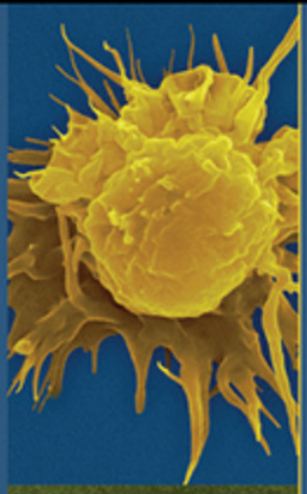

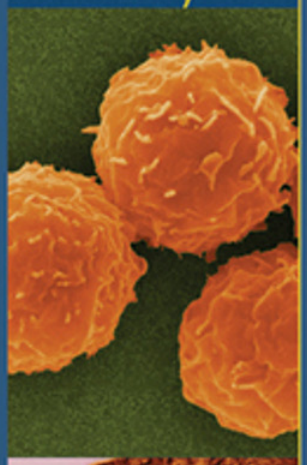

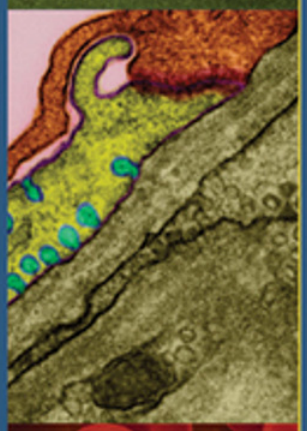

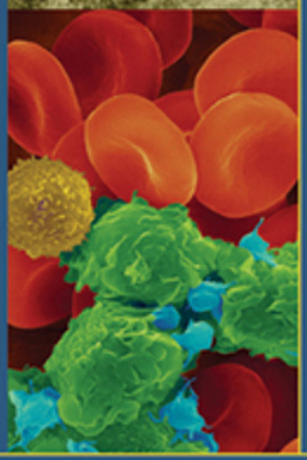

IMMUNOLOGY

CLINICAL CASE STUDIES AND DISEASE PATHOPHYSIOLOGY

Warren Strober and Susan R. S. Gottesman

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Clinical Case Studies and Disease Pathophysiology

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In memory of Hyam Gottesman.

Dedicated to our children, David, Lena, and Matthew.

With special thanks to Richard Coico and the hundreds of medical students, graduate students, and residents at SUNY Downstate Medical Center who have taught me how to teach.

Susan R. S. Gottesman

Dedicated to my wife, Heather Birnie.

Warren Strober

CONTENTS

PREFACE

This book consisting of clinical case studies of immunologically-related diseases is a companion volume to "Immunology, A Short Course" by Coico and Sunshine. The idea for the book grew out of our belief that the understanding of the complicated and sometimes confusing subject of immunology would be greatly facilitated by a study of the diseases of the immune system in which normal immune processes go awry. This view was strengthened by the complementary belief that the diseases of the immune system can be best understood through a firm grasp of normal immune mechanisms. The editors and authors have therefore created reality-based cases of typical patients with immunologic diseases and lead the reader through a detailed and logical clinical course based on both standard of care medical principles and a thorough knowledge of the underlying immunologic defect. In addition, each case is accompanied by an in-depth discussion of the normal and pathological immunological processes related to the disease state being discussed. In effect, a careful reading of the cases as a whole provides the reader with an alternative course in immunology that amplifies and complements the various sections of the companion "Short Course." Overall, we hope this somewhat unique way of learning about immunology and its associated diseases will offer an effective path to the mastery of a difficult subject and, at the same time, deepen your knowledge of clinical medicine.

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INDEX OF LESSONS IN NORMAL IMMUNITY

Each of the case studies contains a discussion of one or more normal immunologic processes that is relevant to the disease under discussion. In this way the abnormalities of the immune response occurring in the individual diseases is contrasted with normal immunologic function. Below is a list of the normal immune functions discussed in the various case studies; these discussions can serve as a review of the principles of immunology.

Miscellaneous:

INTRODUCTION TO CASE STUDIES: A GUIDE TO USING THIS BOOK

This book is designed to pique your interest in and increase your understanding of the immune system in normal individuals by showing you what happens when it goes awry in disease states. This book contains a series of clinical vignettes. Each is based on one or more real patients suffering from one of the main types of immunologic disorders. You follow each patient through the initial workup and laboratory studies to observe how the diagnosis and treatment plan are developed. You then follow the patient's subsequent course to learn how the disease typically evolves and, at times, how it requires changes in therapy.

Each clinical story has been calibrated to illustrate the main features of the disease under study and to discuss the disorders of normal function that result in these features. However, the disease manifestations presenting in a single patient usually do not encompass all of the manifestations of a particular disease entity that can occur in a large number of patients. Thus, where appropriate, we have included a section describing the characteristics of the disease in the entire patient population. Finally, each vignette morphs into a discussion of the normal immune system and the defects that have led to disease, that is, the mechanism of the disease as we now understand it. This provides a platform for discussion of many of the major immunologic processes that collectively make up the immune system.

