Immunology Made Easy: What Every Trial Advocate Should Know

Plaintiffs' experts often try to oversimplify the incredibly complex subject of the immune system and clinical manifestations of dysregulation

By Bruce R. Parker

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THE science of immunology is the study of how the immune system is capable of differentiating between what is part of the host and what is foreign, and "all the rest is technical detail."[1] Understanding the "technical detail" of immunology is nevertheless challenging because it includes the study of all of the mechanisms used by the body to protect itself against agents that are foreign to the body.

THE CONCEPTS

Conceptually, it is helpful to divide the immune system into innate (non-specific) and acquired (specific) immunities. In reality, however, it is difficult to differentiate the innate and acquired immune responses to a foreign body. Many cells and soluble protein molecules participate in both types of immune responses.

Components of innate and acquired immunity generally are capable of recognizing the molecular differences between the host and foreign bodies and then eliminating the foreign bodies. Although the mechanisms by which immune cells recognize and respond to foreign agents has been fairly well described in the immunological literature, it still remains relatively conjectural as to how, during fetal development, the body is able selectively to destroy immune cells that lack the capacity to differentiate self from non-self.

In general, both innate and acquired immune responses involve striking a balance between the immune system aggressively attacking foreign bodies and minimizing damage to healthy host cells during the attack. The balance is achieved by cells of the immune system which turn off the immune attack at the appropriate times so that damage to healthy host tissue from the immune "friendly fire" is minimized.

A major difference between innate and acquired immunities is the lack of specificity and immunologic memory in cells that mediate innate immunity.

"Specificity" refers to the ability of white blood cells (leukocytes) and molecular components of the immune response (i.e., antibody) to discriminate between different molecular species presented to it and respond only to those molecular species against which the immune component has been designed to attack. A non-specific immune response (innate) is one in which the immune components respond randomly to foreign bodies.

"Immunologic memory" is the ability of specific types of leukocytes to remember having seen a foreign body (antigen) on re-exposure. Immunologic memory allows immune cells (i.e., lymphocytes) to respond to subsequent encounters with a "learned" response (i.e., quicker and stronger).

The chart on page 346 lists some of the key features of both types of immunity and their important differences.

A. Innate (Non-Specific) Immune Response

Innate or natural immunity comprises all of those components of the host that are immediately available to protect the host from foreign bodies. Some of the defenses provide physical barriers (skin, mucosal membranes) to the entry of pathogens. Others provide an environment in which some pathogens cannot live—for instance, the ph balance of the digestive system. Others—phagocytic cells and soluble mol-

1. E. BENJAMIN & S. LESKOWSKI, IMMUNOLOGY: A SHORT COURSE 1 (1991), hereinafter IMMUNOLOGY.

INNATE AND ACQUIRED IMMUNE SYSTEMS

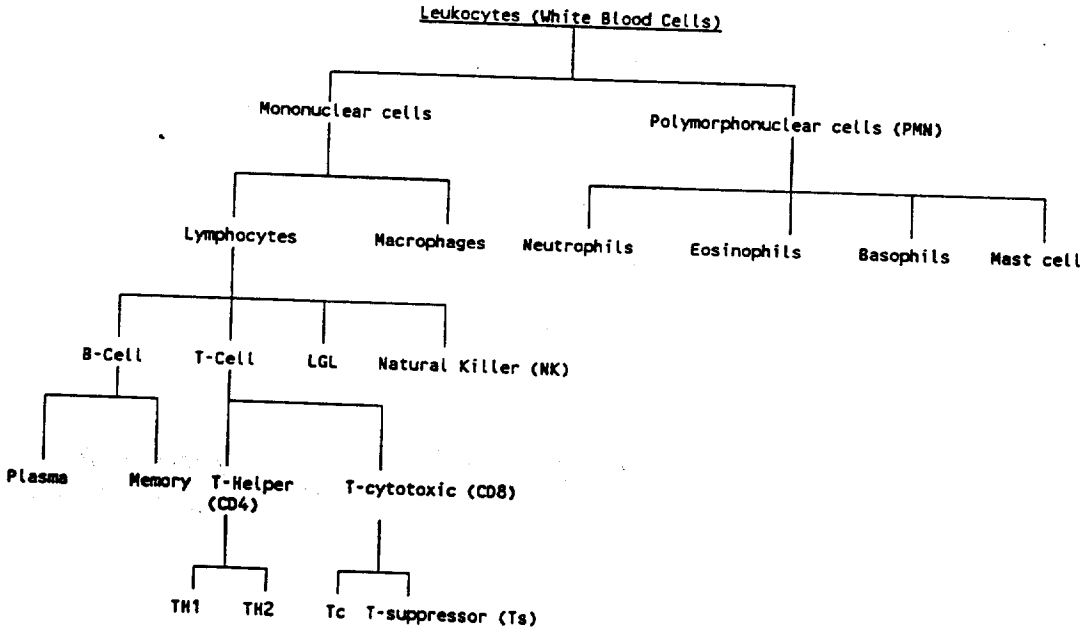
	Innate Immunity	Acquired Immunity
Distinguishing Features	Resistance is not improved by repeated infection, includes physical barriers to pathogens (i.e. skin), does not require immunization	Immunologic memory leads to improved resistance with repeated infections
Soluble Factors	Lysozyme, complement, acute phase proteins (e.g., CRP), interferon	Antibody, complement
Cells	Phagocytes Natural killer (NK) cells	T lymphocytes B lymphocytes

