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Chapter 9

TOXIC RESPONSES OF THE IMMUNE SYSTEM*

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INTRODUCTION

The immune system functions in resistance to infectious agents, homeostasis of leukocyte maturation, immunoglobulin production and immune surveillance against arising neoplastic cells. Cells of the immune system providing these functions are termed leukocytes, and they arise from pluripotent stem cells within the bone marrow, where they undergo highly controlled proliferation and differentiation before giving rise to functionally mature cells. The functionally mature cells are divided into granulocytes, lymphocytes, and macrophages. Lymphocytes can be subdivided into thymus-derived (T lymphocytes) and bursa-equivalent (B lymphocytes) depending on the primary lymphoid tissue where maturation occurs. The interaction of environmental chemicals or drugs with lymphoid tissue may alter the delicate balance of the immune system and result in four types of undesirable effects: (1) immunosuppression, (2) uncontrolled proliferation (i.e., leukemia and lymphoma), (3) alterations of host defense mechanisms against pathogens and neoplasia, and (4) allergy or autoimmunity.

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Traditional methods for toxicologic assessment have implicated the immune system as a target organ of toxic insult following chronic or subchronic exposure to some chemicals and drugs. Alterations in lymphoid organ weight or histology; quantitative changes in peripheral leukocyte counts and differentials; depressed cellularity of lymphoid tissues; and increased susceptibility to infections by opportunistic organisms may reflect potential immune alterations and have been observed in animals exposed to chemicals at doses where overt toxicity was not apparent. Increased incidence of allergy and autoimmunity has also been associated with exposure to chemicals and drugs in both animals and humans. It is becoming increasingly apparent that the immune system represents an important target organ for studying the toxicology of chemical exposure for the following reasons: immunocompetent cells are required for host resistance, and thus exposure to immunotoxicants can result in increased susceptibility to disease; immunocompetent cells require continued proliferation and differentiation for self-renewal and are thus sensitive to agents that affect cell proliferation; the cellular and molecular biology of the immune system is better understood than in many other target organ systems, and thus the mechanism(s) by which toxicants are immunoalterative can be determined; functional assessment or enumeration of leukocytes can be easily achieved using a small volume of blood or lymphoid tissue; and finally, observations obtained in experimental animals can be confirmed in humans using leukocytes obtained by minimally invasive methods (i.e., venipuncture). Now that sensitive and reproducible assays of immune function and host resistance are available, attention has focused on the usefulness of immunotoxicity as an adjunct in the routine safety evaluation of chemicals and drugs under development.

This chapter will provide an overview of the current concepts regarding the organization and function of the immune system and its cellular

* Abbreviations used in this chapter include the following: AIDS = Acquired Immunodeficiency Syndrome; Ab = Antibody; ADCC = Antibody-Dependent Cellular Cytotoxicity; CMI = Cell-Mediated Immunity; CF = Chemotactic Factor; C′ = Complement; Con A = Concanavalin A; Fc = Constant Portion of Ab Molecule; Cy = Cyclophosphamide; CYA = Cyclosporin A; CTL = Cytotoxic T Lymphocyte; DTH = Delayed-Type Hypersensitivity; ELISA = Enzyme-Linked Immunosorbent Assay; GALT = Gut-Associated Lymphoid Tissue; HI = Humoral Immunity; Ig = Immunoglobulin; IFN = Interferon; K Cell = Killer Cell; LPS = Lipopolysaccharide; MØ = Macrophage; MAF = Macrophage-Activating Factor; MIF = Migration Inhibition Factor; MLC = Mixed Leukocyte Culture; NK Cell = Natural Killer Cell; PHA = Phytohemagglutinin; PFC = Plaque-Forming Cell; PWM = Pokeweed Mitogen; PMN = Polymorphonuclear Leukocyte; PGs = Prostaglandins; RAST = Radioallergsorbent Test; RBC = Red Blood Cell; SRBC = Sheep Red Blood Cell; SRS-A = Slow-Reacting Substance of Anaphylaxis.

elements; dysfunctions of the immune system; approaches and methods for assessing immunotoxicity induced by chemicals and drugs; and a partial listing of chemicals, metals, and drugs that have been found to produce immunosuppression, allergy, or autoimmunity.