You will encounter several recurrent themes as you read the vignettes. One theme that will become apparent to you is that immunologic disease of almost any type has some genetic basis. This is quite obvious in the congenital immunodeficiency diseases that are most often due to a single gene mutation. This is also true of autoimmune disease and malignant disease; however, in these cases the diseases are multigenic. It is apparent that the genetic abnormalities associated with autoimmune diseases consist of gene polymorphisms that are also present in large numbers of normal individuals. In order to give rise to disease, these polymorphisms must be present with other, additional gene polymorphisms and the genetic abnormalities in autoimmunity actually consist of multiple polymorphisms acting in tandem. Thus, one of the key challenges of research into the cause of multigenic diseases is to understand how each of the component polymorphisms contributes to a pathologic change in immune function. In malignant transformations of cells of the immune system, the neoplasm is the result of mutations in one or more key genes, usually involved in cell cycle control and thus affecting cell proliferation. The effects of these mutations combine with the individual's inherent (genetic) ability to control that proliferation. In malignant disease, in contrast to the situation in immunodeficiency or autoimmunity, the mutation is a somatic mutation that occurs in a very limited number of

Immunology: Clinical Case Studies and Disease Pathophysiology, By Warren Strober and Susan R. S. Gottesman

at least partially differentiated cells in the body. In immunodeficiency or autoimmune diseases, the genetic mutation or genetic susceptibility polymorphism is inherited in the germline and is therefore present in all the body's cells.

A second theme emerges from the inherent complexity of the immune response. Since the immune system consists of an astonishing array of different cell types and subtypes, understanding immune processes often involves dissecting the functions of each of these cells as well as their complex interactions. In studying immunologic diseases, one finds again and again that the key to understanding the mechanisms of immunologic disorders is to identify the specific cell subtypes initiating disease and how each one functions in the normal immune response as well as in the disease state. A related theme is really a testament to the economy of cell biology in general in that cells of the immune system with different phenotypes and functions often use identical intracellular signaling pathways and transcription control mechanisms. Therefore, characterization of diseases that affect many different cell types frequently leads to the identification of abnormalities of intracellular molecular processes occurring in those cells.

Our third theme, and one that should give clinicians pause in their approach to treating patients, evolves from the realization that the immune system is finely tuned to act as a totality. Therefore, any breakdown or manipulation of one function of the immune system inevitably affects another immune function. A consequence of this is evidenced in the fact that a deficiency in one arm of the immune system may lead to dysregulation of another arm, resulting in an overexuberant response against self or autoimmune disorder or, alternatively, even allowing the proliferation of mutated cells (neoplasms). One should not be surprised therefore to learn that seemingly disparate disease manifestations—immunodeficiency, autoimmunity, and malignancy—not infrequently appear together in the same patient.

The first section of this book (Unit I) is devoted to immunodeficiency diseases. In most cases these are congenital immunodeficiency diseases. In that primary immunodeficiency diseases involve, for the most part, a defect in a discrete component of the immune system, they have provided immunologists with an unparalleled opportunity to study and understand the function of these discrete components in humans. These diseases, some uncommon and some rare, are true "experiments of nature," and by learning about them you will enhance your understanding of the normal development of the immune system. This section also contains a chapter devoted to one of the most important secondary immunodeficiency disorders of our time, acquired immunodeficiency syndrome (AIDS). Analysis of AIDS, a disease in which the immune defect develops predominantly from the elimination of mature cells, has allowed immunologists to assess the immune system as a totality in which most of the immune system becomes dysfunctional. In this book we follow in the footsteps of these immunologists in that we use these various immunodeficiencies to teach about the specific as well as the general functions of the human immune system.

Units II (Immediate Hypersensitivity and Mast Cell Disorders) and III (Autoimmune Disorders) illustrate the consequences of dysregulation of the immune system whether due to responses to outside (exogenous) agents such as allergens or infectious organisms, to internal self-antigens as a result of the breakdown of tolerance, or to a combination of the two.

Lastly, Unit IV Malignancies of the Lymphoid System, demonstrates what happens when the internal cellular controls of lymphocytes fail to properly regulate their life cycle. Here again it becomes apparent that the inability of even a subset of lymphocytes to function normally (because of malignant transformation) leads to poor regulation of the remaining nonneoplastic cells, often resulting in the clinical sequelae of immunodeficiency (infection) and loss of tolerance (autoimmunity).

We envision this book to be used at a variety of levels based on the background and needs of the reader. The presentation in most chapters is based on two current methods of medical school teaching. One is block learning, in which all aspects of an organ system or disease are studied together: pathophysiology, epidemiology, biochemistry, and treatment or pharmacology. The second is the method of case-based learning, in which students take an active role in dissecting, learning, and teaching a case. In many ways this mimics real life, in which you analyze the problem in front of you, be it in diagnosing and treating a patient or in developing a research project to better understand a disease. By incorporating into each chapter up-to-date research and theories and freely admitting what is unknown, the reader can easily appreciate the uncharted intellectual territory ahead and the possibility of personally contributing to new knowledge through further research. We therefore hope that this book will motivate a new generation of scientists to study immunology through the lens of disease. Enjoy!

Susan R. S. Gottesman and Warren Strober

Unit I

IMMUNODEFICIENCY DISEASES

INTRODUCTION: ORGANIZATION OF THE IMMUNE SYSTEM AND EVALUATION OF THE IMMUNODEFICIENT PATIENT

SUSAN R. S. GOTTESMAN and WARREN STROBER

INTRODUCTION

This first unit examines several inherited immunodeficiency diseases as well as the acquired immunodeficiency syndrome (AIDS). As stated in the introduction, the study of congenital immunodeficiency diseases (and their animal models) has provided scientists with an unparalleled opportunity to define the function of the individual components of the immune system. Such an opportunity was in fact encountered by immunologists in the 1960s and 1970s who were conducting the initial series of investigations of patients with X-linked or Bruton's agammaglobulinemia (Case 1), severe combined immunodeficiency diseases (SCID; Case 2), and DiGeorge syndrome (Case 3). In analyzing children with these congenital immunodeficiency disorders and in the development of animal models with similar findings, it became apparent to immunologists that the adaptive immune system consists of two main compartments, one populated by T lymphocytes and the other by B lymphocytes. In this way, one of the main organizing principles of modern immunology became established. Students approaching the study of these rare diseases are encouraged to adopt the approach of the early investigators in realizing that a thorough understanding of these diseases will lead to an in-depth understanding of the immune system as a whole.