SOURCE: RICH, CLINICAL IMMUNOLOGY, PRINCIPLES AND PRACTICE (1995)

ecules, such as enzymes, complement and cytokines—attack foreign bodies that successfully bypass the physical and chemical barriers.

The immunological response to the implantation of a medical device is a good example of how the innate immune system functions. Placing a medical device in the body damages tissue adjacent to the implant. Almost immediately, components of the innate immune system begin to remove damaged cells and attack the foreign body—the implant. The initial stage of this response is called acute inflammation. Additional white cells (leukocytes) circulating in the microvasculature are recruited to the site by chemical messages (cytokines) released by cells at the site. One type of cytokine binds to receptors on cells lining the microvasculature. This causes the microvasculature walls to become more permeable. The increased permeability permits blood plasma to flow from the microvasculature into the extravascular spaces around the injured cells. This fluid migration produces the swelling experienced in acute inflammation. The loss of plasma from the microvasculature slows the leukocytes circulating in the vasculature and allows them to migrate through the walls of the microvasculature into the tissue.

White blood cells (leukocytes) are responsible for directing the innate and acquired immune response. The diagram below depicts the relationship of various types of leukocytes.



The first type of leukocyte that migrates to the damaged area are polymorphonuclear leukocytes (PMN). Neutrophils are the primary PMN that respond during acute inflammation. Neutrophils are phagocytic cells that ingest cellular debris and/or foreign material. Once material is ingested, the neutrophil releases enzymes and superoxide radicals to digest the material. During this process, some of the degradative products are released from the phagocytic cells. When this occurs, adjacent healthy cells are damaged. The pain and redness experienced during acute inflammation is largely due to the cellular injury caused by phagocytic cells releasing degradative enzymes.

Neutrophils live only a few days. During the acute inflammatory stage, which generally lasts two to three days, neutrophils release cytokines, which recruit longer-lived phagocytic mononuclear cells. Macrophages are the primary phagocytic mononuclear cells involved in acute and chronic inflammation. The predominance of macrophages over neutrophils signifies the transition from acute to chronic inflammation.

Once they arrive at the site of inflammation, macrophages continue the process of ingesting and digesting cellular debris and foreign bodies. They are very efficient phagocytic cells and are capable of releasing more than 40 different cytokines. The cytokines released by macrophages help regulate the inflammatory response. Fever is one characteristic of an inflammatory response caused by some of the cytokines released by pyrogenic (fever-producing) macrophages. Such cytokines bind to receptors in the hypothalamus, which controls the set point for body temperature.

Macrophages responding to the presence of a biomaterial will attempt to ingest the implant. If a microparticle from an implant is too large to be ingested by a single macrophage, then several macrophages will fuse together to form a "giant cell" and ingest the particle. However, if the particle is too large for even a giant cell to consume, then macrophages will recruit fibroblasts. Fibroblasts are cells that, once activated, release fibrin, which is deposited around an implant in an effort to wall off the implant. The fibrous capsule (scar) that forms around a biocompatible implant is characteristic of virtually all implantable biomaterials. Once the encapsulation is completed, the chronic inflammatory process subsides.

