible the development of more targeted therapies directed at the specific genetic components, epigenetic modifications, and protein pathways that cause diseases such as cancer.

Immunophenotyping is used to identify cells based on the types of antigens or markers on their surfaces (e.g. WM cells and the surface marker CD20). These markers are usually functional membrane proteins involved in cell communication, adhesion, or metabolism. They are identified in a sample if they bind to specific antibodies that are tagged with a dye or some other substance to make them detectable under a microscope or with other special instrumentation. Immunophenotyping can be done on tissue sections (either fresh or fixed) and on cell suspensions and is very useful in the diagnosis of leukemia and lymphoma. There are two basic types of immunophenotyping:

- Immunohistochemistry – surface antigens on cells in a tissue section can be identified with antibodies that have enzymes attached (usually horseradish peroxidase or alkaline phosphatase). When the tissue is exposed to a special substrate, the enzyme- tagged antibodies bound to these surface antigens will precipitate and cause the substrate to change color. The resulting color change can be detected with a microscope.
- Flow cytometry – surface antigens on cells can be identified with antibodies that are tagged with fluorescent dyes. The antibody- labeled cells are suspended in a stream of fluid. This stream passes through an instrument called a flow cytometer, which is an electronic laser-based detection instrument capable of analyzing thousands of cells per second, identifying and sorting them according to their size, their morphology, and the types of fluorescent dye-labeled surface markers they express.

Whole genome sequencing permits the researcher to decode the entire DNA sequence of an organism – in essence to “read” its DNA “blueprint.” In the case of individual humans, this encompasses some 3 billion DNA nucleotides. The DNA from a cell is extracted, and powerful computers piece together and analyze the gene sequences. Sequencing the whole genome may lead to clues about where specific genes, including cancer genes, are located. Analyzing the results may also enable researchers to understand how genes work together to direct the growth, development and maintenance of cells and indeed of an entire organism. Since whole genome sequencing requires such powerful computing power, it is expensive and not yet widely available, although its cost is decreasing.

Exome sequencing, in contrast, permits researchers to extract and analyze only the protein coding content of DNA, which makes up about 1-2% of the DNA in a cell. Exome sequencing is thus a less expensive and more efficient alternative to whole genome sequencing.

Polymerase chain reaction (PCR) is a technique used to make multiple precise copies of a segment of DNA. PCR is based on the ability of an enzyme called DNA polymerase to synthesize new strands of DNA complementary to the original target DNA. PCR can be used to analyze extremely small amounts of a sample by amplifying a specific sequence of DNA as many as one billion times. A similar method can be used to amplify RNA. PCR is extremely useful in the context of leukemia and lymphoma research and diagnosis and is widely used in other biotechnology, medical, and genetic applications, including forensics, paternity testing, and detection of infectious diseases.

Microarray uses a supporting material (such as a glass or plastic slide) onto which hundreds of molecules of known DNA sequences or proteins are attached in a regular pattern. The DNA or proteins in a sample are labeled with fluorescent dyes and placed onto the microarray slide. Any DNA or proteins present in the sample will bind to a complementary spot on the slide. A researcher then uses a special scanner to measure the fluorescent intensity of each spot. If a particular gene or protein is very active in the sample, it generates a bright fluorescent area. A gene or protein that is less active produces a dimmer spot, and an inactive or missing gene or protein produces no fluorescence. The resulting patterns of gene or protein expression in tumor cells can be compared to those of normal cells, providing the researcher with information about which genes or proteins are important to investigate in a particular cancer.

AFTERWORD

The science of immunology continues to amaze and mystify. New discoveries are made that have resulted in better therapies for the myriad of immune system disorders that exist. Newer and more exciting discoveries are made on an almost daily basis, and newer and safer treatments will surely continue to be developed to help patients with immune system disorders.

The continued study and identification of the cytokine chemical messengers and the possible increased manipulation of the immune system itself promise extremely precise medical therapies. The bewildering advances in molecular genetics, the stunning discovery of the prevalence of the MYD88 L265P mutation in WM patients, and the routine use of advanced research tools and techniques can only lead to more specialized and individualized therapies based on the patient's own genetic blueprint or peculiarities.