GENERAL PRINCIPLES IN THE WORKUP OF IMMUNODEFICIENCY

The following description of the differential diagnosis and workup of patients with possible immunodeficiency is based on broad principles concerning organization of the immune system as well as specific approaches to evaluation of immune function. Nevertheless, it is not designed to give you computer algorithms into which you can simply plug in data and arrive at a correct diagnosis. Much of what is known in medicine, and in immunology in particular, would never have been discovered if physicians had restricted themselves to prescribed algorithms. However, orderly thinking and a framework for problem solving are needed as an approach to understanding these patients, along with the realization that each patient is sufficiently unique that arrival at a diagnosis will usually require some creative thinking.

Primary and Secondary Immunodeficiency Diseases

Immunodeficiency diseases may be divided into two categories: primary and secondary. In ***primary immunodeficiency diseases***, which are either inherited or acquired, the immunodeficiency is the underlying abnormality.

Immunology: Clinical Case Studies and Disease Pathophysiology, By Warren Strober and Susan R. S. Gottesman

Secondary immunodeficiency disorders are those that arise from a nonimmunologic abnormality that has collateral effects on the immune system. This distinction thus separates inherited immunodeficiencies and their usual complication, recurrent infection, from the infectious disease caused by the human immunodeficiency virus (HIV), in which the infection itself is the cause of the immunodeficient state. It should be borne in mind that primary immunodeficiency diseases are infrequent (with one or two exceptions) and thus one must always search for a nonimmunologic cause of frequent infections; for example, one must rule out secondary immunodeficiency before an expensive and difficult immunologic workup is undertaken.

Whether primary or secondary, the immunodeficiency, almost by definition, will lead to impaired host defense and therefore increased susceptibility to infection. This leads to the axiom that an immunodeficiency disease should be suspected in any patient with recurrent infections who does not have predisposing factors and should particularly be suspected in a patient with unusual infections.

Two Systems

The first level of characterization of immunodeficiency disorders can be based on which of the two major branches of the immune system is defective: the innate immune system or the adaptive immune system. The approach to the evaluation of these systems may differ considerably.

Innate Immune System. Our innate immune system starts with the physical or anatomic barriers that protect us from the outside world. These include our skin and epithelial barriers and protective secretory molecules such as mucus. We will not discuss these in this section but do touch upon them in the section on autoimmune diseases (see Unit III).

After breaching these physical barriers, the body stands ready to respond to insult with antigen-nonspecific cells and molecules that do not require prior sensitization or have memory. The former include granulocytes (predominantly neutrophils and eosinophils), macrophages, and natural killer (NK) cells whereas the latter include the complex of molecules comprising the complement system and secreted molecules such as lactoferrin and defensins. The cells of our innate immune system have evolved from similar cells found in lower organisms: phagocytes are present in species as primitive as sponges and sea anemones. However, in contrast to these organisms, our innate immune elements have a dual role. On one hand, they mount a defensive response by themselves while allowing the body the time to generate a specific adaptive immune response to the challenge; on the other hand, they interact with and facilitate the response of the adaptive immune system in both its afferent and efferent phases.

In recent years it has been discovered that the cells of the innate immune system have an elaborate system of receptors with which they recognize and respond to categories of molecules associated with potential pathogens. These molecules, called ***pathogen-associated molecular patterns (PAMPs)***, are general molecular signatures of classes of organisms identifying the type of infectious agent (e.g., bacteria, parasite) invading the body. They are recognized by ***pattern recognition molecules*** which serve as receptors for the PAMPs. The most widely studied of this group of receptors are the ***Toll-like receptors (TLRs)*** of the innate immune system. These receptors recognize and mount responses to the prototypic molecules associated with both pathogens and commensal organisms. Signaling through these receptors initiates activation of intracellular signaling, stimulation of phagocytosis, secretion of small molecules (particularly cytokines of the interferon family), and other innate immune responses. These responses provide host defense on their own and communicate with the adaptive immune system.

Adaptive Immune System. Our adaptive immune system is designed to respond to a specific challenge, usually that of a foreign organism. In addition, it has memory for that antigenic challenge, a property lacking in the innate immune system. Immune memory allows a more rapid and effective response on second encounter with the organism. The adaptive immune system may be subdivided into two arms, the humoral immune response and the cell-mediated immune response (see below).

Two Arms of Adaptive Immunity

The two arms constituting adaptive immunity are the ***humoral*** or ***antibody immune response*** and the ***cell-mediated immune response***. The final effector cell of the humoral response is the B cell or its differentiated counterpart, the plasma cell, and the product of these cells is immunoglobulin or antibody. In contrast, the cell-mediated immune response is mediated in its final step by one of the many types of T cells. In actuality, interactions among different cell types are required for both responses, and these include cells of the innate immune system such as dendritic cells and macrophages, which serve as antigen-presenting cells. Of course, as we will see, the humoral immune response, a prototypic B-cell response, reaches full capability only with assistance (help) provided by T cells. In addition to direct cell–cell interaction, much of the communication among cells is achieved through the local release of small protein molecules, cytokines and chemokines, which are products of cells of both the innate and adaptive immune systems.

Immunodeficiency diseases (Table UI.1) can be categorized on the basis of the branch that is defective: the innate immune system or the adaptive immune system. For

the innate immune system the possible defective components include (1) nonspecific effector cells, for example, phagocytic cells, including defects in their ability to migrate to sites of infection, the TLR signaling pathway, or their soluble products of defense and (2) complement components. For the adaptive immune system the defect may lie within the (3) T-cell or cell-mediated immune response, (4) B-cell or antibody-mediated immune response, or (5) both T and B cells or combined cell-mediated and humoral immunity. Mutations resulting in the defective functioning of cytokines and chemokines (the protein molecules by which the cells communicate) and/or their receptors may underlie some of these diseases and are therefore incorporated into the above listed categories based on which cell lineage is dependent on these molecules for cell differentiation and function.