Further Reading

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Immunology, An Illustrated Outline, by D. Male, Mosby Publications (2d ed. 1993).

Rheumatology, by J. Klippel & P. Dieppe, Mosby Publications (1994).

Textbook of Rheumatology, by W. Kelly, et al., W.B. Saunders Co. (4th ed. 1993).

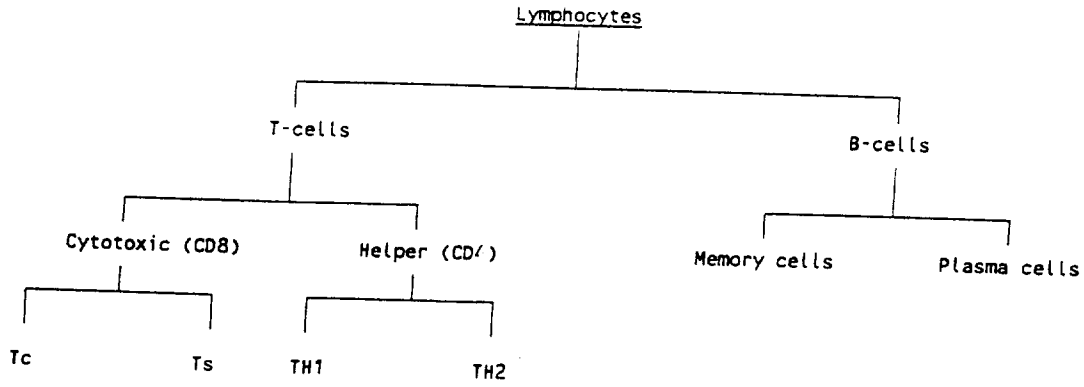
B. Acquired (Specific) Immune Response

1. Overview

Although all living organisms possess an innate immune system, only vertebras have evolved to the point of developing an acquired immune system. Two key features of the acquired immune response are specificity and memory. Both these features permit the immune system to respond faster and more efficiently when the host is re-exposed to an antigen.

Acquired immunity requires the interaction of a number of organs, cells and soluble protein compounds. Lymphocytes are the primary mediators of acquired immunity. Lymphocytes are either T or B-cells. Whether a cell is a T or B is determined by the type of receptor molecules on its surface. The surface receptors also serve as the basis for distinguishing among the classes of T-cells. Surface receptors are designated by the label CD—.

As shown below, T-lymphocytes are either helper (CD4) or cytotoxic/suppressor (CD8) cells. T-helper cells are further divided into TH1 and TH2 cells. TH1 cells promote an inflammatory cell mediated immune response and TH2 cells a humoral (i.e., antibody) response. After receiving the appropriate signal from a T-helper cell, a B-cell will mature into a



plasma cell and produce antibodies. B-cells which do not mature to plasma cells serve as memory cells in the event the host is re-challenged by the antigen. The diagram below depicts the various types of lymphocytes.

In addition to lymphocytes, acquired immunity requires the support of several lymphatic organs. Lymphatic organs are those in which lymphocytes mature, differentiate and proliferate. Primary lymphoid organs are those in which lymphocytes mature. Lymphocytes that are stimulated by antigens, proliferate and differentiate in secondary lymphoid organs.

The primary lymphoid organs are the thymus and bone marrow. T-cell lymphocytes mature in the thymus and B-cell lymphocytes mature in the bone marrow. The secondary lymphoid organs include the spleen and lymph nodes. Together, these organs comprise the reticuloendothelial system (RES), which operates as a sewage system for circulating lymphocytes and phagocytic cells.

Other important components of acquired immunity are protein compounds, which are soluble in blood and tissue fluid. Antibodies are protein molecules made by B-cells. Because of their globular structure, antibodies are called "immunoglobulins" (Ig), and they are divided into five classes—IgM, IgG, IgA, IgD and IgE—based on their structural and biologic properties.

