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**An Overview of the Immune System, Part Two:
The Generation and Evaluation of Immune Responses**
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by

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An Overview of the Immune System Part 2: The Generation and Evaluation of Immune Responses

ABSTRACT

Although immunology is a complex and specialized area of medicine and laboratory science, a basic understanding of how normal immune responses occur can be helpful to anyone in the health care field. In the first course of this two-part series, *The Cells and Cell Surface Molecules of the Immune System*, the basic components of the immune system were reviewed. Here, in the second course, the characteristics of antigen-specific immune responses will be introduced, and the cellular interactions necessary for normal humoral and cell-mediated immune responses will be presented. In addition, techniques that are utilized to assess the immune system and/or to utilize immunologic techniques in the clinical laboratory will be reviewed.

OBJECTIVES

Upon completion of this course, the reader will be able to:

- List the four hallmarks of antigen-specific immune responses
- Identify which innate white blood cells can serve as antigen-presenting cells in adaptive immune responses
- Match the appropriate class of MHC molecule to the T cell subset that requires it for antigen presentation
- Describe the role of CD4-expressing T helper cells in humoral and cell-mediated antigen-specific responses
- List one clinical laboratory technique that enumerates lymphocyte subsets, and at least one additional technique that provides information on immune system function

Preface/Author's Note

This two-part series of immunology courses is dedicated to the memory of Dr. Janis Kuby. Dr. Kuby was an exceptional professor of immunology at San Francisco State University, and the original author of *Kuby Immunology*, one of the most widely-used immunology textbooks throughout the country. Janis Kuby will never be forgotten by anyone who has ever had the benefit of her knowledge and love of immunology, either through her classroom teaching or her outstanding textbook.

LIST OF FREQUENTLY-USED ABBREVIATIONS

WBC	white blood cells
Ig	immunoglobulin; also known as antibody
TCR	T cell receptor
MHC	major histocompatibility complex
CTL	cytotoxic T lymphocyte (CD8-positive T cell)
T _H	T helper cell (CD4-positive T cell)
APC	antigen-presenting cell
DC	dendritic cell
IL-2	interleukin-2

CTL-P	mature CTL precursor
PE	phycoerythrin
FITC	fluorescein isothiocyanate
ELISA	enzyme-linked immunosorbent assay

INTRODUCTION

The immune system is constantly challenged to protect individuals from the enormous array of foreign materials to which they are exposed. The protection provided by the human immune system is a collaborative one, calling on both innate immunity, which is present in some form in all multicellular plants and animals, and adaptive immunity, which is a relatively recent product of evolution found in more advanced vertebrates (1). As reviewed in a previous course (2), the innate immune system prevents foreign materials, known as antigens, from gaining entry to the body through a variety of physical and chemical barriers. If a toxin, foreign protein, pathogen, or any other antigen succeeds in breaching these barriers, it would soon encounter a host of innate system cells and cellular defenses capable of preventing infection and/or eliminating pathogens and antigens from the body. While these cellular defenses can recognize and respond to foreign antigens, the innate immune system is relatively non-specific, i.e., it is not sufficiently specialized to discriminate between different types of foreign molecules. Although it is a highly-effective first line of defense against most infections and toxic exposures, the innate system does not always succeed in preventing disease. When disease is the outcome, the innate immune response serves as the critical first step in generating a more sophisticated and exquisitely specific adaptive, or acquired, immune response. Adaptive immunity is designed to recognize and eliminate a particular antigen in a very specific fashion, and to remember the antigen so as to be able to protect against it in the future. It is this combination of initial adaptive response and development of antigen-specific memory which is responsible for immunity as we know it.

Immunity is defined as “the state of protection from infectious disease” (1). The concept is an ancient one, dating back to an epidemic, “The Plague of Athens,” in 430 BC, when an historian (Thucydides) observed that only those who had recovered from the disease could nurse the sick without becoming sick again themselves. The root of the word immunity is the Latin *immunis*, meaning “exempt,” reflecting the early understanding of exemption or protection from recurrent disease. Over the centuries, especially during the twentieth century and into the twenty-first century, scientists’ understanding of how the immune system accomplishes this feat has become more and more detailed. The primary goal of this course is to describe how the different types of white blood cells (WBC) interact with antigen and with each other to bring about adaptive antigen-specific immunity. In addition, the course will provide an overview of some of the specialized ways in which the status of the immune system can be evaluated in the clinical laboratory.

I. ADAPTIVE IMMUNE RESPONSES

The WBC of the immune system and the function(s) of each cell type have been described in the first part of this series (2). They include granulocytes such as neutrophils, and mononuclear cells such as monocytes/macrophages, natural killer cells, and lymphocytes. It is

the ability of B and T lymphocytes to specifically recognize and remember individual antigens that makes antigen-specific adaptive immune responses possible (1). These cell types are capable of antigen recognition due to the presence of antigen receptors on their cell surfaces. B lymphocytes (also known as B cells) utilize surface-bound antibody or immunoglobulin (Ig) molecules as antigen receptors. T lymphocytes (or T cells) use a different, but antibody-like molecule called the T cell receptor (TCR). Surface Ig and TCR are proteins that are anchored in the cell membrane, and have an antigen-binding “pocket” with a particular three-dimensional shape extending outward. When a cell encounters an antigen that has the proper complementary shape to fit into the pocket (like a key fitting into a lock), the antigen will bind to the receptor molecule in a very specific fashion. The interaction of antigen and receptor on the cell surface initiates a signaling cascade that turns on new gene expression in the nucleus, and ultimately, leads to the activation of the T or B cell.

Adaptive, antigen-specific immune responses share four distinguishing characteristics which set them apart from innate, non-specific responses (1). All of these characteristics are mediated by the antigen receptors on B and T cells. They are *antigenic specificity*, *diversity*, *self/non-self recognition*, and *immunologic memory*.

Antigenic specificity permits the immune system to distinguish differences among antigens. Many antigens differ greatly, making it easy for the immune system to discriminate among them. However, the immune system has such exquisite specificity that it can also discriminate between antigens that differ only by a single amino acid. The specificity of the immune system lies in the antigen receptors on B and T cells. On the surface of a single T or B cell, there are thousands of TCR or Ig molecules. However, on a single cell, every antigen receptor is identical, with the same antigen-binding pocket that is complementary to a specific antigen. This ensures that a given T or B cell will recognize only a certain shape on an antigen, and as long as that antigen does not change its shape, will continue to recognize the same antigen time and time again.

Diversity is the ability of the immune system to generate a tremendous number of different antigen receptors, allowing it to recognize millions of unique sites on antigens. Although each individual B or T cell recognizes only one particular shape on an antigen, collectively all of the B and T cells together have the capacity to recognize more than one hundred million different antigen sites. This is accomplished by an extraordinary system of DNA rearrangements that is unique to B and T cells. By physically cutting, moving, and pasting small portions of the Ig or TCR genes into new combinations, the immune system is capable of generating enormous numbers of different B and T cells from a relatively small amount of genetic material. Each cell thus has a cell surface antigen receptor with a slightly different antigen-binding pocket. This means of generating specificity and diversity through genetic recombination was unprecedented in biology when it was first described for Ig genes in 1976 by S. Tonegawa. Tonegawa’s work revolutionized the field of immunology, and the importance of his contribution was recognized with a Nobel prize in 1987. It took until the 1980s for scientists to identify TCR genes, which proved to have similar structure and undergo the same genetic rearrangement as Ig genes. While the structure of Ig and TCR genes and the highly ordered manner in which they rearrange is well-established, there are some aspects of antigen-receptor generation (such as some details of the signals and enzymes involved in the cutting and pasting

of DNA) that remain to be elucidated. The story of immune system gene rearrangements continues to be a fascinating one, and can be read about in detail in reference 1 and many other immunology and genetics texts.

Self/non-self recognition is the ability of the immune system to distinguish self components from non-self (foreign) antigens and cells. This ensures that the immune system mounts responses only to non-self antigens or cells, preventing (under normal circumstances) autoimmune responses. Self/non-self recognition of cells is carried out by the TCR on T cells, and utilizes the major histocompatibility complex (MHC) molecules. These molecules, also known in humans as Human Leukocyte Antigens (HLA) or the transplantation antigens, are identical on the cells within a single individual, but differ from individual to individual. There are two major classes of MHC molecules, class I (HLA-A, B, C) and class II (HLA-D), that are utilized in different types of immune cell interactions (as described below). Discrimination between self and foreign antigens by B and T cells is carried out by their respective antigen receptors through a process known as tolerance that occurs during lymphocyte development.