The above categorization of immunodeficiency is simplistic since it does not fully incorporate the fundamental reality that the immune system has an elaborate network of intercommunication as well as some redundancy in the use of small molecules. For example, a "pure" T-cell defect may affect both the humoral and cell-mediated immune responses. Even both the innate and adaptive immune systems can be perturbed by a deficiency in a single molecule. This cross–talk and interdependence are part of what drives the need for an orderly approach to the analysis of the patient with possible immunodeficiency.

PRACTICAL APPROACH TO THE WORKUP OF IMMUNODEFICIENCY PATIENTS

History and Physical Examination

When a patient presents with recurrent and/or unusual infections, the types of infections usually govern the approach to analysis. However, the details of the history and physical examination are of paramount importance in the investigation of possible immunodeficiency. The obvious first consideration is the *patient's age*. Many of the more severe immunodeficiency diseases are congenital (present at birth) and/or genetic (inherited), and patients present as infants or young children. Other primary immunodeficiencies are influenced by environmental factors and may thus become evident at a later age (teens or twenties) for unclear reasons. Of course, secondary immunodeficiencies are the consequence of non–immune system abnormalities and their age at presentation is dictated by the underlying disease.

Another age-related distinction arises from the fact that the infectious consequences of humoral immunodeficiencies do not become apparent until after six months of age when the maternal antibodies protecting the newborn disappear. On the other hand, cell-mediated immunodeficiencies will present at birth or very soon thereafter and may result in failure to thrive. Finally, patients with defects in phagocytes or the complement system may present at any age.

Given the fact that most primary immunodeficiencies have a genetic component, a *family history* is another important element in the analysis of these diseases. A relevant family history may reinforce the perception that this child is having more than his or her share of the usual childhood infections and may also suggest a pattern of inheritance, either autosomal or X-linked. Questions about early deaths in an extended family, particularly those from repeated infections, need to be specifically asked since an inherited immunodeficiency disorder may have gone previously unrecognized.

An essential aspect of the patient's history is the *pattern* (location and type) *of infections* that the individual has experienced since this will help identify the particular arm of the immune system which would be required for host defense against that category of organism (Table UI.2). In particular, it is critical to know if the patient has been subject primarily to bacterial infections or to viral, protozoan, and fungal infections. Patients with recurrent infections caused by ***pyogenic*** (pus-forming) bacteria, usually targeting the respiratory or gastrointestinal tract, will most often be suffering from a B-cell or humoral immune abnormality, since the primary means of fighting extracellular bacterial organisms rests with the synthesis of specific antibodies to that organism. By contrast, patients with viral, protozoan, and fungal infections are more likely to have a T-cell-mediated immunodeficiency since T cells are the primary defenders against intracellular organisms and are also required for defense against large fungal organisms. These include ***opportunistic infections***, the clinical consequences of organisms that do not cause disease in immunologically intact individuals. Finally, recurrent skin infections and impaired wound healing will be more suggestive of a phagocytic cell deficiency, whereas systemic infection with encapsulated organisms and autoimmunity in older individuals will prompt consideration of a complement deficiency (Table UI.2).

The physical examination of the patient will likely yield two pieces of information. First, it will direct your attention to the presence of a currently ongoing acute or chronic infection. In addition, examination may provide evidence of preceding infections, that is, their location, severity or frequency, and consequences, which the patient (or in the case of children, the patient's parents) may have forgotten to mention. For example, repeated severe ear infections (otitis media) can result in scarring of the tympanic membrane. Second, a complete physical examination will provide information on possible structural abnormalities and their underlying causes. The sizes of the ***secondary lymphoid organs***—the lymph nodes, spleen, and tonsils—are of particular importance. A clinical "pearl" is the observation of underdeveloped tonsils in any patient inherently incapable of generating germinal

TABLE UI.1. Summary of Major Immunodeficiency Disorders

Adaptive Immune System

Severe combined immunodeficiency diseases
- Cytokine receptor γ-chain defect: X-linked SCID
- JAK3 deficiency
- Interleukin-7Rα chain defect
- Recombination activating gene defect
- Artemis deficiency
- Adenosine deaminase deficiency
- Purine nucleoside phosphorylase deficiency
- Wiskott–Aldrich syndrome
- Ataxia telangiectasia
- SLAM-Associated Protein (SAP) mutation (X-linked lymphoproliferative syndrome)

T-cell deficiencies
- DiGeorge syndrome
- Zap-70 deficiency (presents as SCID)
- CD3 chain defect
- Bare lymphocyte syndrome (on antigen-presenting cells; some may present as SCID)
  - MHC[a] class 1 deficiency
  - MHC class 2 deficiency
- Fas mutation (autoimmune lymphoproliferative syndrome)

B-cell deficiencies
- X-linked agammaglobulinemia: Bruton's agammaglobulinemia
- Hyper-IgM syndromes
  - Type I: X–linked: CD40L defect
  - Type II: activation-induced cytidine deaminase defect
  - Type III: CD40 defect
  - Type IV: NEMO defect
  - Type V: Uracil–DNA glycosylase defect
- Common variable immunodeficiency disorder
- IgA deficiency
- IgG subclass deficiency

Innate Immune System

Neutrophil disorders
- Leukocyte adhesion deficiency
  - Type 1: integrin β-chain defect
  - Type 2: selectin ligand defect
  - Type 3: integrin signaling defect
- Chronic granulomatous disease
- Hyper IgE syndrome