CELLS OF THE IMMUNE SYSTEM AND THEIR FUNCTION

The immune system is a highly evolved organ system involved in rejection of foreign tissue grafts and host defense against infectious agents and neoplastic cells. These functions are provided by two major mechanisms: a *nonspecific* or *constitutive* mechanism not requiring prior contact with the inducing agent and lacking specificity; and a *specific* or *adaptive* mechanism directed against and specific for the eliciting agent (Table 9–1). Mononuclear phagocytes (i.e., blood monocytes and tissue macrophages) and granulocytes are phagocytic cells involved with nonspecific resistance. Lymphoid cells, as well as macrophages, are responsible for specific host resistance.

Pluripotent stem cells comprise a unique group of cells that are unspecialized and have renewal capacity. During fetal development, pluripotent stem cells are found in the blood islands of the yolk sac in the embryo, in the liver of the fetus, and later in the bone marrow. The pluripotent stem cell differentiates along several different pathways giving rise to erythrocytes, myeloid series cells (i.e., macrophages, and granulocytes or polymorphonuclear leukocytes [PMNs]), megakaryocytes (which produce platelets), or lymphocytes. Maturation generally occurs within the bone marrow. Lymphoid progenitor cells are, however, disseminated by the vasculature to the primary lymphoid organs where they differentiate under the influence of the microenvironment of these organs (Figure 9–1).

Nonspecific and Specific Mechanisms of Immunity

Two categories of phagocytic leukocytes, the polymorphonuclear phagocyte or granulocyte and the mononuclear phagocyte or macrophage (MØ), are involved with nonspecific mechanisms of host resistance. Both cell types originate from the same myeloid progenitor in bone marrow, pass through several developmental stages, and enter the bloodstream where they circulate for one to three days. PMNs can traverse blood vessels and represent the primary line of defense against infectious agents. Both PMNs and MØs exhibit phagocytic activity toward foreign material, especially in the presence of specific opsonic antibodies and complement (see below for description), and can destroy most microorganisms. In the event that PMNs either cannot contain or are destroyed by the infectious agent, as is the case with certain bacteria such as *Listeria monocytogenes*, macrophages are recruited to the site. Macrophages can be activated to a state of enhanced bactericidal activity by soluble mediators (lymphokines) produced by T lymphocytes sensitized to a specific microbial antigen. Macrophages are unique since they can adhere to glass or plastic, can be recruited by sensitized T lymphocytes to a specific tissue location, and can be activated to become more efficient killers of intracellular microorganisms and tumor cells.

The immune response involved with adaptive host resistance represents a series of complex events that occur after the introduction of a foreign material (i.e., antigen) into an immunocompetent host. There are two major types of immune responses: (1) *cell-mediated immunity* (CMI), which is a response by specifically sensitized, thymus-dependent lymphocytes and is generally associated with delayed-type hypersensitivity, graft rejection, and resistance to persistent infectious agents (e.g., certain viruses, bacteria, protozoa, and fungi); and (2) *humoral*

Table 9–1. DIFFERENCES BETWEEN NONSPECIFIC AND SPECIFIC MECHANISMS OF HOST RESISTANCE

PARAMETER	NONSPECIFIC	SPECIFIC
Exogenous stimulation	Not required	Required
Specificity of reaction	None	High degree
Cell types involved	Polymorphonuclear leukocytes Monocytes/macrophages (effector cells)	T lymphocytes B lymphocytes Monocytes/macrophages (accessory cells)