Immunologic memory is the phenomenon that gives rise to rapid and protective antigen-specific immunity upon a second or subsequent encounter with the same antigen. As a result of a first-time exposure to a specific antigen (i.e., a particular pathogen), the adaptive immune system mounts a specific primary response targeted against that antigen. This response tends to be slow to develop (7-10 days to peak response in the case of an antibody response), and is of limited magnitude and duration (1). If the faster-acting but relatively non-specific innate immune response is unable to eliminate a pathogen, an exposed individual typically gets sick the first time he or she encounters a pathogen. In other words, the pathogen wins the race the first time around because the innate response is insufficient and the primary adaptive response is slow. However, during the primary adaptive response, a pool of antigen-specific memory B and T cells is produced which may persist for years or even decades. These memory cells are primed to respond to a particular antigen, and so when they encounter the identical antigen a second time, they are able to respond much more rapidly (3-5 days for peak antibody response), more vigorously (100-1000 times higher peak antibody levels), and for a longer period of time. Memory cells can be described as fulfilling the Olympic motto: "*Citius, Altius, Fortius*" (faster, higher, stronger)! It is this olympian effort that enables the immune system to win the race over a pathogen during a memory or secondary response, as it can swiftly and effectively contain and eliminate the pathogen before the onset of disease. It is immunologic memory, which is a function of the specificity of B and T cells, that enables the development of specific immunity as a result of natural exposures to pathogens. Vaccination takes advantage of this characteristic of antigen-specific immunity by replacing the natural primary exposure to a pathogen with exposure to a harmless form of the same antigen(s). Therefore, upon the first exposure of a vaccinated individual to the actual pathogen, an effective (and hopefully protective) secondary response is generated.

The immune system encounters antigens within the body in two forms. Soluble antigens are found floating freely in the liquid portion of the blood and in the lymph fluid that drains the tissues and filters through lymph nodes. Examples of soluble antigens include bacteria introduced through a break in the skin, or a bacterial protein product like diphtheria or tetanus toxin. Cell-associated antigens are foreign proteins or altered self proteins that have been

synthesized within a cell, such as viral proteins in virally-infected cells, antigens made by intracellular bacteria, and tumor-associated mutant self proteins on cancerous cells. In order to effectively deal with both soluble and cell-associated antigens, the adaptive immune system can mount two distinctly different types of antigen-specific responses.

The humoral immune response, mediated by antibodies, has evolved to deal with soluble antigens. The term “humoral” refers to body fluids, which were known as “humors” in the late 1800s when it was demonstrated that immunity could be transferred via fluids such as serum (1). The surface Ig that acts as the antigen receptor on B cells can bind soluble antigens, which sets off a chain of events (described in more detail below) that eventually gives rise to antibody-secreting plasma cells. The secreted antibody, which circulates in the blood and lymph, binds to the specific soluble antigen that stimulated the response. The resulting antibody-antigen complex can be cleared by the kidneys, lysed by the complement cascade, and/or removed by phagocytic cells such as monocytes and macrophages. In the case of biologically active antigens like toxins or free floating bacteria or viruses, the binding of antibody may neutralize the antigen, i.e., prevent it from interacting with cells, thus preventing toxic effects or infection.

Cell-associated antigens are the product of cells that are defective in some way (mutated and/or cancerous), infected by intracellular organisms (viruses, intracellular bacteria, some parasites), or foreign cells that have entered the body. Mutated self antigens or foreign antigens produced within a cell are digested into small pieces, which become physically associated with self MHC molecules also produced within the cell. The digested antigen-MHC complex then migrates to the cell surface, where it could be detected by the adaptive immune system. In most cases, the digested antigen on the cell surface cannot be removed by circulating antibodies. More importantly, if the antigen is a product of an intracellular pathogen, the pathogen itself is sheltered within the cell, beyond the reach of the humoral immune response. It is necessary, therefore, for the immune system to be able to rid the body of a defective, infected, or foreign cell as a whole. The antigen-specific cell-mediated or cytotoxic response, which is capable of killing whole cells by inducing cell lysis, has evolved to meet this need. Adaptive cytotoxic immune responses are carried out by CD8-expressing cytotoxic T lymphocytes (CTL), as described below.

II. THE COOPERATION OF INNATE AND ADAPTIVE IMMUNE RESPONSES

In a healthy immunocompetent individual, i.e., a person with intact, properly functioning innate and adaptive immune systems, antigen-specific immune responses are generated as a result of a series of interactions between different types of WBC. A schematic illustration of these interactions is shown in Figure 1. Although humoral responses are ultimately carried out by antibody-secreting B cells, and cell-mediated cytotoxic responses are executed by CTLs, both types of responses require the same collaborative first step. The critical cell types in this initial step are antigen-presenting cells (APCs) and the CD4-expressing subset of T cells known as T helper (T_H) cells.

In the example illustrated in Figure 1, the antigen (represented by asterisks) is found on a virus, which can be both free-floating in body fluids (when first entering the body as well as when released by infected cells), and located inside infected cells (the rectangular cells along the left side of the figure). In order to initiate an adaptive immune response, it is necessary for this

viral antigen to be recognized by T_H cells. Unlike B cells, T cells cannot recognize free-floating or soluble antigens. Rather, the TCR on a T cell recognizes digested antigen on the surface of another cell, but only when the antigen is part of an antigen-MHC complex. As previously described (1, 2), there are two classes of MHC molecules: Class I, which is expressed on virtually all cells throughout the body, and Class II, which is expressed only on select cells within the immune system. When cell-associated antigens are produced within any type of cell (such as the virally-infected cells in Figure 1), the digested antigens are complexed with MHC Class I molecules. However, the initiation of either a humoral or cytotoxic adaptive immune response depends on the ability of T_H cells to recognize antigen plus MHC Class II molecules. This critical recognition event requires the participation of APCs, most of which belong to the innate immune system.

Since the expression of MHC Class II molecules is strictly controlled, only certain immune system cells can act as APCs for T_H cells. An APC is capable of taking up virtually any protein antigen from outside the cell, digesting it, and displaying an antigen-MHC Class II complex on its surface for interaction with T_H cells. The most effective APCs for interacting with and stimulating T_H cells in a primary immune response are dendritic cells (DC). DCs are innate, macrophage-like cells, usually found in tissues and/or lymph nodes, that collect antigens, digest them, and present them to T cells with a high concentration of MHC Class II molecules. Other innate APCs are monocytes (circulating in the blood) and macrophages (monocytes that have moved into tissue), which become activated after phagocytosis of antigen and express MHC Class II molecules. The final type of APC that can interact with T_H cells is B cells, which can capture and internalize antigen via cell surface Ig antigen receptors, and then process and present antigen-MHC Class II complexes on the cell surface.

In Figure 1, an APC that has encountered some free-floating viruses is shown at the top of the figure. In this example, the APC could be a DC, macrophage, or monocyte, using innate Toll-like receptors for the recognition of pathogens (1, 2). Upon interaction with the viral particles, the APC becomes activated, internalizes and processes the virus particles, and presents viral antigens plus MHC Class II to the T_H cell shown to its right. This illustrates how innate system cells serve as a bridge to the initiation of adaptive responses, i.e., facilitating the antigen-specific interaction of the TCR on a T_H cell with antigen plus MHC Class II. It is important to remember that this particular APC will not simply interact with the first T_H cell it encounters. Rather, it will be able to interact only with those T_H cells that carry a TCR with an antigen-binding pocket that is a match for the relevant viral antigens. In many cases, APC- T_H cell interactions will occur in lymph nodes and other lymphoid tissues where antigens are trapped and concentrations of APCs (especially DCs) and T cells are high, maximizing the likelihood of a successful match between antigen and its antigen-specific T cell.

The specific binding of the TCR to the viral antigen-MHC Class II complex on the APC sends a signal to the nucleus of the T_H cell. Although not shown in Figure 1, all APCs interacting with T cells must also provide an additional stimulatory signal via cell-to-cell contact with the T_H cell in order to complete the activation of the T cell. When a T_H cell receives the correct combination of signals from the TCR/antigen/MHC Class II interaction plus cell-to-cell contact, it becomes fully activated, capable of driving all other aspects of adaptive immune responses.