The prestigious research publication, *Science* deemed cancer immunotherapy the scientific Breakthrough of the Year in 2013. This has led to the use of new medications to activate the immune system and new treatments using modified immune cells to treat cancers including malignancies of lymphocytes and plasma cells. This research has found that the immune system can recognize and eliminate cancer. Clinical trials using these approaches are demonstrating the potential for long-term safe disease control and may potentially be curative therapy.

More recently there has been a breakthrough in genomic editing (also called gene editing), a group of technologies that enable change in an organism's DNA. These technologies allow genetic material to be added, removed, or altered at particular locations in the genome. A recent one, known as CRISPR-Cas9 or clustered regularly interspersed short palindromic repeats and CRISPR-associated protein 9 is a fast, cheap, accurate and efficient method of genome editing that has generated a lot of excitement.

CRISPR-Cas9 was adapted from a naturally occurring genome editing system in bacteria. The bacteria capture snippets of DNA from invading viruses and use them to create DNA segments known as CRISPR arrays. The CRISPR arrays allow the bacteria to "remember" the viruses (or closely related ones). If the viruses attack again, the bacteria produce RNA segments from the CRISPR arrays to target the viruses' DNA. The bacteria then use Cas9 or a similar enzyme to cut the DNA apart which disables the virus.

The CRISPR-Cas 9 system works similarly in the laboratory. Researchers create a small piece of RNA with a short "guide" sequence that attaches (binds) to a specific target sequence of DNA in a genome. The RNA also binds to the Cas9 enzyme. As in bacteria, the modified RNA is used to recognize the DNA sequence and the Cas9 enzyme cuts the DNA at the targeted location. Once the DNA is cut, researchers use the cell's own DNA repair machinery to add or delete pieces of genetic material or to make changes to the DNA by replacing an existing segment with a customized DNA sequence.

Genome editing is being explored in research on a wide variety of diseases, including single-gene disorders, such as cystic fibrosis, hemophilia, and sickle cell disease. It also holds promise for the treatment and prevention of more complex disease, such as cancer, heart disease, mental illness, and human immunodeficiency virus (HIV) infection. ghr.nlm.nih.gov/primer/genomicresearch/genomeediting

I hope that the interested reader has come away from this expanded review of basic immunology in WM with a renewed sense of hope. I hope as well that this booklet has perhaps encouraged many WM patients and caregivers to continue to seek further understanding and knowledge about the complexities and wonders of the immune system. Please support research into this fascinating and often mysteriously perplexing disease. Researchers now tell us that the cure is on the horizon!

ACKNOWLEDGEMENTS

I wish to thank the International Waldenstrom's Macroglobulinemia Foundation (IWMF) and in particular past IWMF presidents Ben Rude and Judith May, current IWMF president Carl Harrington, the astonishingly dedicated past and present IWMF Board of Trustees, and all the wonderful volunteers in this vibrant WM community for the opportunity to help my fellow WM friends – may we continue to thrive in our journey towards survivorship.

This booklet, or any of the IWMF booklets that I have been involved with for that matter, could not have been possible without the essential and much appreciated and sorely needed editorial assistance of my friends and fellow volunteers, Sue Herms, Alice Riginos, Linda Nelson, and Drs. Kyle and Ansell for their revisions . Thank you! Thank you!

Many thanks as well to my past and future teachers.

My family is my inspiration, and I am grateful for their acceptance and love.

Guy Sherwood MD Spring 2014, Winter 2018

GLOSSARY OF SELECTED TERMS

Acquired immunity (adaptive immunity): Immunity resulting from the development of active or passive immunity. It involves the activation of white blood cells and the generation of antibodies.

Acute phase reactants (APRs): Proteins that rise and fall with acute inflammation. Examples of APRs include C-reactive protein, C3 complement protein, fibrinogen, haptoglobin, and transferrin.

Affinity: A measure of the binding force, or strength, of a single antigen with its antibody.

Agglutination: The aggregation of antigen by antibodies. Agglutination applies to red blood cells as well as bacteria and inert particles covered with antigen.

Alleles: One of a number of alternative forms of the same gene that produce different effects.

Allergen: An antigen responsible for producing allergic reactions by inducing IgE formation.

Amino acids: The basic structure of proteins, these molecules are made up of carbon, nitrogen, hydrogen, and oxygen and can form long chains called polypeptides, which are the building blocks of proteins.