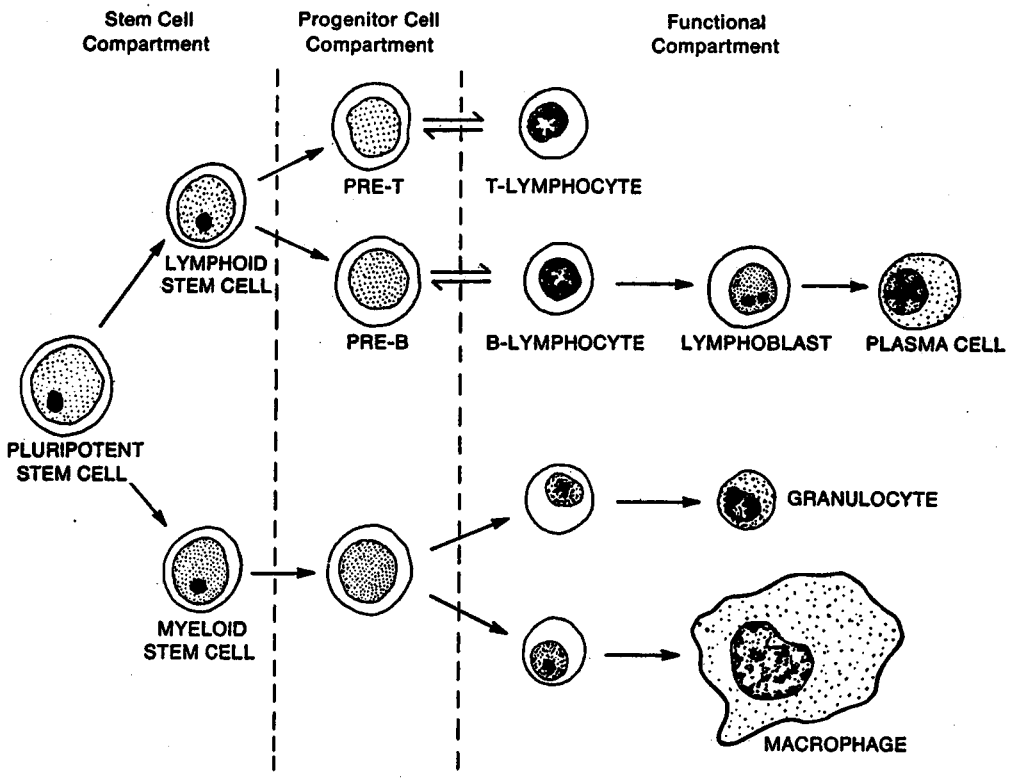

Figure 9–1. Differentiation pathways of lymphomyeloid pluripotent stem cells.

immunity (HI), which involves the production of specific antibodies (immunoglobulins) by bursa-equivalent lymphocytes or plasma cells following sensitization to a specific antigen.

Lymphocyte Differentiation

The primary lymphoid organs include the thymus in all vertebrates and the bursa of Fabricius in birds, or bursa-equivalent tissue in mammals. Primary lymphoid organs are lymphoepithelial in origin, derived from ectoendodermal junctional tissue in association with gut epithelium. During the second half of embryogenesis (days 12 to 13 in the mouse), stem cells migrate into the epithelia of the thymus and bursa-equivalent areas and begin their differentiation into T cells and B cells, respectively (Figure 9–2). The development of lymphocytes in the primary lymphoid organs is independent of antigenic stimulation.

Lymphocytes that differentiate from lymphoid stem cells in the thymus are termed thymus-dependent lymphocytes (T cells). The thymus, which is derived embryologically from the third and fourth pharyngeal pouches, is an organization of lymphoid tissue located in the chest (above the heart). Thymus development occurs during the sixth week of embryologic development in humans and day 9 of gestation in the mouse. The thymus reaches its maximum size (approximately 0.27 percent of body weight) at birth or shortly thereafter in most mammals and then begins a gradual involution until at 5 to 15 years in humans it represents only 0.02 percent of body weight.

Histologically, the thymus consists of many lobules, each containing a cortex and medulla. Lymphocyte precursors from bone marrow proliferate in the cortex of the lobules and then migrate to the medulla, where they further differentiate under the influence of thymic epithelium into mature T lymphocytes before emigrating to secondary lymphoid tissues. The neonatal/postnatal thymus has an endocrine function associated with the nonlymphoid thymic epithelium cells. These cells are believed to produce hormones essential for T-lymphocyte maturation and differentiation. A role for the adult thymus as an endocrine organ responsible for maintaining immune system homeostasis is also speculated.