One of the major functions of antigen-activated T_H cells in immune responses is the secretion of cytokines. Cytokines are secreted proteins which send signals or messages between cells. They were first described as messenger molecules involved in signaling between different types of WBC (also known as leukocytes), and so some cytokines bear the name “interleukin,” meaning “between white cells.” Cytokines are produced by one cell in response to a stimulus, and then, depending on the cytokine, act on the same cell or adjacent cells to influence their behavior. Cytokines are produced by both innate and adaptive immune system cells, and are very important in both types of responses, but for the purposes of this course, will be discussed only in the context of adaptive responses. As shown in Figure 1, cytokines act at a variety of steps throughout both humoral and cytotoxic immune responses.

The newly-activated T_H cell at the top of Figure 1 begins to make an essential cytokine, originally known as T cell growth factor, but now known as interleukin-2 (IL-2). IL-2 produced by an activated T_H cell can act on itself, as well as on other T cells. IL-2 is absolutely required for the antigen-activated T_H cell to divide and proliferate. This proliferation dramatically increases the number of antigen-specific activated T_H cells, which is necessary to ensure an immune response of sufficient magnitude. (It also contributes to the swelling and tenderness of lymph nodes during an acute immune response.) In addition to proliferation, the T_H cells begin the process of differentiation, in which cells fully mature and/or acquire new function. In the case of T_H cells, differentiation allows the production of a variety of cytokines in addition to IL-2. It also permits the expression of additional cell surface molecules that will deliver cell-to-cell signals, and the development of a pool of antigen-specific memory T_H cells. It is through the secretion of cytokines, as well as providing co-stimulatory signals, that T_H cells “help” both antibody- and cell-mediated immune responses to develop.

III. GENERATION OF AN ANTIBODY RESPONSE

In Figure 1, while an innate APC is processing and presenting soluble viral antigens, a B cell that is specific for the same viral antigens is recognizing and binding to free-floating virus particles. B cells are capable of recognizing soluble antigens prior to processing, and more importantly, without the participation of MHC molecules. The B cell binds to the virus particles using the surface Ig molecules which serve as the B cell receptors for antigen. Similar to TCR binding, binding of antigen to B cell receptors delivers a signal to the nucleus of the B cell, initiating a cellular activation process. Back at the cell surface, the bound virus particle and Ig molecule are internalized by the B cell and processed in much the same way as in an innate APC, resulting in the digested viral antigen plus MHC Class II being displayed on the B cell surface. The B cell is then ready to interact with one of the newly-generated activated T_H cells that is specific for the same antigen, in order to complete the activation process.

When a B cell displaying viral antigen plus MHC Class II encounters an activated T_H cell that recognizes the same viral antigen, the two antigen-specific cells form a pair with the antigen serving as a bridge between them (as shown on the right side of Figure 1). These antigen-specific B-T cell pairs form in lymph nodes and other secondary lymphoid tissues, where T cell-rich and B-cell rich zones meet. Cell-to-cell contact with an activated T_H cell, which ensures the proper delivery of co-stimulatory signals, is required for the B cell to become fully activated.

Upon activation, the B cell expresses cytokine receptors that will enable it to receive cytokine

signals from the T_H cell. The interaction also brings the T_H cell into close proximity to the B cell so that it can effectively secrete additional cytokines to stimulate the B cell. These cytokines, which include IL-4, IL-5, and IL-6, are collectively called “B cell stimulatory cytokines,” and drive the activated B cell through the steps necessary to become an antibody-secreting plasma cell.

As was seen with the T_H cell, the newly-activated B cell responds first to cytokine signals from the T_H cell by proliferating in order to increase the number of antigen-specific B cells. This B cell proliferation also contributes to lymph node swelling during acute immune responses. Following several rounds of B cell division, there will be a pool or clone of B cells, all with the same antigen specificity as the original B cell that was activated by antigen and the T_H cell. Once the activated B cells have stopped dividing, additional cytokine signals from the T_H cell stimulate them to differentiate.

Differentiation for B cells takes two paths. One path generates a pool of antigen-specific memory B cells, some of which may persist for the life of an individual. It is these memory B cells, in concert with memory T_H cells, that will facilitate the rapid, high quality secondary antibody response upon subsequent exposure(s) to the identical antigen. The other path of B cell differentiation results in the Ig molecule, which originally served as the cell-surface antigen receptor, being actively secreted by the B cell. Fully differentiated B cells that secrete Ig (also known as antibody) are known as plasma cells (shown at the bottom right of Figure 1). Therefore, in the example shown, the very same virus-specific Ig that was anchored in the surface of the B cell is now being produced in a soluble form by a clone of plasma cells, to deal with free-floating virus particles in body fluids. Secreted Ig circulates in the blood and lymph, where it binds to the appropriate antigen and facilitates antigen clearance and/or neutralizes its activity, i.e., preventing the virus particles from infecting new cells.

IV. GENERATION OF A CELL-MEDIATED RESPONSE

In the case of a viral infection, humoral responses are effective in blocking viral infection and eliminating free-floating virus particles. However, cell-associated viral antigens and the virus itself within infected cells are not easily eliminated by circulating antibody. Instead, such cells (and associated antigens and pathogens) need to be eliminated by cell-mediated or cytotoxic immune responses. While innate cell-mediated immunity, involving macrophages and natural killer cells, serves as an important first line of defense (1, 2), the remainder of this course will focus on adaptive cell-mediated immune responses, which involve CD8-expressing T cells (CD8 T). Also known as cytotoxic T lymphocytes (CTL), this subset of T cells is responsible for antigen-specific cytotoxicity, which results in the lysis and death of whole cells in an antigen-specific manner.

CD8 T cells in the circulation or tissues are mature T cells that carry antigen-specific TCR on their surfaces, but are not yet capable of killing. An example of one of these cells, referred to as a CTL precursor (CTL-P), is shown on the upper left of Figure 1. Like T_H cells, the TCR on CTL-P cells will recognize only digested antigen plus MHC molecules on the surface of cells. However, in a very important difference from T_H cells, CTL-P cells recognize antigen plus MHC class I molecules, which are found on all nucleated cells in the body, including the APCs that also can facilitate MHC Class II-associated antigen presentation. For

the activation of a CTL-P, the TCR on the CD8 T cell interacts with an antigen-MHC Class I complex, usually presented by DCs or other APCs (as shown on the left side of the APC at the top of Figure 1). APCs are necessary for the initial activation of CTL-P due to a requirement for additional co-stimulatory signals. However, once activated and fully differentiated, CD8-expressing CTLs can respond to antigen plus MHC Class I on virtually any cell type in the body as described below.

The further activation of the CTL-P is driven by cytokines produced by nearby antigen-activated T_H cells. It is not clear whether a CTL-P and a T_H cell have to interact with the same APC at the same time, or if a T_H activated by a nearby APC will suffice (1). In either case, an activated CTL-P cell expresses the receptor that will permit it to respond to IL-2, the cytokine necessary for any T cell to proliferate. Activated CD8 T cells make little or no IL-2, therefore they must rely on activated T_H cells to help them by providing IL-2. This illustrates once again the collaborative nature of the innate and adaptive immune systems, and the critical role for T_H cells in both humoral and cytotoxic immune responses. Ultimately, the help provided by T_H cells to CTL-P and CTLs is similar to that provided to B cells—the secretion of cytokines that provide proliferation and differentiation signals. However, there is one major difference between B and CD8 T cell help. In order to become fully activated, B cells require cell-to-cell contact with an activated T_H in addition to the secretion of cytokines. In contrast, neither the CTL-P nor the CTL appears to require physical contact with the T_H cell, but depends instead on its secreted cytokine signals.

Upon receipt of the IL-2 signal from a T_H , the activated CTL-P proliferates, increasing the number of potential antigen-specific CTLs. As the CTL-P cells proliferate, they continue to respond to IL-2 as well as to additional cytokines secreted by T_H and possibly even by APCs. These cytokine signals allow the CTL-P cells to differentiate into fully-functional, lethal CTLs, as well as into memory CTLs. All of these CTLs bear the same TCR as the original CTL-P cell that was activated by viral antigen, but now have the ability to kill and/or remember any cell bearing the same viral antigen.

All proteins manufactured within a cell show up on the cell surface in digested form as an antigen-MHC Class I complex, regardless of whether the proteins are normal self, mutated self, or foreign. This process occurs in nearly all cells in the body, but the levels of MHC Class I expression vary among cell types, with the highest levels of expression on lymphocytes. CD8 T cells develop tolerance to self antigens during immune system development, and under normal circumstances, do not respond to self-antigen plus self-MHC complexes. However, when foreign proteins are being synthesized inside a pathogen-infected cell, or mutant proteins are being produced that are perceived as foreign, it is the combination of foreign antigen plus self-MHC Class I that successfully engages the TCR of a fully-mature CTL and initiates cell killing. In Figure 1, some of the virally-infected cells along the left side of the figure are shown as expressing viral antigen plus MHC Class I on their surfaces. They are probably not immune system cells, do not express MHC Class II, and so cannot act as APC for CD4+ T_H cells or CTL-P cells. They are, however, poised to serve as targets for fully-functional CTLs.