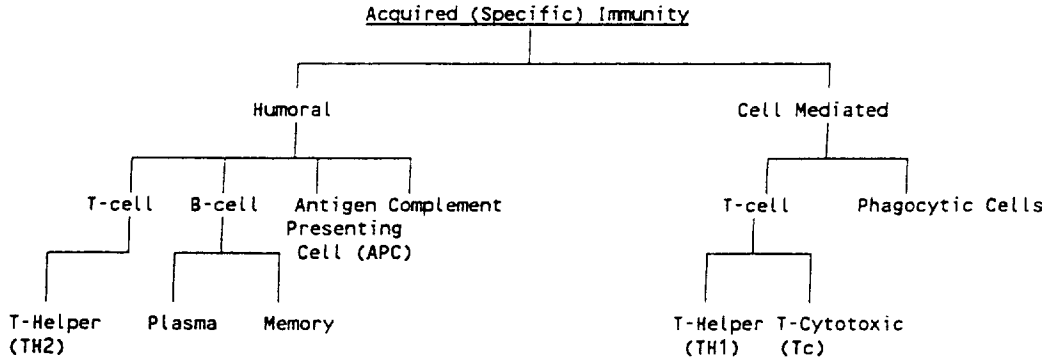
Each antibody is created to bind structurally to a specific molecular configuration on the receptor on the surface of a antigen. In general, antibodies neutralize toxins and viruses, immobilize micro-organisms and agglutinate or clump micro-organisms by forming immune complexes with antigens, which activate, complement and are phagocytized and destroyed by phagocytic cells.

"Complement" is a generic term that refers to a number of serum proteins made by the liver. Once activated, the protein compounds enter the membrane of the target cell. This disturbs the osmotic equilibrium in the cell and allows water to enter the cell. As the cell membrane swells, macromolecules escape, resulting in the death of the target cell.

Activation of the acquired immune system requires the presence of an immunogen/antigen. An antigen is defined functionally as something capable of binding specifically to an antibody. Antigen and antibody binding can be viewed as a lock and key mechanism. Just as one key is specific to one lock, each antibody has a specific molecular configuration at its end, which permits it to bind to one antigen.

An immunogen is an antigen that has sufficient size and chemical complexity to stimulate lymphocytes to proliferate. Although a molecule may be capable of structurally binding to an antibody, it may not be of sufficient size or chemical sophistication to stimulate lymphocytes to proliferate. In such cases, an antibody will bind to the antigen, but the acquired immune system will not be stimulated to respond to the antigen with an enhanced cell mediated or humoral response. For purposes of simplicity, the term antigen is used in this article to mean it is immunogenic.

Acquired immunity is mediated by cells (cell-mediated) and soluble proteins (humoral). Although it is helpful to look at both systems independently for purposes of understanding their operation, an antigen generally will stimulate both responses in vivo. A schematic of acquired immunity is shown on the next page.



2. Cell-mediated Immune Response

Acquired cell mediated immunity is, in some respects, very similar to innate immunity. When phagocytic cells, such as macrophages, encounter an antigen, particularly one to which an antibody has attached or complement has coated, the macrophage will ingest and digest it. A portion of the digested protein antigen is transported to the surface of the macrophage by a molecule called the major histocompatibility complex (MHC). Depending on their configurations, MHC molecules are identified as MHC I or MHC II.

The structure of the antigen segment will determine whether it is presented by an MHC I or MHC II molecule. If the antigen segment is presented by an MHC II molecule, it will attract a T-helper cell whose surface receptor binds to the antigen segment and the MHC II molecule. Cells such as macrophages, which present antigen segments to T-cells, are functionally referred to as "antigen-presenting cells" (APC). If the T-helper cell attaches to the APC and it receives a second stimulatory signal from the APC, the T-helper cell will begin releasing a number of different cytokines. Some cytokines, by binding to surface receptors on nearby T-cells and macrophages, will cause the T-cells and macrophages to migrate to the area for antigen ingestion (phagocytosis) and presentation to the T-cells.