In birds, B-cell differentiation occurs in the

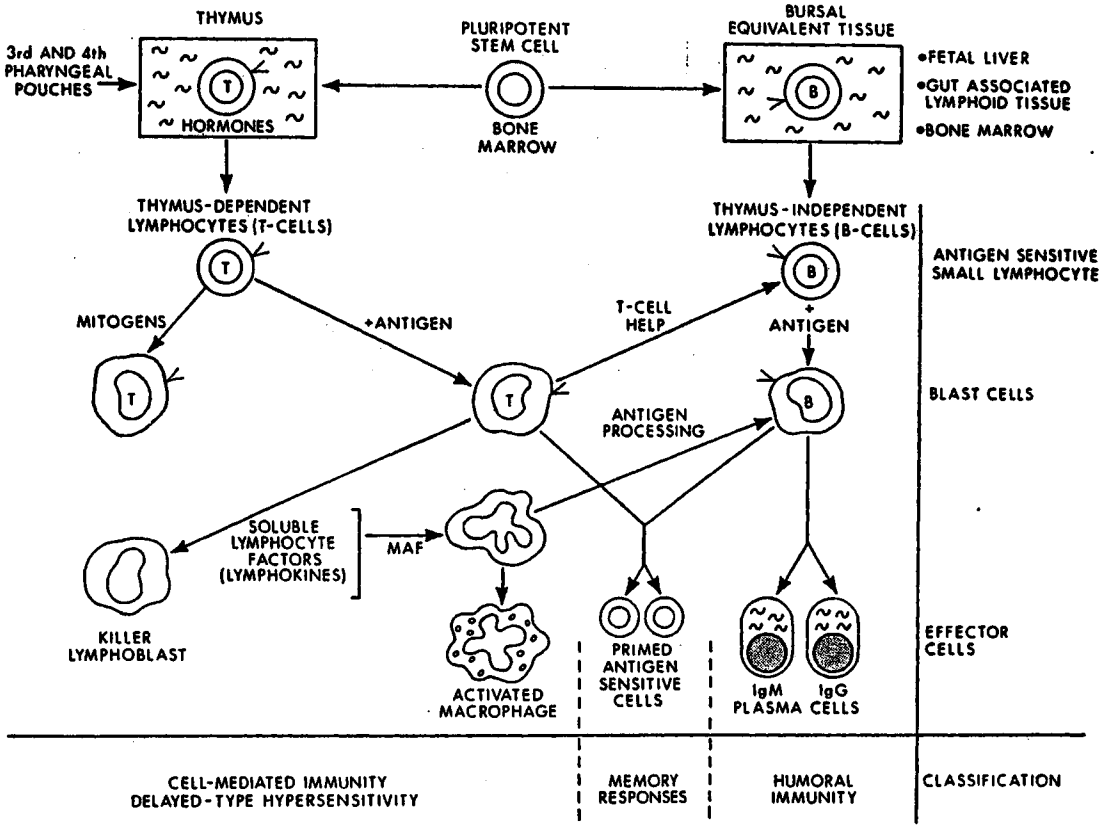

Figure 9–2. Development, interactions, and effector cells of the immune system.

bursa of Fabricius, a lymphoepithelial organ that develops from a diverticulum of the posterior wall of the cloaca. It is divided into a medullary region, containing lymphoid follicles, and a cortical region. Bursectomy in young birds results in impairment of germinal center formation (see below), plasma cell formation, and immunoglobulin production. The mammalian bursa-equivalent is believed to be the fetal liver, the neonatal spleen, gut-associated lymphoid tissue, and adult bone marrow. Mature B lymphocytes migrate from the bursa-equivalent tissue to populate the B-dependent areas of the secondary lymphoid tissues.

Neonatal removal or chemical destruction of primary lymphoid organs prior to the maturation of lymphocytes into T or B cells or prior to their population of secondary lymphoid tissue dramatically depresses the immunologic capacity of the host. However, removal of these same organs in adults has little influence on immunologic capacity. In addition, neonatal thymectomy in mammals dramatically impairs the development of CMI, but does not generally influence the generation of immunoglobulin-producing cells involved in humoral antibody responses unless they require T lymphocyte help for induction of antibody production. In contrast to the removal of primary lymphoid organs, removal of secondary lymphoid organs does not inhibit the development of immunocompetence although it may suppress the magnitude or alter the tissue location of the responsive cells (Table 9–2).