As illustrated on the left side of Figure 1, a CTL utilizes its TCR to bind to a target cell in an antigen-specific fashion. Once it has bound to its target via cell-to-cell interactions, the CTL usually uses one of two ways to kill the offending cell. It can secrete a pore-forming protein

known as perforin, that inserts itself in the membrane of the target cell and permits the delivery of toxic granules into the target cell. Within five minutes of contact with a CTL, the contents of the granules begin to induce target cell death through a suicide process known as apoptosis. Alternatively, in the absence of perforin, CTLs can induce apoptosis via an interaction between two membrane proteins, Fas and Fas ligand, on the surfaces of the target cell and CTL, respectively. After either one of these interactions, the CTL releases the mortally-wounded target cell (shown as a hatched cell in Figure 1), leaving it to die and ultimately break apart within a few hours or less. Interestingly, the CTL is resistant to its own means of killing other cells, so this killing can be repeated again and again. Therefore, not only does the original CTL precursor proliferate to increase the number of antigen-specific CTLs, but each of those CTLs is capable of killing many target cells. Upon the death of target cells, pathogens (such as more viruses) and/or soluble antigens may be released into the circulation. If cell-mediated responses occurred in the absence of humoral responses, this release of pathogens and antigen might be more harmful than helpful. However, many pathogens and antigens are soluble at some point in their journeys through the body, and so may have stimulated both humoral and cell-mediated responses. Then, upon the death of infected cells as a result of CTL activity, newly-released organisms and antigens could be bound and cleared by circulating antibody being produced concomitantly by plasma cells.

V. IMMUNOLOGIC ASSESSMENTS

In the first part of this series, the cells and cell surface molecules that make up the immune system were reviewed (2). In this course, the cellular interactions necessary to generate antigen-specific responses that battle primary exposures and ensure long-term immunity have been presented. The course will conclude with an examination of some of the laboratory assays that are used to evaluate the immune system as well as a discussion of immunologic techniques (3). In particular, the ability of flow cytometry (Figure 2) and ELISA testing (Figure 3) to contribute information about the immune system will be emphasized. Although immunology laboratories and specialized immunologic techniques are often found only in large institutions and/or reference laboratories, such techniques are becoming more common in many areas of the routine clinical laboratory. Even if a clinical laboratory does not perform any of these assays, clinical scientists at all levels may be called upon to facilitate the performance of such testing. It may be helpful, therefore, to be familiar with some of these techniques.

A traditional method of testing immune cell function is the delayed-type hypersensitivity skin test, where a small dose of antigen is introduced intradermally, then the injection site is observed for 24-48 hours for evidence of a localized immune response (3). Health care workers are familiar with this type of test being used to determine whether or not an individual has been previously exposed to a particular antigen or pathogen, such as the purified protein derivative (PPD) skin test for tuberculosis. However, skin testing is also used clinically to evaluate the ability of an individual's immune system to make a cell-mediated memory response to common antigens. Such a response requires many of the cellular interactions shown in Figure 1, and so can provide an indication of the overall status of the immune system. Therefore, in situations where the immune system is likely to be suppressed, (i.e., a person with HIV infection, undergoing cancer treatment, or receiving an organ transplant), the ability of an individual to

respond to one or more commonly-encountered antigens in a skin test can be a valuable means of assessing overall immunocompetence (3). The information provided by skin testing is limited due to its qualitative nature, i.e., it can only show if an individual does or does not respond. There are other, more quantitative means of evaluating the immune system, some of which can be performed in the typical clinical laboratory, and others that are likely to be performed only in highly specialized hospital, research, or reference laboratories.

Physicians have long relied on the hematology laboratory and the complete blood count to provide them with critical information regarding the status of the immune system. The total WBC count and the white cell differential count (whether manual or automated) provide insight into the possible presence or absence of infection and/or immunologic disorders. The serology laboratory can provide additional clues, ranging from quantitative measures of serum immunoglobulin levels to more specific diagnostic information such as antibody titers against a particular pathogen. However, as the practice of medicine has become more sophisticated in its understanding of the immune system, it has been necessary for the clinical laboratory to provide more detailed immunologic analyses. Out of this need has evolved the field of flow cytometry, which has taken the best and brightest technology that hematology has to offer, and coupled it with the powerful specificity of antigen-antibody reactions of the serology lab.

Flow cytometry permits the discrimination of subsets of white blood cells, especially lymphocytes, that cannot be distinguished simply on the basis of morphology under a light microscope or in an automated hematology analyzer (3). As our understanding of the different roles played by the T_H , CTL, and B cells has increased, so has the need increased to be able to enumerate these lymphocyte subsets in much the same way that a white cell differential enumerates neutrophils, lymphocytes, and eosinophils. Although all lymphocytes share the same basic mononuclear cell morphology, lymphocyte subsets can be distinguished by the presence (or absence) of particular proteins expressed on each cell's surface. The power of flow cytometry lies in its ability to detect, one cell at a time, specific antibodies that recognize and bind to known cell surface proteins. These antibodies serve to classify morphologically-identical lymphocytes into their immunologically-distinct subsets.

The antibody reagents that make flow cytometric analysis possible are known as monoclonal antibodies. A monoclonal antibody preparation is a homogenous solution of identical antibody molecules, all of which recognize and bind to the same, known cell surface antigen (1). A standard nomenclature has been developed for monoclonal antibodies, as reviewed briefly in the first part of this series (2). All monoclonal antibody preparations that recognize the same cell surface antigen are grouped into a "Cluster of Differentiation" or CD, which corresponds to the antigen recognized. For example, all monoclonal antibodies which recognize a TCR-associated protein that has been assigned the name "CD3," are referred to as "anti-CD3" or simply as "CD3 antibodies." As of December 2004, CD designations had been assigned up to CD339 (4). The CD antigens typically assessed in lymphocyte subset analyses are CD3 (all T cells), CD3+CD4 (T_H cells), CD3+CD8 (CTL-P, CTL), and CD19 or 20 (B cells). In some cases, CD14 (monocytes) and CD16+CD56 (NK cells) would also be included as part of an immune system assessment.

Monoclonal antibodies used in flow cytometry must be labeled so that they can be detected if they are bound to the surface of a cell. As will be described below, flow cytometry

instruments utilize laser light for cell analyses (Figure 2). Therefore, a monoclonal antibody reagent is labeled with a fluorescent tag which can be detected after exposure to a particular wavelength of light. The two most common tags which are frequently used simultaneously are phycoerythrin (PE) and fluorescein isothiocyanate (FITC), which fluoresce red and green, respectively. Additional tags are sometimes used, including peridinin chlorophyll protein (PerCP) and Texas Red (3).

Prior to analysis in a flow cytometer, aliquots of whole blood or purified WBC are incubated with labeled monoclonal antibodies, to allow binding of the antibodies to the appropriate cell surface antigen (if present). It is these “stained” cells that are then subjected to flow cytometric analysis. In the schematic illustration of a flow cytometer shown in Figure 2, the WBCs being analyzed were stained simultaneously with two different monoclonal antibodies, each recognizing a different cell surface antigen. One of the monoclonals was tagged with a FITC label, and the other with a PE label. This has resulted in a mixed population of cells in solution, which are shown entering the flow cytometer at the top of the figure. This population includes cells that are not stained by either monoclonal (i.e., do not have either antigen on their surface), cells stained with FITC only or PE only (have only one antigen), and cells stained with both FITC and PE (have both antigens, or “double-positive”). The task for the flow cytometer is to analyze and record cell characteristics that will allow enumeration of WBC and/or lymphocyte subsets based on the expression (or lack of expression) of the cell surface antigens recognized by the monoclonal antibodies used for staining.

Similar to hematology analyzers, flow cytometers perform their analyses on thousands of individual cells which pass through the instrument one at a time. As shown in Figure 2, cells are carried through the cytometer in a focused fluid stream barely wide enough for a single cell, so that they will pass in front of a light source one cell at a time. Virtually all flow cytometers currently use a laser as the light source, which produces high intensity light at a specific wavelength (3). As each cell passes in front of the laser, it disrupts the laser beam. Each disruption is detected by several different types of light detectors, strategically placed around the fluid stream. These detectors make possible the multi-parameter measurements that give the flow cytometer its analytic power.