The cell-mediated immune response is most efficient when the antigen exists extracellularly (i.e., bacteria). However, some antigens, such as viruses, live within cells (i.e., intracellular). With intracellular antigens, the antigen segment is sent to the APC surface and presented to a T-cytotoxic cell by an MHC I molecule. Once stimulated, the T-cytotoxic cell will kill the cell that has presented the antigen to it. By killing the host cell, the virus also is destroyed.

3. Humoral Immune Response

The initial stages of humoral immunity are very similar to those of cell-mediated immunity. Antigens are phagocytized and segments are presented by an MHC II molecule to a T-helper cell with receptors specific for the antigen segment and MHC II molecule.

B-cells also can present antigen segments to T-helper cells. The surface of B-cells are lined with antigen receptors. Unlike T-cells, B-cells are capable of recognizing and binding to antigens in their whole (undigested) or native configuration. If an antigen receptor on a B-cell binds to a molecule on an antigen, the antigen will be internalized and a segment of the protein antigen will be presented by an MHC molecule to a T-helper cell.

Regardless of whether the T-helper cell is activated by antigen presentation from a B-cell or macrophage, the T-helper cell (TH2) will release cytokines that bind to surface receptors on adjacent B lymphocytes. The B lymphocytes respond by maturing into plasma cells. Once matured into a plasma cell, the cell begins generating and releasing antibodies specific to the antigen which was presented to the T-helper cell.[2]

In general, IgM is the first type of antibody produced by plasma cells in response to antigenic stimulation. IgM production reaches its

2. Some antigens are capable of binding directly to surface receptors on B-cells and stimulating the B-cell to produce antibodies without T-cells activation. Such antigens are referred to as "T-cell independent antigens." The vast majority of antibodies are produced only after presentation to a T-cell and T-cell stimulation of B-cells.

peak after approximately seven to ten days. Plasma cells then will switch to producing IgG antibodies, whose production typically peaks at approximately 14 days. It is important to note that although a B (plasma) cell is capable of producing different classes of antibody (e.g., IgM and IgG) to a specific antigen, all the antibodies will have the same binding specificity. If the lymphyocytes are subsequently re-exposed to the same antigen, lymphocytes with memory to the first exposure will produce a quicker and stronger humoral response. The second response is characterized by B-cells producing IgG as the primary class of antibodies, with considerably greater quantities produced in a shorter period of time.

The specific binding of an antibody to antigen is called an immune complex. Since an antibody has multiple antigen binding sites, antibodies that bind to antigens often form a lattice. The creation of an immune-complex lattice activates complement, which enhances phagocytosis of cells coated by complement and circulating immune complexes.

IMMUNOLOGICAL TESTING

Immunological tests (assays) are conducted for a wide variety of clinical and research purposes. However, in general, immunological assays done for clinical purposes are designed to determine the presence of high (positive) levels of antibody that are specific to antigens of particular interest.

There are several types of assays that allow a laboratory to assess the level (i.e., "titer") of antibodies to particular antigens. It is interesting to note that contrary to the heavily regulated drug and medical device industries, there is surprisingly little regulation over laboratory tests offered to clinicians. Laboratory equipment is subject to federal regulation and inspection under the Clinical Laboratory Improvement Act.[3] In addition, if a laboratory sells a diagnostic test kit to physicians for their use, the Food and Drug Administration considers the kit to be a medical device under the Food, Drug and Cosmetic Act and thus subject to federal regulation.[4] However, if a laboratory performs an assay as a service for physicians, then it is unclear whether the FDA can compel the laboratory to demonstrate the efficacy of the assay before it is marketed.

The FDA has sent regulatory letters to a number of laboratories that offer "silicone antibody" testing. The letters have advised the laboratories that it is impermissible under federal statutes and regulations to claim that an assay has diagnostic or clinical value unless the clinical efficacy of the test has been established.[5] Consequently, when confronted by a test whose clinical efficacy has not been established, one should never assume that the assay is a reliable indicator of what it purports to show. Several texts are available that evaluate laboratory techniques, some of which are listed in the bibliography with this article, "Further Reading," on page 347.

Since one of the cardinal features of the acquired immune system is lymphocytic memory of antigens to which the host has been exposed, healthy persons will have low (normal) levels of antibodies to a large number of antigens circulating in their blood. Through large statistical sampling, ranges of normals have been established for validated immunological assays.