Markers of Differentiation

T lymphocytes, B lymphocytes, and MØs can be identified by a distinct pattern of cell surface-associated markers and receptors found on each of these cell types (Table 9–3). B cells, for example, have a high density of immunoglobulin on their surface, whereas T cells lack immunoglobulin. Conversely, B cells lack specific alloantigens that are found on T cells at different stages of differentiation. In humans, but not in mice, T lymphocytes can also be distinguished by their ability to form rosettes with sheep erythrocytes, while B cells lack this characteristic. Macrophages, granulocytes, killer cells, and plasma cells possess a receptor for the Fc region of antibody molecules.

Table 9–2. ORIGIN AND CHARACTERISTICS OF LYMPHOID ORGANS

PARAMETER	PRIMARY LYMPHOID ORGANS	SECONDARY LYMPHOID ORGANS
Lymphoid organs	Thymus Bursa of Fabricius (birds) Fetal liver (mammals) Adult bone marrow	Spleen Lymph nodes Gut-associated lymphid tissue (GALT)
Embryonic origin and development	Ectoendodermal junction Thymus—day 9 to 10 mouse, week 6 man Bursa-equivalent—day 10 to 13 mouse, week 10 man	Mesoderm
Lymphoid cell proliferation	Independent of antigenic stimulation	Dependent on antigenic stimulation
Germinal center formation	Nonexistent	Occurs after antigenic stimulation
Cells repopulating after depletion	Stem cells only	Differentiated lymphocytes
Early surgical or drug removal	Depressed numbers of T and B cells, depressed immune responses	No significant effect on immune function

Table 9–3. DIFFERENTIAL CHARACTERISTICS OF LYMPHOID CELLS

PARAMETER	T CELLS	B CELLS	MACROPHAGES
Phagocytosis	No	No	Yes
Adherence	No (blasts only)	No (plasma cells)	Yes
Surface receptors:			
Antigens	Yes	Yes	No
Fc region of Ig	Some	Yes	Yes
Complement	No	Yes	Yes
Differentiation antigen:			
Mouse	Thy-1 (pan-T cell) Lyt-1 (helper) Lyt-2,3 (suppressor, cytotoxic)		MAC-1
Man	OKT-3 (pan-T cell) OKT-4 (helper) OKT-5,8 (suppressor, cytotoxic)	Ig	—
Proliferation to:			
Phytohemagglutinin	Yes	No	No
Concanavalin A	Yes	No	No
Lipopolysaccharide of gram-negative bacteria	No	Yes (mouse only)	No
Allogeneic leukocyte antigens in mixed leukocyte culture	Yes	No	No
Effector functions:			
Immunologic memory	Yes	Yes	No
Tumor cell cytotoxicity	Yes	No	Yes
Bactericidal activity	No	No	Yes
Immunoglobulin production	No	Yes	No
Cytokine production	Yes (lymphokines)	No	Yes (monokines)

In contrast to T and B lymphocytes, macrophages and blood monocytes have the ability to phagocytose bacteria and other foreign particles. A group of mononuclear cells has been described that lack well-defined cell surface markers and are nonphagocytic. These cells possess a receptor for the Fc region of the immunoglobulin molecule and, when mixed with antibody and tumor target cells, are able to lyse the tumor target cells. They have been termed killer cells (K cells) and are believed to mediate cytolytic reactions against tumors and foreign tissue grafts in the presence of antibody, a process termed antibody-dependent cellular cytotoxicity (ADCC) (Perlmann *et al.*, 1975). Other subpopulations of lymphocytes have been described that possess spontaneous cytolytic activity toward neoplastic cells, but not normal cells. These are termed natural killer (NK) cells (see review by Herberman and Holden, 1978) and natural cytotoxic (NC) lymphocytes (Stutman and Cuttito, 1981).

Organization of Secondary Lymphoid Organs

The organized areas of secondary lymphoid tissues consist of the lymph nodes, spleen, and gut-associated lymphoid tissue (Table 9–2). The anatomic organization of these tissues provides a microenvironment for functional development of lymphocytes.