First and foremost, the passage of a cell through the path of the laser beam registers the presence of a cell and is the basis of the cell count. Secondly, the manner in which the laser light is scattered provides information regarding the physical parameters of each cell. The forward light scatter (FSC) and side light scatter (SSC) are a function of the size and intracellular complexity of the cell passing in front of the beam, respectively (3). These two parameters, especially the side scatter, can be used to distinguish between granulocytes, monocytes or macrophages, and lymphocytes. Finally, the laser light will excite the fluorescent tag(s) (such as FITC and PE in this example) on any labeled monoclonal antibody bound to the surface of the cell. This is illustrated in Figure 2, where the double-positive cell currently in the laser light path is emitting both red and green fluorescence, which are detected by their respective detectors. When a flow cytometer is set up to detect two colors simultaneously as shown in this example, the analysis is sometimes referred to as “five parameter” – cell count, forward scatter, side scatter, and two colors (usually red and green). As each of the following cells passes through the path of the laser, they will be evaluated in the same manner. All of the information collected on

each cell is recorded and stored electronically, then analyzed to provide the desired data regarding the entire cell population.

As an extension of the type of analysis shown in Figure 2, some flow cytometers are capable of physically sorting cells according to their fluorescence. This procedure is called “fluorescence-activated cell sorting” or FACS®, hence flow cytometry analyses were sometimes referred to as “FACS analyses.” However, since FACS® is actually a registered trademark of Becton-Dickinson, its use as a generic term for flow cytometric analysis, regardless of the instrument used, has been discouraged recently. This sorting technology can be extremely useful for separating cells according to cell surface markers, for further analysis or experimental use. However, it is not necessary in the majority of flow cytometry analyses where an enumeration of cell types is all that is desired.

Analysis by flow cytometry determines the relative percentages of each cell type within the population of interest. For example, an analysis could be performed that collected information on 5,000 lymphocytes that had been stained with a PE-labeled antibody for CD3, an antigen which is present on all T cells and only on T cells, and a FITC-labeled antibody for CD19, an antigen that identifies B cells. Out of those 5,000 lymphocytes, 81% were detected as CD3-positive (red only), 12% were CD19-positive (green only), and 7% were found to be unstained (negative for CD3 and CD19). Therefore, of the lymphocytes, 81% are T cells, 12% are B cells, and the remainder are “non-T, non-B” lymphocytes (usually a class of cells known as “natural killer cells”). As another example, lymphocytes could be stained for CD3 (to identify T cells) and CD4 (to identify the T_H subset of T cells), and the percentage of cells that were double-positive would indicate the percentage of lymphocytes that were of the T_H type.

Flow cytometry laboratories can differ widely in their reporting format, but most do include the percentages actually obtained by the flow cytometer. However, clinicians are often more familiar with absolute cell numbers, i.e., the numbers of B cells or T_H cells per mm³ of blood. Therefore, even the sophisticated flow cytometer must, in most cases, still rely on a basic complete blood count, which would provide an absolute lymphocyte count, in order to calculate lymphocyte subsets in absolute numbers (3). For instance, an individual with 50% of lymphocytes registering as CD3+/CD4+ T_H cells by flow cytometric analysis, and an absolute lymphocyte count of 1800 cells/mm³ in a hematology analysis, would have an absolute T_H cell count of 900 cells/mm³. An example of reference ranges for both percentages and absolute numbers of lymphocyte subsets can be found in the first part of this series (2).

What is to be gained by flow cytometric analysis over a complete blood count? It is the ability to determine in relative percentages, and calculate in absolute numbers, the different types of lymphocytes that are critical for different immune cell functions. While this level of discrimination is not required for the majority of patients, it can be critical in the diagnosis and care of persons whose medical conditions are directly related to the status of their immune system (3). If a person has been found to have below-normal levels of circulating immunoglobulin, it may be essential to determine if he or she has a normal number and/or percentage of B cells. If an abnormally high percentage or number of lymphocytes has been seen and a hematologic malignancy is suspected, lymphocyte subset analysis specifically designed for detection of leukemia or lymphoma could be invaluable in confirming or ruling out such a condition. In the continuing era of the HIV/AIDS pandemic, lymphocyte subset analysis

enumerating CD4-positive T_H cells, the cells which are lost as a result of HIV infection, is considered part of routine care for HIV-infected persons.

Functional assessment of B cells as Ig-producing cells is relatively straightforward, since Ig is secreted by plasma cells into the surrounding lymph fluid and the blood. Therefore, clinically-relevant information can be obtained regarding B cell function simply by measuring the levels or types of Ig present in the serum or plasma of peripheral blood samples (3). Such measurements may be performed using sophisticated automated clinical laboratory instruments. However, quantitative and qualitative determination of Ig levels, as well as levels of many other immune system proteins (such as cytokines) and other biological molecules of interest, can be performed utilizing relatively simple Enzyme-Linked ImmunoSorbent Assay (ELISA) testing. Interestingly, ELISA testing can be used to assess the immune system, while at the same time, it is also an example of a procedure that uses immunological techniques. It harnesses the ability of antibodies to bind with great specificity to an unprocessed antigen of interest, without any need for APCs, enabling the detection and/or quantitation of either soluble molecules (usually proteins) or antigen-specific antibodies in biological fluids (3).

A schematic illustration of the most common type of ELISA, known as an antibody capture or sandwich assay (3), is shown in Figure 3. This type of assay is designed to detect and quantitate molecules of interest that are soluble in serum, plasma, or any other fluid solution. The technique is called “immunosorbent” because antibodies of known antigen-specificity, usually monoclonal antibodies like those described above, are adsorbed or adhered to a plastic surface. Many ELISAs are set up in plastic 96-well microwell plates, with monoclonal antibodies coating the bottom of each well; a schematic of one such well is shown in Figure 3. There are variations, however, that include antibodies coated onto latex beads or membrane surfaces rather than fixed to the bottom of a 96-well plate. A solution or liquid biological sample containing an assortment of molecules, including the molecule of interest (represented in Figure 3 by the indicated five-sided shape), is added to a well that has been coated with an antibody specific for that particular molecule of interest (top of Figure 3). After an incubation to ensure antigen-antibody binding, the original sample solution and all other molecules are washed away, leaving only the molecule of interest bound in the well by the monoclonal antibodies (middle of Figure 3). A second antibody, which is specific for the same molecule but is not blocked by the binding of antigen to the first (coating) antibody, is added to the assay well. This second antibody binds to the antigen retained in the well by the coating antibodies, forming a sandwich (antigen on the inside, two different antibodies on the outside) (bottom of Figure 3). The second antibody is known as the detection antibody, because it is labeled with an enzyme molecule that will catalyze a color-development reaction that can be easily detected and quantitated. It is the labeling of the detection antibody by chemically linking or coupling an enzyme molecule to it that gives rise to the “enzyme-linked” portion of the assay name.

Detection in an ELISA is accomplished by adding to the assay well the appropriate liquid substrate for the enzyme label on the detection antibody. Upon interaction with the enzyme label retained in the antigen/antibody sandwich, the substrate changes color, and the intensity of the color is directly proportional to the starting concentration of the molecule of interest. The color intensity or optical density is easily determined by spectrophotometric instruments that are specific for the appropriate wavelength of light. There are a number of ELISA instruments

available that are capable of detection in the standard 96-well plate format as well as other formats, and accompanying computer-based software permits both qualitative (i.e., positive or negative for the presence of the molecule of interest) and quantitative analyses (based on comparison to standards of known concentration).

Historically, ELISAs were preceded by a very similar technique, known as radioimmunoassay (RIA), which utilized a radioactive isotope label instead of an enzyme label for detection (1, 3). RIAs were originally more sensitive than ELISAs, but with improvements in sensitivity and the appeal of the elimination of the use of radioactive materials, ELISAs have, for the most part, replaced RIAs as the technique of choice for determinations of this kind. This valuable antibody-based technology, however, continues to evolve, with enzyme labels being replaced by chemiluminescent or fluorescent labels, and fixed, single-analyte assays in 96-well plate formats being adapted to bead-based, multiple-analyte assays performed in solution utilizing flow cytometry for detection.