A positive level (titer) of antibodies to a particular antigen means that the person is either fighting an antigenic challenge, recently has been exposed to the antigen or the test result is a false positive. High titers of antibodies are not diagnostic of any disease in the absence of clinical symptomatology. Certain viruses, such as the Epstein-Barr Virus (EBV), are ubiquitous. As a result, it is not uncommon for patients who are asymptomatic to have positive titers to EBV since they are repeatedly exposed to the virus. In the absence of symptoms, a patient with no symptoms and a positive titer of EBV antibodies cannot be said to be suffering from EBV disease.

Asymptomatic people also have low (normal) levels of circulating antibodies to molecular components of their own cells. Such antibodies are called autoantibodies. The most common autoantibody is rheumatoid factor ("RF"). Rheumatoid factor is created when an IgM antibody mistakenly sees an IgG antibody as foreign and binds to it. Rheumatoid factor is often seen in patients following bacterial infections. It is thought that the structural configuration of a particular IgG antibody is substantially similar to the antigenic receptors (epitope) on some pathogens. When this occurs, IgM antibody, which has been made to the foreign anti-

3. 42 C.F.R. § 493 (1988).
4. 21 U.S.C. § 321(h).
5. 21 U.S.C. § 352.

gen, loosely binds to IgG antibody through a process known as molecular cross-reactivity.

Cross-reactivity also explains how antibodies are able to specifically bind to a number of protein components within the host's cells (i.e., "self-antigens"). Antibodies that bind to self-antigens within a cell are called antinuclear antibodies (ANA). Several antinuclear antibodies have been identified.

For the most part, positive levels of autoantibodies are not diagnostic of autoimmune diseases. Approximately 5 percent of the healthy population have low positive titers of ANAs. In addition, ANA levels increase with age regardless of symptoms or disease. Positive ANA titers also are more common in asystomatic women than men. Finally, a number of factors other than autoimmune diseases cause ANA levels to increase.

Although circulating autoantibodies, in the absence of supporting clinical symptomatology, are not diagnostic of autoimmune diseases, epidemiological studies have demonstrated a high correlation between positive titers of particular autoantibodies and some autoimmune connective tissue diseases. Therefore, positive titers of specific autoantibodies can confirm a suspected diagnosis of some autoimmune diseases. Conversely, a negative or normal titer of autoantibodies may be strong evidence that a patient does not have a suspected autoimmune disease.

IMMUNE DISEASE

A. Introduction

Immune diseases are of two basic types. The first type are immune deficiency diseases. These occur when components of the immune system are suppressed. Autoimmune diseases are the other type of immune disease. Autoimmune diseases occur when the immune system, after response to an antigenic challenge, does not shut down and begins to attack organs and tissues of the host.

B. Immune Deficiency Diseases

Patients with immune deficiency diseases, such as AIDS, suffer from recurrent infections since their immune systems are not capable of effectively eradicating an antigenic challenge. Patients with a deficiency in either the number of B-cells or their proper functioning, or both, often suffer from recurrent bacterial infections such as otitis media, bronchitis, pneumonia, meningitis and dermatitis. T-cell deficiencies often make patients susceptible to viral, fungal and protozoal infections. Since B-cell functioning is largely governed by T-cells, dysfunctional T-cells also will leave a patient susceptible to those diseases characterized by B-cell deficiencies.

Immune deficiency diseases also are caused by suppression of phagocytic cells, which are essential for antigen presentation to T-cells. Defects in the metabolism of a phagocytic cell prevents the cell from properly metabolizing foreign bodies it has ingested. The survival of microorganisms within the phagocytic cells may lead to the development of granuloma disease.[6]

C. Autoimmunity or Immune Enhancement Diseases

Some cancers and rheumatic connective tissue diseases are caused by abnormal proliferation or functioning of the acquired immune system. A monoclonal gammopathy is the abnormal proliferation of B and plasma cells. This condition may be a precursor to multiple myeloma, which is a cancer resulting from the malignant proliferation of plasma cells. The myeloma may be found in a number of organ systems, including the skeletal and nervous systems.