Angiogenesis: The formation of new blood vessels. Tumor angiogenesis, whereby the growth of new blood vessels to supply the tumor cells is generated by a soluble chemical released by the tumor cells themselves, is increasingly becoming an important target for biological cancer therapy.

Antibodies (Abs): Also called immunoglobulins. Any of the structurally related molecules formed by B-cells that are specific to antigen; divided into five basic classes or isotypes (IgG, IgM, IgA, IgE, IgD) on the basis of structure and biologic activity.

Antigen: Any foreign molecule that reacts with pre-formed antibody and specific receptors on T- and B-cells; also used loosely to describe materials used for immunization.

Antigen-antibody complexes: Compounds formed by the attachment of an antibody to an antigen; most such complexes are harmless, but some may cause tissue damage by activation of the immune system or by inciting an inflammatory reaction.

Antibody-dependent cell-mediated cytotoxicity (ADCC): A phenomenon in which target cells, coated with antibody, are destroyed by specialized killer cells (and other effector cells such as macrophages) which bear receptors to the Fc portion of the coating antibody. These receptors allow the effector cells to bind to the antibody-coated target and destroy it.

Antigen-processing cells: A specialized type of cell, bearing cell surface class II MHC antigens, involved in processing and presenting antigen to T-helper cells.

Apoptosis: The process of programmed cell death.

Autoantibody: An antibody directed against a self-antigen, i.e., against a normal tissue component. The IgM antibody that causes peripheral neuropathy is considered an autoantibody.

Autocrine action: The ability of a cytokine to act on the cell that produced it.

Autoimmune hemolytic anemia: Hemolysis, or destruction of red blood cells, by autoantibodies, as seen in certain diseases including lymphomas, after use of certain drugs, and for often-unexplained reasons. Cold agglutinin disease is an autoimmune hemolytic anemia seen in some WM patients.

Avidity: The summation of multiple affinities, for example when an antibody binds to an antigen at multiple areas.

Basophils: White blood cells that stain blue with specific basic dyes, they are involved in the release of histamine and serotonin when stimulated, usually in an allergic reaction.

Bifunctional: In the case of antibodies, this means having two functions (e.g., binding to antigen at the Fab ends of the antibody and activating immune system cells or complement at the Fc end of the antibody).

B- (bone marrow-derived) cells/B-lymphocytes: White blood cells formed in the bone marrow by hematopoietic stem cells, they are the precursors of antibody-forming terminally differentiated plasma cells. B-cells carry antibody and class II MHC antigens on their cell surfaces. WM is a cancer of the B-cells.

Bone marrow: Spongy tissue occupying the hollow central cavity of bones that is the site of hematopoiesis. Following puberty, the marrow located in the spine, ribs, breastbone, hips, shoulders, and skull is most active in blood cell formation. In the adult, the bones of the hands, feet, legs, and arms are filled with fat cells rather than active marrow.

CAR T cell therapy: A type of cancer immunotherapy that works with your immune system by using your T cells (or fighter cells). CAR T cell therapy is created by adding a new receptor (or hook) to your T cells. This receptor is called chimeric antigen receptor, or CAR. Your body's T cell with the new CAR added is now called a CAR T cell. The new CAR T cells then work within the body to find their match on special cells to attack the malignant cells. Because most CAR T cell therapies use your own cells to create cancer fighting cells, each therapy is individualized with its own set of side effects.

CD (cluster of differentiation) markers: Cell surface molecules of leukocytes and platelets that are identifiable with monoclonal antibodies (e.g. CD20 and rituximab) and may be used to differentiate cell populations.

CD4: The cell surface receptor protein of T-helper cells and other white blood cells. CD4 causes T-cells to proliferate in response to antigens, and it causes B-cells to produce antibodies. CD4 also serves as the receptor for the AIDS virus.

CD4/T-helper/Th cells: This is a functional subclass of T-cells, expressing the CD4 marker on their surfaces, which help trigger B-cells to make antibody. CD4 cells also facilitate generation of T-cytotoxic (Tc) cells. CD4 cells recognize antigen in association with class II MHC molecules.

CD8: The cell surface receptor protein that is a marker for T-cells with suppressor and cytotoxic activity; binds to class I MHC antigens on antigen-presenting cells.