Complement deficiencies
- Early components:
  - C1, C4, or C2 defect
  - C3 deficiency
- Late components: C5–C9
- Glycosyl phosphatidyl inositol deficiency: leads to red blood cell lysis
- Hereditary angioedemia: C1 esterase inhibitor deficiency; leads to uncontrolled complement activation

[a] MHC: major histocompatibility complex

TABLE UI.2. Major Clinical Manifestations of Immune Disorders

Deficiency	Associated Diseases
B-lymphocyte deficiency: deficiency in antibody-mediated immunity	Recurrent bacterial infections, e.g., otitis media, recurrent pneumonia
T-lymphocyte deficiency: deficiency in cell-mediated immunity	Increased susceptibility to viral, fungal, and protozoal infections
T- and B-lymphocyte deficiency: combined deficiency of antibody and cell-mediated immunity	Acute and chronic infections with viral, bacterial, fungal, and protozoal organisms
Phagocytic cell deficiency	Systemic infections with bacteria of usually low virulence; infections with pyogenic bacteria; impaired pus formation and wound healing
NK cell deficiency	Viral infections, associated with several T-cell disorders and X-linked lymphoproliferative syndromes
Complement component deficiency	Bacterial infections; autoimmunity

centers (the structure necessary for high-affinity antibody formation). The presence of other anomalies, such as craniofacial or cardiac malformations or abnormalities of pigmentation, will suggest certain associated immunodeficiency syndromes. Failure to thrive, a symptom particularly prominent in T-cell defects, can be determined simply from the infant's pattern of growth in height and weight.

While a thorough history and physical examination are critical in suggesting and guiding evaluation of an immunodeficiency syndrome, in the final analysis, with a suspicion of an immunodeficiency disorder based on recurrent and/or unusual infections, a laboratory workup of the patient must be undertaken to confirm and identify the immune defect.

Laboratory Investigation

To evaluate a patient one can construct a flow chart with the different branches of the immune system

Adaptive Immunity		**Innate Immunity**	
Humoral Immunity	Cell-Mediated Immunity	NK Cells/ Phagocytes	Complement

During the development of humoral immunity, the major effector cell, the B cell, synthesizes its product, immunoglobulin (antibody), in response to antigen associated with pathogenic organisms, particularly encapsulated bacteria. In contrast, during the development of cell-mediated immunity, the T cell is the major effector cell, either as a $CD4^+$ T cell (acting through the release of cytokines or via cell–cell interactions) or as a $CD8^+$ cytotoxic T cell that will lyse virally infected target cells. However, it is important to remember that both types of immune responses depend on T helper cells and antigen-presenting cells such as macrophages or dendritic cells for the generation of the effector cells. Even complement components, which act as chemoattractants, opsonins, and as part of the antibody-mediated lytic process, impact the humoral and cell-mediated immune responses. In practice, therefore, one starts the evaluation of the patient with quantitating the final product or cell type in each arm of the immune system, evaluating them functionally and then, if necessary, working backward in the activation and developmental scheme until the block or deficiency is identified. In patients with combined immunodeficiencies (both humoral and cell mediated), the block could occur in a mechanism early in development and be common to both T and B cells or it could simply be present in T cells and interfere with help in both antibody production and cytotoxic T-cell maturation.

Quantitative Assays. The simplest initial test is the quantitation of products and cells that are present in the peripheral blood. Even when the clinical presentation points to a specific arm of the immune system, it is common to perform the simplest quantitative tests to establish that those parameters are normal in the patient. We can assign each quantitative test to an arm of the immune system as follows:

Adaptive Immunity		**Innate Immunity**	
Humoral Immunity	**Cell-Mediated Immunity**	**NK Cells/ Phagocytes**	**Complement**
B Cells Immunoglobulin isotypes	$CD4^+$ T Cells $CD8^+$ T Cells	NK Cells Monocytes (macrophages) Neutrophils/ Eosinophils/ Basophils	C3, C4 C1esterase inhibitor

Cellular Composition of Peripheral Blood. The first step in such quantitation is a ***complete blood count (CBC)***, which results in the quantitation of the white blood cell types (differential count) present in the circulation; neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

Further quantitation of immune cells in the blood involves analysis of the ***lymphocyte subsets*** by flow cytometry (see Unit V for assay descriptions) to determine the number and percentage of mature $CD4^+$ T cells, $CD8^+$ T cells, B cells, NK cells, and other cellular subsets in the circulation. This analysis is based on expression of characteristic cell surface markers (***CD: clusters of differentiation***) and is usually undertaken even if the number of lymphocytes in the peripheral blood is normal.

Immune-System Related Molecules in Peripheral Blood. Quantitation of the secreted protein products that are essential for immune function is also conducted on peripheral blood samples. For suspected B-cell deficiencies, assays are performed to determine the levels of total ***serum immunoglobulins*** along with individual isotype quantitation and analysis (IgM, IgG, IgA, and IgE). Levels of ***complement components***, generally C3 and C4, are also part of a standard initial evaluation. Quantitation of protein molecules is generally performed by enzyme-linked immunosorbent assay (ELISA; see Unit V), which allows detection of low levels of specific proteins.

Analysis of Results of Quantitative Assays. Clearly, the absence of a specific cell type (such as mature B cells) or a molecule (such as an immunoglobulin isotype) will direct your further analysis. In particular, the absence of a mature cell population will now require analysis of the immature cells of that lineage, cells which may only be accessible from the primary lymphoid organs. Additional quantitative analysis may be undertaken subsequently as indicated by results of qualitative or functional assays. This might include further characterization of activation and memory markers on lymphocytes or adhesion molecules on granulocytes as examples. A number of immunodeficiency syndromes are characterized by defects in surface molecules that impair cell function but which do not result in altered cell numbers.