Depending on the monoclonal antibodies utilized, sandwich ELISAs, as illustrated in Figure 3, can be designed to detect total serum Ig, or serum levels of the subclasses of Ig (IgM, IgG, IgA, IgE). In a slightly different type of ELISA, known as an indirect assay, a specific antigen and/or pathogen is coated to a plate, serum or plasma is added, and the ELISA determines the presence/absence and/or titer of antigen- or pathogen-specific antibodies in the blood. Both of these types of analyses can shed light on the status of humoral immunity in an individual. Further functional analysis of B cells at the cellular level is usually beyond the scope of clinical laboratory practice, but may be part of clinical trials or other research protocols. The techniques utilized may include specialized flow cytometry analyses or a technique that has combined ELISA technology with cell culture which is known as an ELISPOT assay (1, 3).

Laboratory assessment of CTL function poses a much greater challenge, both immunologically and technically. Since CTLs must have antigens processed and presented to them in association with self MHC molecules, it is a considerable challenge to provide a “self” target cell. In order to prepare true self target cells for CTL assessments, an individual would typically need to be available to provide fresh blood samples on at least two occasions, or WBC from a single blood sample must be frozen for future use. Additional technical challenges include the consistent measurement of target cell killing. These obstacles restrict the performance of CTL assessments almost exclusively to research settings, where the time and expertise is available to carry out such complicated assays. With the advent of more sophisticated flow cytometry techniques, assessment of ability of T cells from an individual to recognize and respond to a particular antigen has become possible using complexes of four synthetic MHC molecules (known as MHC tetramers) matched to the MHC type of the individual being assessed, and synthesized peptides that mimic processed antigens (1, 3). Details regarding CTL assessments can be found in multiple chapters of reference 3.

As shown in Figure 1, an important early step in humoral and cytotoxic adaptive immune responses is the activation and proliferation of the antigen-specific T and/or B cells. Therefore, the extent of activation and/or proliferation of lymphocytes can be used as a semi-quantitative indicator of the level of function of the immune system (3).

There are a number of well-characterized cell surface molecules that can act as markers of activation on lymphocytes (1, 3, 4). They tend to be expressed on the cell surface at low or

undetectable levels on resting cells, but increase significantly following stimulation of cells. Monoclonal antibodies that recognize and bind to activation antigens can be used to perform flow cytometry analysis on B and T lymphocytes to determine the extent of cellular activation. New markers and techniques for flow cytometry-based activation assays continue to be introduced and evaluated (3).

There are various ways to evaluate cellular proliferation, usually by measuring the extent of cellular division. Techniques range from determining increases in cell numbers by counting live cells manually under a light microscope, to new flow cytometry-based assays, where the intensity of staining of a cell membrane dye is reduced by half during each cell division. However, the classic means of determining the amount of cell division depends on the fact that all cells must duplicate their DNA chromosomes before dividing. In such an assay, a constant number of immune system cells is cultured in medium that contains all four nucleoside building blocks needed for making new DNA: adenosine, cytosine, thymidine, and guanine. As each activated lymphocyte prepares to divide, it makes a new copy of its DNA by assembling these four nucleosides in the proper order. In order to detect the presence of this newly-synthesized DNA (which indicates cell division and proliferation), one of the nucleosides in the medium is labeled with a tag that can be quantitated in some way. Thymidine (T) is usually the labeled nucleoside, and the label used in the traditional assay is tritium ($[^3\text{H}]$), a radioactive isotope of hydrogen (3). Hence, this assay is referred to as a tritiated thymidine ($[^3\text{H}]\text{-T}$) DNA incorporation assay; if performed on lymphocytes, it may be referred to as a lymphocyte proliferation assay (LPA). Cultured cells are harvested onto a glass fiber filter, which captures the newly-synthesized radioactively-labeled DNA. The amount of labeled DNA present is determined by liquid scintillation counting, and the amount of radioactivity present is directly proportional to the number of cell divisions which have occurred. As is the case in all areas of the laboratory, it is desirable to reduce or eliminate use of radioactive isotopes, so non-radioactive alternatives have been developed for LPAs. For example, one non-radioactive DNA incorporation assay utilizes bromodeoxyuridine (BrdUrd) as the labeled nucleoside, which can be detected with a monoclonal antibody, while another assay uses a colorimetric, ELISA-like assay that detects mitochondrial enzyme activity (3). Once again, additional details regarding cellular proliferation assays can be found in reference 3.

Although most clinical laboratory scientists may never perform any of the assays described above, this brief survey of immunologic techniques and assessments has been presented in order to integrate the details of the innate and adaptive cellular interactions necessary for the establishment and maintenance of immunity into the scope of work performed in clinical laboratories. As immunology continues to play a prominent role in clinical medicine, a broader understanding of the complex, yet collaborative nature of immune responses should assist the clinical laboratory scientist, regardless of his or her own area of expertise, in participating in the complex and collaborative delivery of clinical care.

REFERENCES

1. Kindt TJ, Goldsby RA, Osborne BA. 2006. *Kuby Immunology, 6th Ed.* W.H. Freeman and Company, New York.

2. Breen EC. Feb. 2007. An Overview of the Immune System, Part 1: The Cells and Cell Surface Molecules of the Immune System. *CAMLT Newslines*, Vol. 33 No. 1.
3. Rose NR, Hamilton RG, Detrick B, eds. 2002. *Manual of Clinical Laboratory Immunology*, 6th Ed. American Society for Microbiology Press, Washington, DC.
4. Human Cell Differentiation Molecules, <http://www.hlda8.org/HLDAtoHCDM.htm>