CD8/T-cytotoxic/Tc cells: This is a functional subclass of T-cells that express the CD8 marker on their surface. CD8 cells can kill malignant or virally infected cells that have antigenic fragments presented by class I MHC molecules on their cell membranes.

CD34: The cell surface receptor characteristic for hematopoietic stem cells (HSCs); useful for the identification of HSCs in flow cytometry and in isolating HSCs.

Centromere: The constriction point of a chromosome that links its two arms. The centromere plays an important role in DNA duplication during cell division.

Chemokines: Cytokines produced by specialized immune cells that have cell-activating properties and encourage migration or attraction of a target cell toward the concentration gradient of the chemokine in question.

Chromosomes: The condensed rod-shaped structures inside the cell nucleus that contain the genes.

Class I major histocompatibility complex molecules (class I MHC): Proteins expressed on the surface of virtually all cells that are used to present antigenic material to CD8 T-cytotoxic cells. Class I MHCs are therefore important in the recognition of self by the immune system and for the identification of a virally infected or malignant cell.

Class II major histocompatibility complex molecules (class II MHC): Proteins expressed on the surface of B-cells, macrophages, dendritic cells, and other auxiliary immune system cells. Class II MHCs are characterized by their ability to stimulate lymphocytes.

Class switch recombination (CSR): The process by which an individual B-cell or its progeny can link immunoglobulin heavy chain constant (C) genes to its recombined variable (V) genes to produce a different class (or isotype) of antibody with the same specificity. This process is irreversible (change from IgM to IgG production, but not the reverse).

Codons: The basic three-unit nucleotide structures that code for a particular amino acid.

Cold agglutinin disease: An autoimmune hemolytic anemia caused by IgM autoantibody that binds to red blood cells at temperatures reached in the capillaries of the skin and subcutaneous tissues, causing red blood cell destruction (hemolysis).

Colony-stimulating factors (CSFs): A group of cytokines which control the differentiation of hematopoietic stem cells.

Complement-dependent cytotoxicity (CDC): The mechanism of cell destruction by activation of the complement protein cascade initiated by the formation of antigen-antibody complexes.

Complement proteins: A group of serum proteins involved in the control of inflammation, the activation of phagocytes, and the attack on cell membranes causing cell lysis. The system can be activated by interaction with the antibodies of the immune system.

Constant region: The terminal portion of an antibody's heavy and light chains, which does not vary in distinct antibody classes and which attaches to the effector cells and complement proteins of the body's immune system.

CRISPR-Cas9: A unique technology that enables geneticists and medical researchers to edit parts of the genome by removing, adding or altering sections of the DNA sequence.

Cryoglobulinemia: Clinical disease characterized by cryoglobulins in the serum; often associated with immune complex antigen- antibody deposits in the kidneys and other tissues. Three types of cryoglobulinemia have been described: Type I (monoclonal); Type II (mixed monoclonal-polyclonal) was first noted in WM and can be seen as well in autoimmune disorders; and Type III (mixed polyclonal) can be seen in autoimmune diseases, infections, and other disorders.

Cryoglobulins: abnormal antibody proteins detected in the laboratory by chilling serum to below 32° Celsius where the proteins become insoluble and form a precipitate. At the normal body temperature of 37° Celsius, cryoglobulins are soluble. Serum specimens from patients with cryoglobulins must be kept warm until testing.

Cryoprecipitable immunocomplex: Precipitate formed when an antibody-cryoglobulin immune complex is exposed to temperature below the normal body temperature of 37° Celsius. Clinical findings include joint pain, red non-blanching rashes, cold intolerance (particularly of the extremities such as the fingers, toes, and nose), and other symptoms.

Cytokines: This is a generic term for non-antibody proteins released by one cell population which act as intracellular facilitators, as in the generation of an immune response.

Dendritic cells: A set of immune cells present in tissues which capture antigens and migrate to the lymph nodes and spleen where they are particularly active in presenting the processed antigen to T-cells.

DNA (deoxyribonucleic acid): the molecule that contains the hereditary material in humans and almost all other living organisms.

Domain: A compact segment of an antibody molecule, made up of about 100-110 amino acids around a disulfide bond and encoded by a unique segment of DNA.

Effector cells: Lymphocytes or phagocytes that produce the end effect of antigen destruction or neutralization.