All laboratory results are compared with those from patients of similar age (as well as sex and sometimes ethnicity). In fact, immunoglobulin levels will be affected by both the patient's age as well as his or her prior exposure to antigen (vaccination history). In a young child, levels of some immunoglobulin isotypes are normally quite low and, as such, an inability to produce an effective immune response may not be apparent from these quantitative studies but will require a specific challenge.

Functional Assays and Further Quantitative Assays. Even the presence of normal numbers of mature circulating cells does not guarantee their functional capability. Depending on which arm of the immune system one suspects has a deficiency, several functional assays may be undertaken *in vitro* (in the test tube) and/or *in vivo* (in the patient).

B-Cell Functional Assays

PROLIFERATION ASSAYS. The ability of the cell to proliferate (divide) *in vitro* in response to nonspecific stimuli (or mitogens) is a cross between a functional assay and quantitative assay and in humans is usually performed using peripheral blood cells. This test does not evaluate the cell's ability to perform its differentiated function; rather, it measures a preliminary step that is necessary for most lymphocytes, T cells, and B cells to go through in the course of activation. B cells may be incubated with pokeweed mitogen (PWM), *Staphylococcus aureus* Cowen's I, and/or stimuli directed at the B-cell receptor (BCR). In the latter case, the assay is designed to evaluate the functionality of pathways normally taken to stimulate the division of B cells during an immune response. These include use of anti-μ antibody (the μ heavy chain is part of the BCR for antigen) and anti-CD40, a molecule crucial to the activation of the B cell. Thus, the selective proliferation of the B cell in response to some but not other signals is an important determinant of the ability of the B cell to respond to stimulation specific for a variety of pathways.

SPECIFIC ANTIBODY PRODUCTION *IN VITRO*

Retrospective Study (Prior Immunization). Antibody titer that exists as the result of prior exposure, whether to common antigens in the environment or to vaccination, is a simple way to assess B-cell function. However, the usefulness of these studies depends on the age and history of the patient. For instance, positive titers would be expected to antigens in vaccines administered during childhood and thus antibody levels may be measured for specific antigens, such as measles, mumps, rubella, tetanus, and diphtheria. One expects low levels of specific IgM and IgG antibodies to such antigens even in normal individuals unless they have received a recent challenge. In addition to these organism-specific antibodies, all individuals are expected to have ***isohemagglutinins***, "natural" IgM antibodies to blood group antigens not expressed in the patient. These are also believed to be stimulated by organisms and appear to be the result of environmental exposure to organisms expressing antigens cross-reactive to human blood group antigens. Therefore, levels of isohemagglutinins are expected to be low in children less than one year of age.

Prospective Study (Active Immunization). For a more definitive assessment of B-cell function, one can administer a vaccine and then perform sequential measurements

of specific antibody titers. However, because patients with immunodeficiency may develop active infections if given live attenuated viral vaccines, only nonliving antigens can be used to evaluate such patients. Evaluation for titer and immunoglobulin isotype at two and four weeks following immunization will provide information on the ability to respond to antigen and to switch immunoglobulin isotypes (see Case 4). Pneumococcus and *Haemophilus influenzae* vaccinations are commonly used as polysaccharide antigens and tetanus and diphtheria as protein antigens. The latter type will be more dependent on T-cell help to generate an effective response.

SPECIFIC ANTIBODY PRODUCTION *IN VITRO*

The B Cells on Their Own. Many of the nonspecific mitogens used to stimulate a proliferative response will result in the production of immunoglobulin by the B cells. These products may be measured in the culture media and quantitated by ELISA according to the isotype produced: IgM, IgG, and IgA.

T–B Collaboration. B cells may also be stimulated to produce immunoglobulin by coculture with T cells activated with anti-CD3/anti-CD28. Depending on the combination of patient versus control T cells, patient versus control B cells, or both, the function of each cell type and their ability to cooperate with one another can be measured. The assay also allows the evaluation of the function of T helper cells of the T_H2 subset. This parallels the natural situation of antibody response to T-dependent antigens.

T-Cell Functional Assays

PROLIFERATION ASSAYS. As with the B cell, nonspecific mitogens, in this case phytohemagglutinin (PHA) and concanavalin A (Con A), are used *in vitro* to test the ability of T cells to respond with cell division. A more physiologically relevant stimulation test employs cross-linking anti-CD3 antibody or anti-CD3 plus the cytokine interleukin-2 (IL-2), a T-cell growth factor, to stimulate the T cells.

T-CELL FUNCTIONAL ASSAYS *IN VITRO*. T helper cell function for antibody production was discussed above.

Cytokine Assays. The ability of T cells to produce and secrete their cytokine products after stimulation can be measured by harvesting the culture media and quantitating the specific cytokines in ELISAs. The cytokine products are characteristic of specific T helper cell subsets.

Mixed Lymphocyte Culture (MLC) and Cell-Mediated Lysis (CML) Assays. As will be discussed in some of the case studies, T cells recognize foreign molecules in the context of the individual's major histocompatibility complex (MHC) antigens and also recognize foreign MHC itself. The recognition of foreign MHC *in vitro* can be used as a means of determining the ability to generate cytotoxic T cells and the ability to provide the help necessary for that generation, in this instance the T helper 1 subset. In the MLC assay, T cells are incubated with fixed cells (so that they do not divide) from an unrelated individual and the proliferation or cytokine production by these T cells is measured after several days. For the cytotoxicity assay, the responding cells are transferred from the first culture and incubated with labeled target cells that originate from the individual used for stimulatory cells in the first culture. The killing of those target cells is then a measure of the function of the cytotoxic T cells ($CD8^+$ T cells).