Autoimmune rheumatic connective tissue diseases are caused by the exaggerated and chronic immune response to an antigen, during which the body looses its ability to distinguish what is foreign from what is self.

There are four types of hypersensitivity reactions:

Type I: Anaphylactic reactions. These reactions result from IgE antibodies binding to mast cells. When stimulated by IgE, the mast cells release chemicals that are responsible for allergic symptoms.

Type II: Cytotoxic reactions. This condition occurs when IgM or IgG antibodies are produced in excess quantities and bind or stick non-specifically to the surface of healthy cells. The healthy cells then become targets for phagocytic cells and/or the coating of complement.

Type III: Immune complex reactions. Normally, immune complexes are cleared from the

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6. *See generally* IMMUNOLOGY, *supra* note 1, at 214-15, 217, 220.

body through phagocytosis. However, when excessive quantities of immune complexes are formed and deposited in tissue, the tissue often is injured from the activation of complement and phagocytosis.

Type IV: Cell-mediated immunity. This type of hypersensitivity reaction is also called delayed type hypersensitivity reaction (DTH). Symptoms generally appear one to two days after the event that triggers the reaction. Unlike Types I, II and III, which are mediated by antibodies, Type IV is mediated by T-cells. When T-cells become excessively stimulated, they produce increased levels of cytokines, which activate excess numbers of macrophages, which in turn produce local tissue damage. The symptoms typically include the skin becoming red, raised and thickened. If the macrophage activity does not abate, the macrophages will fuse together to form giant cells and eventually granulomas. In extreme cases, granulomas can be harmful because they replace normal tissue.

Autoimmune diseases present diagnostic challenges to physicians since they can be organ specific or systemic. In addition, most autoimmune diseases are not exclusively antibody or T-cell-mediated diseases but rather they typically involve an increased reaction by both.

Below is a partial list of some of the more well-known autoimmune diseases:

- **Autoimmune hemolytic anemia.** Characterized by reduced levels of circulating red blood cells resulting from their destruction by antibodies binding to self-antigens on the surface of the red blood cell.
- **Myasthenia gravis.** Antibodies bind to self-antigens on the acetylcholine receptors located at the neuromuscular junction. This blocks nerve impulse reception and leads to muscle weakness and eventually death owing to insufficient respiration.
- **Graves disease.** Autoimmune disease of the thyroid. Antibodies are thought to be directed against hormone receptors in the thyroid, which leads to increased hormone production and a hyperthyroid condition.
- **Hashimoto's thyroiditis.** Autoimmune disease of the thyroid. This disease produces a state of hypothyroidism. The cause is unclear, although antibodies have been identified to protein components of the thyroid cells.
- **Systemic lupus erythematosus.** Lupus is a systemic disease that affects many organs and produces a multitude of symptoms. Most clearly understood are the lesions occurring in the kidneys as a result of immune complex deposition.
- **Rheumatoid arthritis.** This disease is thought to be the result of an inflammatory process in the joints resulting from the deposition of rheumatoid factor.
- **Multiple sclerosis.** This disease is caused by an autoimmune attack on the myelin sheath around the nerves of the central nervous system.
- **Systemic sclerosis (scleroderma).** Scleroderma occurs in limited and diffuse forms. Although both forms may produce internal organ damage and skin changes, diffuse scleroderma is generally more progressive and offers worse prognosis than limited scleroderma. The most characteristic clinical finding of this disease is skin thickening on the hands.
- **Sjogren syndrome.** This disorder is characterized by the immune system attacking the salivary and lacrimal glands. Autoimmune attack on these glands leads to clinical symptoms of dry eyes and mouth.
- **Sarcoidosis.** This disease is multi-systemic and characterized by the development of granulomas in the lungs, lymph nodes, spleen, skin, eyes, salivary glands, liver and central nervous system.

CONCLUSION

The immune system and the clinical manifestations of its dysregulation are incredibly complex subjects. Not surprisingly, plaintiffs' experts often oversimplify their explanations of immunologic mechanisms to the point that their testimony is extremely misleading. The challenge to defense counsel is to be able to understand and teach a jury the difference between immunological facts, reasonable immunological theory and the nonsense so often articulated by plaintiffs' expert witnesses.