Effector functions: In the context of the immune system, this term refers to the end results of activated immune system cells, including fixation of complement proteins and phagocytosis.

Eosinophils: White blood cells that stain red with specific acidic dyes, they are involved in reactions against parasitic worms and in some hypersensitivity reactions involving IgE.

Epigenome: Consists of chemical compounds that modify, or mark, the genome in a way that tells it what to do, where to do it, and when to do it. The modifications, which are not part of the DNA itself, can be passed on from cell to cell as cells divide, and from one generation to the next.

Erythrocytes (red blood cells): These cells contain hemoglobin which binds oxygen when the cells pass through the lungs and then releases it to the tissues of the body. Red blood cells make up a little less than half the volume of blood in healthy individuals.

Erythropoietin (EPO): A hormone, produced mainly by the kidneys, which is required for the normal production of red blood cells. Released into the bloodstream in response to decreased levels of oxygen in the blood (as in anemia), EPO interacts with the EPO receptors on red blood cell progenitors to increase red blood cell production. Epoetin alfa (Epogen, Procrit) and darbepoetin alfa (Aranesp) are laboratory-made forms of EPO that can be used to treat anemia.

Fab: The fragment of antibody containing the binding site to antigen, consisting of a light chain and part of the heavy chain.

Fc: The fragment of antibody containing the binding site to effector cells and complement, consisting of part of the heavy chains.

Fc receptors: Surface molecules on a variety of effector cells that bind to the Fc region of antibodies. They are antibody class-specific.

G-CSF (granulocyte-colony stimulating factor): A cytokine that stimulates the bone marrow to produce granulocytes and stem cells and release them into the bloodstream.

Half-life/antibody half-life: A measure of the mean survival time of antibody molecules following their formation, usually expressed as the time required to eliminate 50% of a known quantity of antibody from the body – varies from one antibody class to another.

Heavy chains: The larger of the chains that comprise a normal antibody molecule.

Hematopoiesis: The process of blood cell formation.

Hematopoietic stem cells (HSCs): Residing in the bone marrow, the HSCs are the single common ancestor to all the functional cells found in the blood and immune system. The HSCs represent less than 0.01% of bone marrow cells in adults and give rise to a larger, intermediately differentiated population of progenitor cells. These progenitor cells in turn divide and differentiate further through several stages into mature cells responsible for specific tasks. The stem cells are also able to renew themselves; this potential for unlimited life span and future proliferation is their most important defining property.

Hinge region: The portion of an antibody heavy chain between the Fab and Fc regions which permits flexibility within the molecule and allows the two combining sites to operate independently.

Hypervariable regions: Portions of the light and heavy antibody chains that are highly variable in amino acid sequence from one antibody molecule to another and that together constitute the antigen-binding site of an antibody molecule.

Immunity: The condition of being immune; the protection against infectious disease conferred by the immune response generated by immunization, by previous infection, or by other nonimmunologic factors.

Immunization: The induction of immunity by either (1) the stimulation of the immune system and subsequent production of antibody by exposure to an antigen in order to confer protection against disease (e.g. active immunity by administration of a vaccine) or (2) the conferring of specific immune reactivity on previously non-immune individuals by the administration of sensitized lymphoid cells or serum from immune individuals (e.g. passive immunity by intravenous IgG administration).

Immunogen: A substance capable of inducing an immune response, in most contexts synonymous with antigen (but not always).

Immunoglobulins (Igs): See Antibodies.

Innate immunity (non-adaptive immunity): Consists of protective elements with which an individual is born and that are always present and available at very short notice to protect the individual from infection. Examples of innate immunity include the skin barrier, the mucous membranes of the upper respiratory system, the cough reflex, the acidic pH of the stomach, and tears. Internal elements also play a role in innate immunity, including fever, specialized proteins found in the blood, certain chemicals, and certain immune cells that act as non-specific security guards to any foreign invader.

Interferons (IFNs): Any of a family of immune regulatory proteins produced primarily by T-cells in response to DNA, viruses, antigens, and other substances usually associated with infected or malignant cells. Interferons increase the killing activities of macrophages.

Interleukins (ILs): A family of factors produced by lymphocytes, monocytes, and other cells that induce growth and differentiation of lymphoid cells and hematopoietic stem cells.