T-CELL FUNCTIONAL ASSAYS *IN VIVO*

Delayed-Type Hypersensitivity. This assay is commonly referred to as an assay of cell-mediated immunity. However, as will become apparent from its description, it actually measures the ability of T helper cells (and not T cytotoxic cells) and antigen presenting cells (APCs, e.g. macrophages) to collaborate. The reaction is dependent on the presentation of antigen in the context of MHC by the APCs in addition to the interaction of surface molecules and their receptors on the two cell types. An antigen to which the individual should have been previously exposed, such as antigen from *Candida*, a common environmental fungus, is injected intradermally in the forearm. The development of redness and swelling is evaluated 48–72 h later. The requirement for APCs to present antigen to the T cell and produce chemokines attracting additional cell types to the region illustrates the interaction between the innate and adaptive immune systems in both the afferent and efferent phases of this specific immune response. In fact, the results of this test are also dependent on vascular changes at the site, demonstrating both the potential difficulty of interpreting *in vivo* testing and the complexity of responses as they occur naturally in the body.

Allogeneic Skin Graft Rejection. Although used in the past, the ability to reject a skin graft from an unrelated donor is no longer a test employed in humans (although still acceptable in animal models). This assay would be the *in vivo* equivalent of the MHC–CML assay.

Phagocytic Cell Functional Assays

PHAGOCYTOSIS. The ability of cells to phagocytize (ingest or engulf particle through the formation of a vacuole) may be measured using a variety of small, usually labeled particles. Their ingestion can be detected by either flow cytometry (see Unit V) or microscopy. Killed bacteria, yeast, or synthetic particles may be labeled and used as the targets for phagocytosis.

ROLLING, ADHESION, AND MIGRATION. These are evaluated by flow cytometric detection of the surface molecules required for each function.

OXIDATIVE BURST. Two assays are employed to measure the oxidative burst of phagocytic cells.

Nitroblue Tetrazolium (NBT) Assay. This assay tests the oxidative burst capacity of neutrophils by measuring their ability to convert NBT, which is yellow, to formazan, which is blue/violet and detectable by microscopy.

Dihydrorhodamine Assay. Stimulated neutrophils loaded with dihydrorhodamine will oxidize the dye with H_2O_2 and demonstrate increased fluorescence, which is detectable by flow cytometry.

Evaluation of NK Cell Function. Although the function of NK cells can be evaluated by an *in vitro* test similar to the T-cell cytotoxicity assay, using susceptible targets and without presensitization, in actuality the functioning of these cells is rarely measured in the clinical situation. Deficiencies in NK cells may be part of a larger deficiency in the T-cell lineage and will be reflected in the peripheral blood NK cell numbers. The rarity of an NK cell defect as an isolated abnormality suggests that the NK cell's individual capabilities are compensated for, that these patients have yet to be described, or both.

Complement Function Assay. The CH50 assay is a functional assay for complement that generates a kinetics curve for the rate of lysis of antibody-coated sheep red blood cells and is reported at the 50% point. The assay measures the presence and function of C1–C9.

SUMMARY

Given identification of a quantitative or functional defect, the specific disorder may need to be further defined. For the quantitative tests, such as finding the absence of a surface molecule on neutrophils, the results of the assays and identification of the immunodeficiency disorder will be straightforward; one only needs to think to look for the specific defect. In analyzing of the results of functional studies, particularly those involving lymphocytes, the multitude of interactions among the components of the adaptive as well as the innate immune systems must be considered. Figure UI.1 attempts to illustrate these interwoven parts by placing arrows between the components of the branches and arms of the immune system that we described at the start of this chapter (Fig. UI.1). From this figure it is easy to appreciate how a defect in one cell may affect many functions or how a defect in one cell may be compensated for in the individual by the intact components of their immune system.

Once a quantitative or functional defect is identified, the underlying pathophysiology must be elucidated. For some of the immunodeficiency diseases, familiarity with the development and differentiation of the immune cells will be required. Figure UI.2 is a flow chart of the development and interaction of T and B lymphocytes and the positions of the blocks in differentiation and function that are seen in a variety of immunodeficiency disorders; use of such a chart is a good approach to the problem if the adaptive immune system is deficient. The diagnosis of the specific disorder (Table U1.1), whether it requires further functional studies