Figure 1

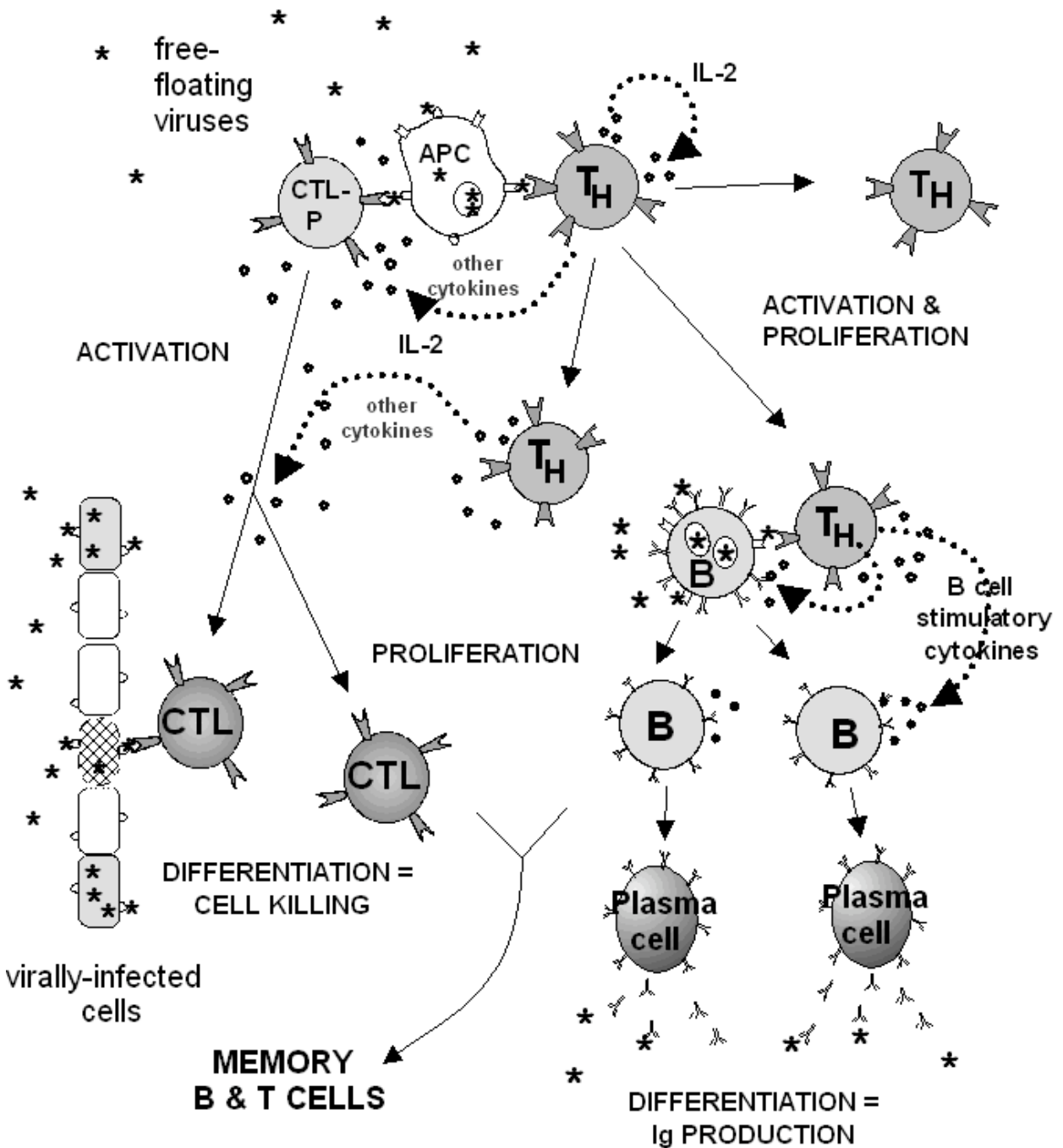


Figure 1: The Generation of Humoral and Cell-Mediated Immune Responses

A schematic illustration of the cellular interactions necessary to generate adaptive, antigen-specific immune responses. The antigens shown are viral particles (*), which can be both free-floating and in infected cells; processed viral antigen is shown on cell surfaces in association with MHC Class I (rounded) and/or MHC Class II (rectangular) molecules. Abbreviations are as follows: antigen-presenting cell (APC), T helper cell (T_H), interleukin-2 (IL-2), B cell (B), immunoglobulin (Ig), CD8-positive cytotoxic T cell precursor (CTL-P), cytotoxic T lymphocyte (CTL)

Figure 2

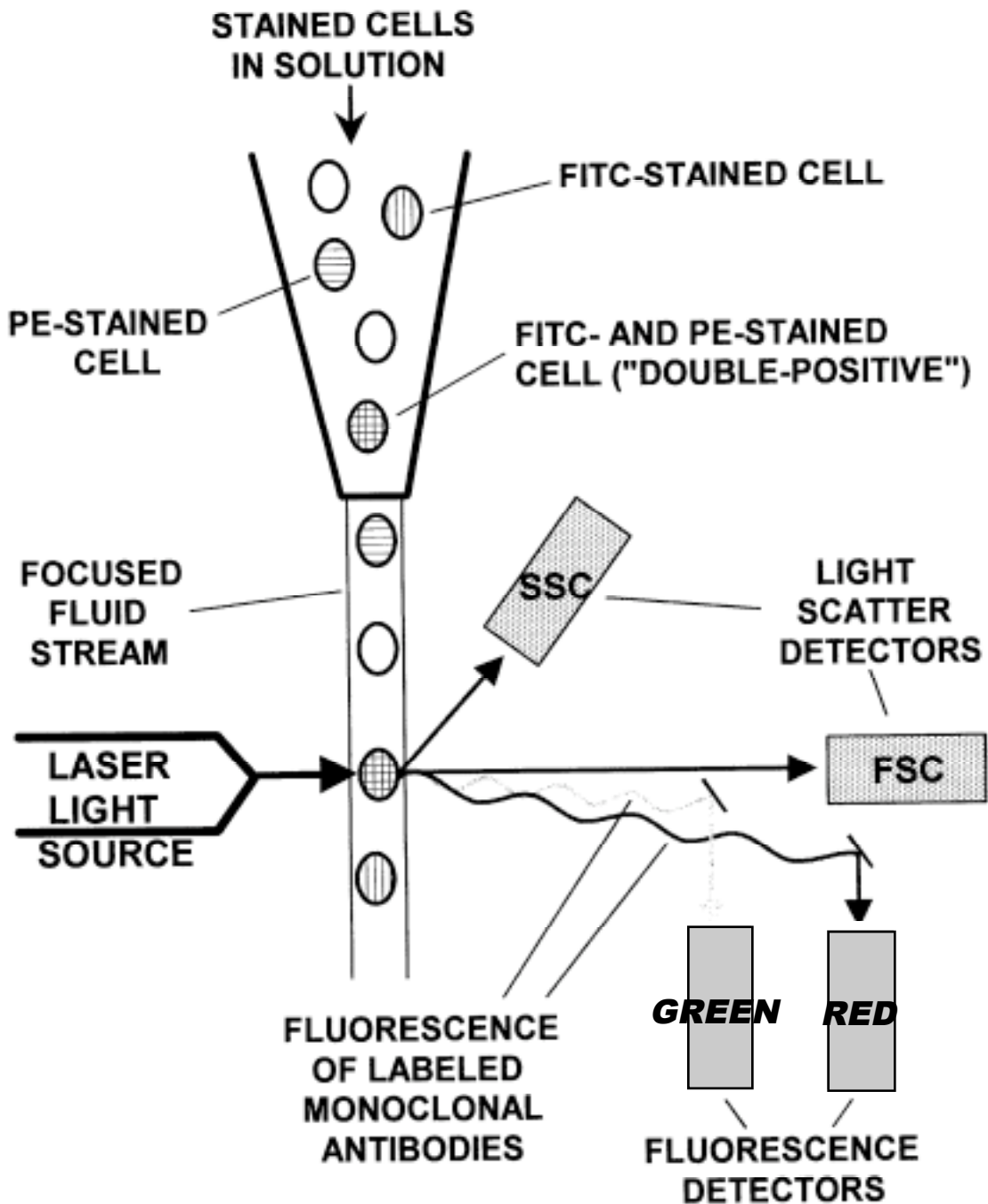


Figure 2: Cellular Analysis by Two-Color Flow Cytometry

A schematic representation of a laser-based flow cytometer analysis of cells stained with fluorescent-tagged monoclonal antibodies that recognize two different cell surface antigens. Phycoerythrin (PE)-stained cells are shown with horizontal hatching; fluorescein isothiocyanate (FITC)-stained cells are shown with vertical hatching; cells which stain with both antibodies ("double positive") are cross-hatched. Other abbreviations: side light scatter (SSC), forward light scatter (FSC)

Figure 3

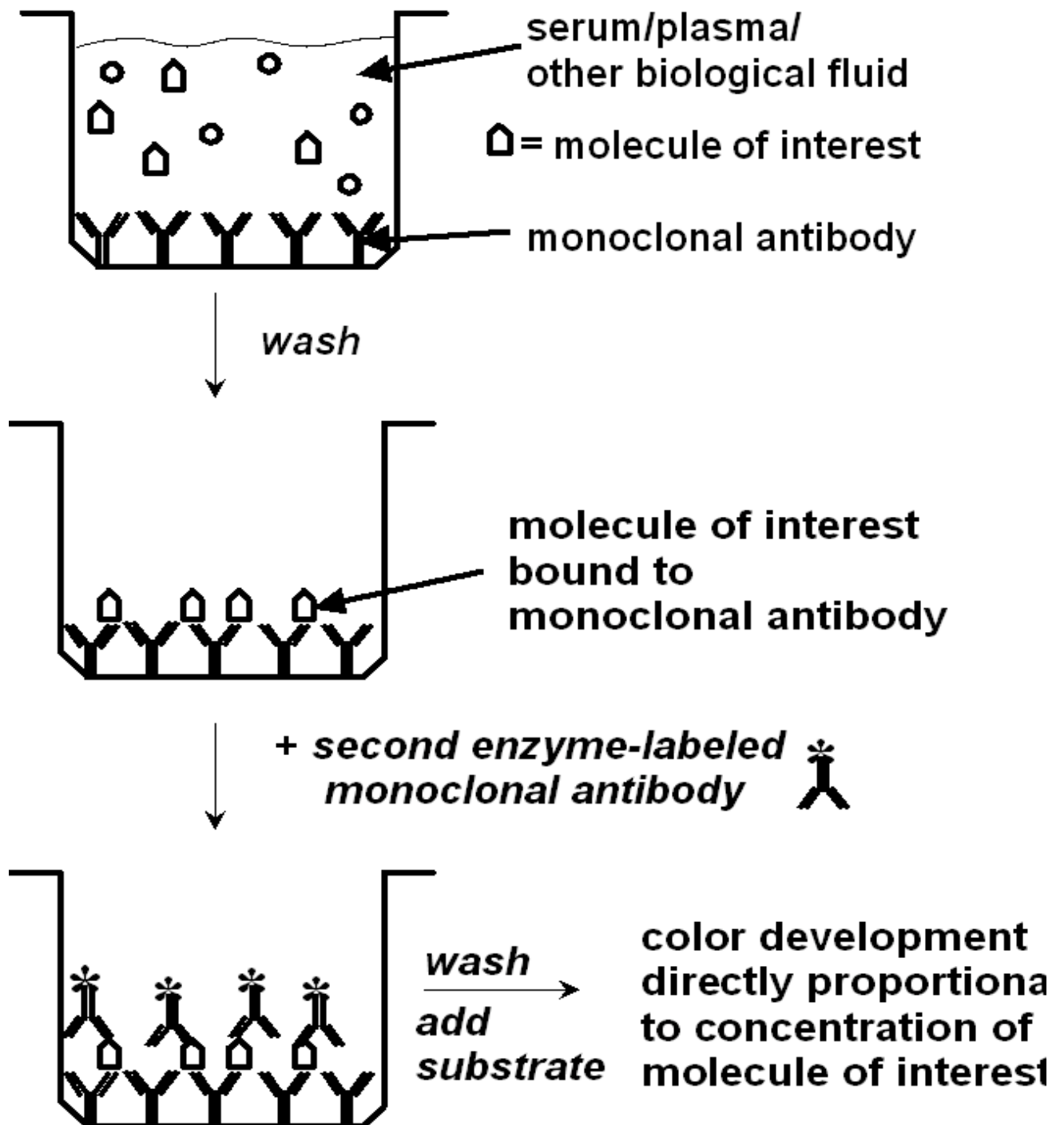


Figure 3: Sandwich Enzyme-Linked Immunosorbent Assay (ELISA)

A schematic illustration of the steps in an ELISA designed to detect and quantitate a specific soluble molecule of interest (five-sided shape) in a biological fluid sample.