Isohemagglutinins: The naturally occurring IgM and IgG antibodies against the red blood cell antigens of the major blood groups.

Isotypes: In the context of antibodies, these are the classes of antibodies present in all normal individuals (e.g. IgG, IgM, IgA, IgE, IgD).

Kappa light chain: One of the two light chain types found in the basic antibody molecule. Both types of light chains are present in all individuals, and either of the kappa or lambda light chain types may combine with any of the heavy chain types, but in any one antibody molecule both light chains are of the same type and both heavy chains are of the same type. Light chains are also found as two-unit structures (dimers) in the urine in certain abnormal conditions, particularly in multiple myeloma, and are called Bence-Jones proteins.

Lambda light chain: See Kappa light chain.

Leukocytes: White blood cells formed in the bone marrow; include lymphocytes, phagocytes, and certain auxiliary cells.

Light chains: The smaller of the chains that comprise a normal antibody molecule.

Lymph nodes: Part of the secondary lymphoid system; bean-shaped organs found in the underarms, groin, neck, and abdomen that act as filters for the lymph fluid as it passes through them. The lymph nodes are major sites of antigen trapping by lymphocytes, which in turn can activate an immune response.

Lymphoid stem cell: Progenitor stem cell for lymphocytes.

Lymphokines: A term for cytokines produced by lymphocytes. Interferon, interleukins, and colony-stimulating factors are lymphokines.

Lysozyme: An enzyme found in saliva, tears, and other body fluids that has anti-bacterial activity.

Macrophages: White blood cells that interact with antigens and present these antigens to T-cells, thus activating the T-cells. Macrophages that circulate in the blood are called monocytes, whereas those that reside in certain tissues are called tissue macrophages. Macrophages are capable of phagocytosis, and they secrete various substances that enhance the immune response to infectious agents and malignant cells.

Mast cells: Non-mobile cells distributed near blood vessels in most tissues; these cells are full of granules containing inflammatory facilitators and are often associated with allergic reactions.

Memory cells: Long-lived B-cells which have already been primed with their antigen but have not undergone terminal differentiation into plasma cells. They react more readily than naïve lymphocytes when re-stimulated with the same antigen.

Monoclonal: A group of cells derived from a single ancestral cell through repeated division.

Monocytes: Macrophages that are mobile and present in the bloodstream and that comprise 2-5% of circulating white blood cells.

Monokines: A term for cytokines produced by macrophages that act as facilitators of immune responses not involving antibodies or complement.

Mucosa-associated lymphoid tissue (MALT): Generic term for lymphoid tissue associated with the gastrointestinal tract, bronchial tree, and other mucosal tissue.

Mutation: a change in the nucleotide sequence of a gene. A germline mutation is inherited while a somatic mutation is acquired during the lifetime of an individual.

Natural killer cells (NK cells): White blood cells that have an intrinsic ability to kill various targeted cells.

Neutrophils: The most abundant type of granulocytes, they are short-lived and mobile and are part of the innate immune system.

Nucleotide: The subunits of DNA and RNA, these are composed of nitrogen bases, a sugar (either deoxyribose or ribose) and one or more phosphate groups.

Paracrine action: Denotes a type of function in which a substance such as a hormone or a cytokine is synthesized by a cell and released, affecting the function of other nearby cells.

Peripheral neuropathy (PN): A clinical symptom that occurs because of a transient or permanent problem with the functioning of the nerves outside the spinal cord. The symptoms of peripheral neuropathy may include numbness, weakness, burning pain, and loss of reflexes. The pain may be mild or severe and disabling.

Phagocytes: Term referring to cells of the immune system (macrophages and neutrophils) that are able to ingest microorganisms and other antigen particles coated with antibody or complement. This process is facilitated by specific cell surface receptors called Fc receptors.

Phagocytosis: The process by which cells engulf material and enclose it within a special area (phagosome) inside the cell.

Plasma cells: Terminally differentiated white blood cells of the B-cell lineage that produce antibodies. In multiple myeloma, the plasma cell becomes malignant and produces in most cases large amounts of IgG antibodies.

Plasmapheresis: The removal, treatment, and return of blood components. This procedure is used as therapy in several types of blood disorders, including WM.

Platelets: Cells formed in the bone marrow from hematopoietic stem cells that circulate in the blood and are needed to help the blood clot and control bleeding.