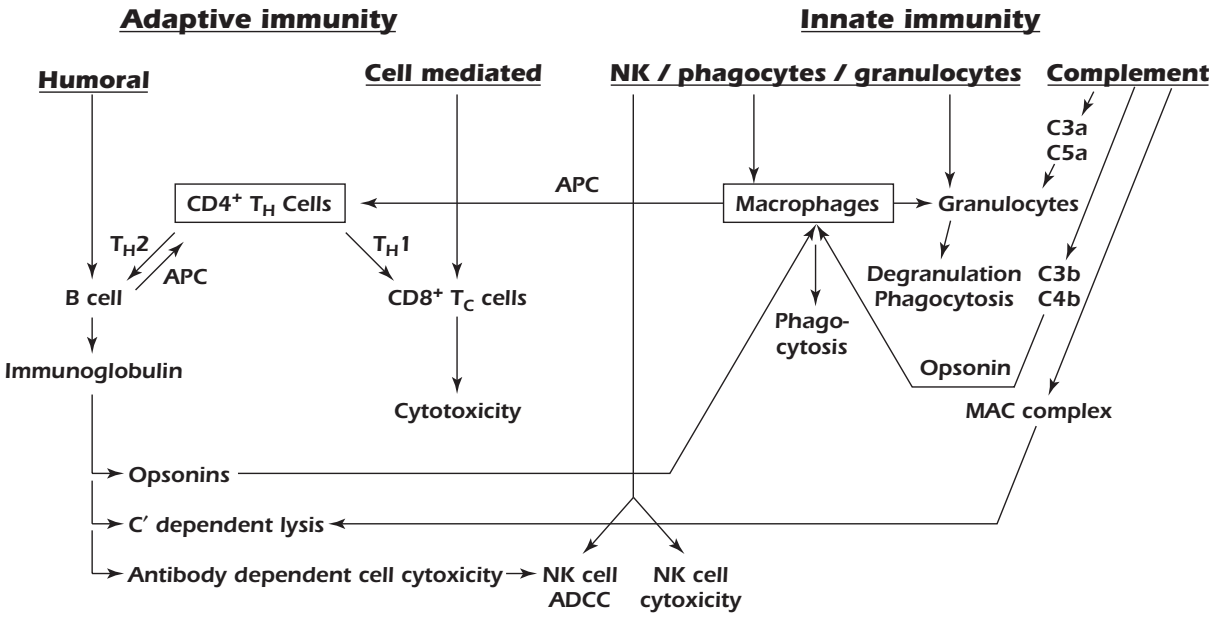

Figure UI.1. Flow chart depicting interactions between arms of immune system.

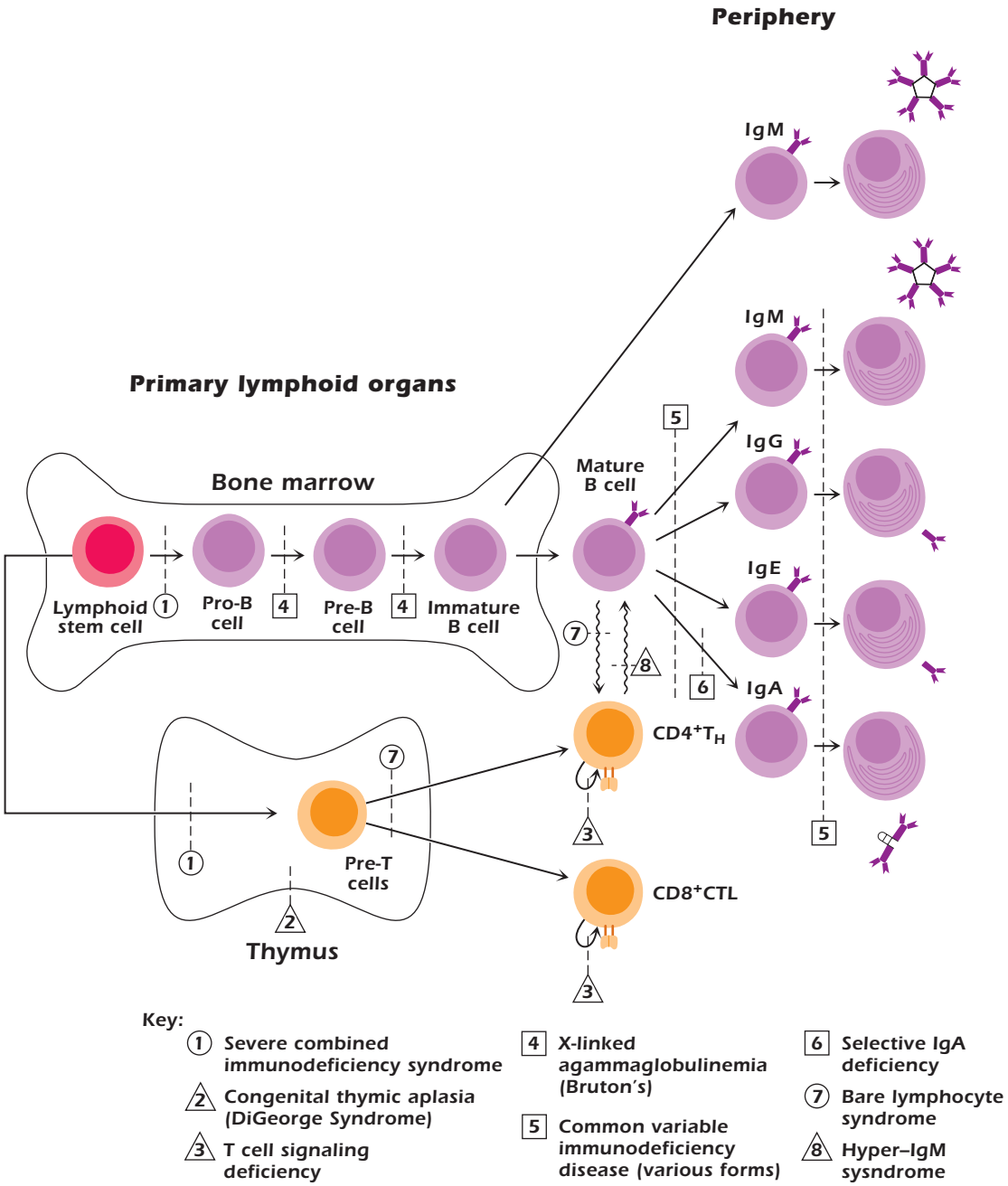

Figure UI.2. Flow chart depicting B- and T-cell development with locations of immunodeficiency diseases. Circles denote diseases with severe combined immunodeficiency presentation; triangles are for diseases with predominantly T cell deficiencies and squares are for B cell defecent disorders.

and flow cytometric analysis of surface molecules, enzymatic studies as in adenine deaminase (ADA) deficiency, or even gene sequence analysis, is discussed in the individual cases presented in this unit. It is apparent that we are lucky to have the footprints of investigators who came before us to follow in unraveling this complex set of diseases.