REVIEW QUESTIONS

Course #DL-981

Choose the **one** best answer.

1. Observations of immunity were first recorded:
 - a. during an epidemic in 430 BC
 - b. as part of the first description of smallpox vaccination
 - c. by Louis Pasteur following his experiments with anthrax
 - d. among the survivors of the influenza epidemic of the early 1900s
2. Antigen-specific immune responses are possible due to:
 - a. the presence of molecules on the surface of lymphocytes that act as antigen receptors
 - b. antigen-specific recognition of pathogens by monocytes and macrophages
 - c. the phagocytosis of antigen by neutrophils
 - d. the ability of the skin to act as a barrier to antigen
3. Which of the following is not true about immune system genetic recombination?
 - a. it is mediated by enzymes that physically cut and paste DNA
 - b. it is restricted solely to T and B cells
 - c. it was first described for TCR genes
 - d. it contributes to both specificity and diversity
4. Secondary immune responses:
 - a. permit the invading pathogen to win the race and cause disease
 - b. are facilitated by a pool of antigen-specific memory B and T cells
 - c. are slower to occur than the primary response to the same antigen
 - d. occur more rapidly, but are not as vigorous as the primary response
5. Cell surface immunoglobulin molecules serve as:
 - a. toll-like receptors on monocytes
 - b. antigen receptors on T cells
 - c. pathogen detection receptors on natural killer cells
 - d. antigen receptors on B cells
6. Which one of the four distinguishing characteristics of antigen-specific responses utilizes MHC molecules?
 - a. antigenic specificity
 - b. diversity
 - c. self/non-self recognition
 - d. immunologic memory
7. The adaptive immune system is able to specifically rid the body of infected or foreign cells by:
 - a. utilizing CTLs to induce target cell apoptosis
 - b. neutralizing the offending cells with antibodies
 - c. reducing MHC Class I expression on potential target cells
 - d. enhancing phagocytosis by dendritic cells
8. The humoral or antibody response has evolved to deal with:
 - a. tumors
 - b. cell-associated antigens
 - c. tissue transplantation
 - d. extracellular antigens

9. The adaptive immune cell type which is necessary for the initiation of both humoral and cytotoxic immune responses is:
 - a. a CD4-positive T cell
 - b. a CD8-positive T cell
 - c. a CD19-positive B cell
 - d. an antigen-presenting cell

10. The major difference in the recognition of antigen by T cells vs. B cells is:
 - a. T cells recognize antigen in its native form; B cells recognize processed antigen
 - b. T cells recognize processed antigen plus MHC molecules; B cells recognize antigen only
 - c. B cells must have antigen presented to them by APC
 - d. B cells can recognize only protein antigens

11. The ability of the adaptive immune system to specifically recognize millions of unique sites on antigens describes:
 - a. antigenic specificity
 - b. diversity
 - c. self/non-self recognition
 - d. immunologic memory

12. Cytokines are:
 - a. always retained within the cytoplasm of a cell until its death
 - b. toxic proteins released by CTLs
 - c. secreted proteins that send signals or messages between cells
 - d. markers of self and non-self

13. The maturation of an antigen-activated B cell into a plasma cell is known as:
 - a. apoptosis
 - b. proliferation
 - c. differentiation
 - d. redistribution

14. Which of the following is not a property of interleukin-2 (IL-2)?
 - a. drives the proliferation of T_H cells
 - b. only cytokine necessary for the differentiation of B cells
 - c. stimulates the division of CTL-P
 - d. produced by antigen-activated T_H cells

15. A plasma cell secretes antibody that:
 - a. will bind to virtually any soluble antigen encountered in the circulation
 - b. has the same antigen specificity as the Ig originally on the cell's surface
 - c. can only deal with cell-associated antigens
 - d. remains sequestered in the lymph node in which it was produced

16. The characteristic of antigen-specific immune responses that is the basis for vaccination is:
 - a. antigenic specificity
 - b. diversity
 - c. self/non-self recognition
 - d. immunologic memory

17. An antigen-activated CTL-P proliferates in order to:
- begin secreting antigen-specific antibodies
 - to provide help by secreting cytokines
 - increase the number of antigen-presenting cells with the appropriate MHC expression
 - increase the number of potential CTLs with the desired antigen specificity
18. A fully mature CTL can kill:
- virtually any cell in the body expressing the appropriate antigen plus MHC Class I
 - virtually any APC presenting the appropriate antigen plus MHC Class II
 - only non-self cells expressing foreign MHC
 - only other CTLs expressing MHC Class I
19. In order for T_H cells to provide help to other cells, they must do all of the following except:
- express MHC molecules
 - provide co-stimulatory signals
 - secrete cytokines
 - recognize processed antigen
20. Delayed-type hypersensitivity testing with commonly-encountered antigens:
- is not possible in persons who have a positive TB skin test
 - provides a quantitative measure of memory cells present for a given antigen
 - is useful as a qualitative indicator of immune system function
 - is always positive, regardless of immune system status
21. Flow cytometric analysis:
- is useful only when standard hematology analysis is unavailable
 - utilizes a spectrophotometric instrument
 - is unable to distinguish between morphologically-identical B and T cells
 - permits enumeration of lymphocyte subsets
22. For use in flow cytometry analyses, a monoclonal antibody must:
- be inherently reactive to laser light
 - recognize several different antigens on a single cell
 - be labeled so that it can be detected when bound to cells
 - reflect only white light
23. Of the four distinguishing characteristics of adaptive immune responses, which one enables the immune system to discriminate between proteins that differ only by a single amino acid?
- antigenic specificity
 - diversity
 - self/non-self recognition
 - immunologic memory
24. Which of the following is not a parameter measured by a flow cytometer as a cell passes through its laser beam?
- electrical resistance
 - forward scattered light
 - side scattered light
 - presence/absence of fluorescence

25. The status of an individual's humoral immunity is easily assessed by:
- enumeration of CD3+ cells by flow cytometry
 - determination of serum levels of Ig
 - induction of apoptosis in target cells
 - measuring secretion of IL-2
26. Plate-based ELISA technology utilizes:
- MHC-matched self target cells
 - a radioactive isotope as its label
 - a laser-based instrument
 - enzyme-linked antigen-specific detection antibodies
27. Which of the following would not be necessary in order to assess CTL function?
- self target cell
 - MHC class I molecules
 - CD8 T cells
 - soluble antigen
28. The classic techniques for measurement of lymphocyte proliferation depend upon:
- the generation of non-dividing memory T and B cells
 - the processing and presentation of self DNA by APCs
 - the synthesis of new DNA prior to cell division
 - the incorporation of radioactive thymidine into cell membranes
29. All of the following are alternative techniques for measuring lymphocyte proliferation or activation except:
- flow cytometry-based assay utilizing a cell membrane dye that is reduced by half during each cell division
 - flow cytometry analysis utilizing monoclonal antibodies that recognize activation antigens
 - indirect ELISA utilizing cellular DNA to determine the titer of anti-DNA antibodies
 - non-radioactive DNA incorporation assay utilizing bromodeoxyuridine
30. The generation of immune responses:
- has no bearing on the delivery of clinical care
 - has little or no impact on the practice of medicine today
 - can only ever be assessed by research scientists
 - is a collaborative effort of the adaptive and innate immune systems