Polyclonal: Derived from different cells. Normal IgM is polyclonal since it is derived from many different B-cells, as opposed to monoclonal IgM produced by the WM cell.

Polymorphism: In genetics, this refers to the occurrence in the same population of two or more genetically determined phenotypes. Sexual dimorphism (difference in appearance between the sexes) is one example.

Polymorphonuclear neutrophils: See Neutrophils.

Primary lymphoid organs (central lymphoid organs): Lymphoid organs in which lymphocytes complete their initial maturation steps; in the adult these are the bone marrow and thymus.

Progenitor cells: Cells derived from hematopoietic stem cells that in turn serve as interim stem cells for the other blood cell types that develop during the process of cell maturation and differentiation.

Proteasomes: Protein complexes inside the cell whose function is to degrade unneeded or damaged proteins.

Ribosomes: Large intra-cellular molecular structures composed of two subunits that are the site of protein synthesis.

RNA (ribonucleic acid): A nucleic acid that plays an important role in the coding, decoding, regulation, and expression of genes.

Secondary lymphoid organs and tissues: These comprise well-organized encapsulated organs such as the spleen and lymph nodes and non-encapsulated accumulations of lymphoid tissue; usually the site of first encounter of immune cells with antigen. In general, lymphocytes are generated in primary lymphoid organs and function in secondary lymphoid organs and tissues.

Serum viscosity (SV): The physical property of serum as it relates to its "thickness." The serum viscosity is affected by the concentration of various constituents in the serum.

Somatic hypermutation: A process occurring during B-cell maturation and affecting the antibody gene region, which permits refinement of antibody specificity.

Spleen: The largest structure in the lymphoid system, the spleen is a gland-like organ situated in the left upper abdomen. It serves as a reservoir of blood, produces lymphocytes and plasma cells, and functions as a "filter" for the blood by removing damaged red blood cells from the circulation.

T- (thymus-derived) cells/T-lymphocytes: These are probably the most complex cells of the immune system, given the diversity of T-cell types; the wide range of cytokines, growth factors, and immune modulators produced by activated T-cells; the complexity of T-cell interaction with antigens; and the complexity of T-cell maturation in the thymus.

T-cell receptors (TCRs): Structurally related to antibodies, the T-cell receptors on the surface of T-cells interact with class I or class II MHC molecules presented to them by antigen-presenting cells of the immune system. Activation of the TCRs leads to various functions performed by T-cells. TCRs are unable to recognize free unbound antigen.

T-cytotoxic cells (Tc cells): CD8 T-cells that respond to class I MHC presentation of viral or tumor antigens on the surface of a target cell, leading to destruction or lysis of the infected or malignant target cell by the Tc cell.

T-helper-1 cells (Th1 cells): CD4 T-cells that produce cytokines which are associated with cell-mediated inflammatory reactions, activation of complement and/or macrophages, and antibody-dependent cell-mediated cytotoxicity.

T-helper-2 cells (Th2 cells): CD4 T-cells that produce cytokines which provide optimal help for strong antibody and allergic responses.

Thymus: The major site of T-cell differentiation, the thymus is considered a primary lymphoid organ and is located in the thoracic cavity over the heart.

Transcription: The first step of gene expression, in which a particular segment of DNA is copied to messenger RNA by the enzyme RNA polymerase.

Translation: The process by which ribosomes create proteins.

Transposition: A genetic event where a segment of DNA is moved to another position or is replaced and/or exchanged for another genetic segment.

Variable region: The portion of an antibody's light and heavy chains that is primarily responsible for antigen binding. This region is subject to frequent genetic manipulation/mutation.

White blood cells (WBCs): See Leukocytes.

IWMF Vision Statement

Support everyone affected by Waldenstrom's macroglobulinemia while advancing the search for a cure.

IWMF Mission Statement

To offer mutual support and encouragement to the Waldenstrom's macroglobulinemia community and others with an interest in the disease.

To provide information and educational programs that address patients' concerns.

To promote and support research leading to better treatments and ultimately, a cure.

Published by the International Waldenstrom's Macroglobulinemia Foundation (IWMF)

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IWMF is a 501(c)(3) Tax Exempt Non-Profit Organization, Fed ID # 54-1784426.