Lymph Nodes. Lymph nodes are discrete, organized secondary lymphoid organs and serve as filtering devices for lymphatic fluid. Lymph nodes are divided structurally into three areas: the cortex, paracortex, and medulla (Figure 9–3). Each lymph node is served by several afferent lymphatic vessels collecting lymphatic fluid from distal sites, and this fluid or lymph may contain foreign antigens. The efferent lymphatic vessel, which drains lymph from the node, contains antibodies, lymphokines, and lymphocytes produced in response to foreign antigenic stimulation. The cortex is located beneath the subcapsular sinus and receives the afferent lymph. It is the major site of B-lymphocyte localization. In the absence of antigenic stimulation, the cortex consists of a narrow rim of small lymphocytes. Also located in the cortex are aggregations of small lymphocytes, termed lymphoid follicles, which contain dendritic reticulum cells capable of retaining antigens on their plasma membranes. When the lymphocytes comprising the lymphoid follicles are stimulated by antigen, they undergo proliferation giving rise to dense aggregations of lymphocytes, termed germinal centers, which serve as sites for differentiation of B lymphocytes to plasma cells capable of antibody production. Following antigenic stimulation, germinal centers are easily detectable as spherical or ovoid structures containing many large and medium-sized lymphocytes. Histologically the germinal center is predominantly a B lymphocyte area and contains three principal regions termed the densely populated, thinly populated, and lymphocyte cuff regions. In the densely populated region, the lymphocytes are actively mitotic, while in the thinly populated area one finds an accumulation of large to medium-sized lymphocytes. The cuff

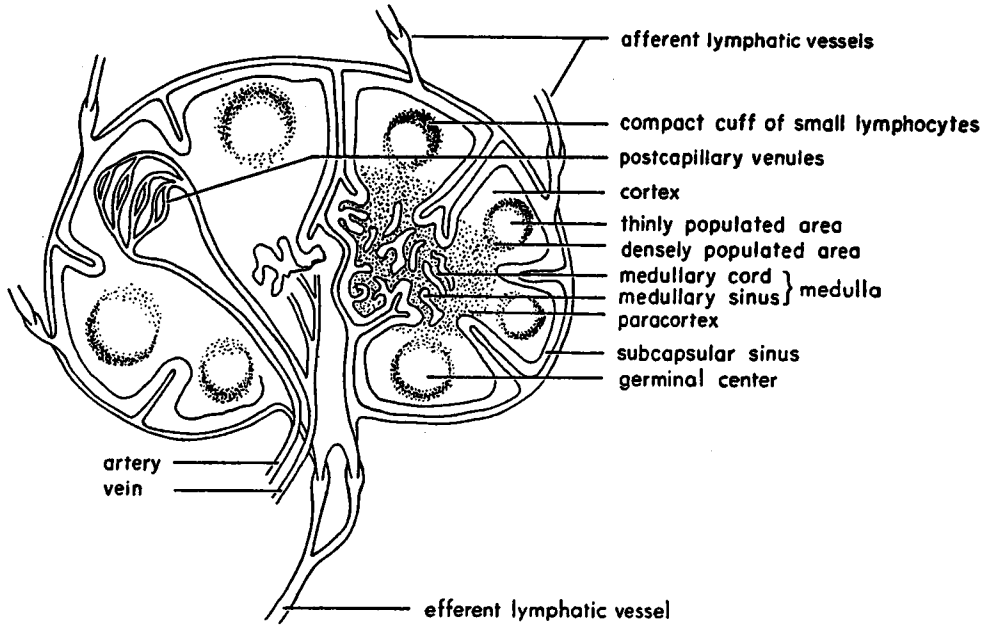

Figure 9–3. Diagrammatic cross-section of lymph node.

contains many small to medium-sized lymphocytes that are part of the recirculating B lymphocyte memory cell pool.

The paracortex, lying between the cortex and the medulla, is predominantly a T-lymphocyte area (Figure 9–3) and is a major area of macrophage/T-cell interactions. Neonatal thymectomy or short-term lymphocyte depletion by thoracic duct cannulation reduces paracortical lymphocytes, leading to depressed immune capacity. In addition, the paracortex contains specialized blood vasculature, termed postcapillary venules, which serve as points of entry for recirculating lymphocytes from the bloodstream.

The medulla of the lymph node is primarily composed of networks of cords and sinuses. The sinuses are continuations of the subcapsular space passing through the cortex and medulla and are interspersed between the medullary cords. They ultimately merge in the hilus of the lymph node to form an efferent lymphatic vessel (Figure 9–3). The medullary cords consist of a structural network of dendritic cells surrounded by dense aggregations of lymphocytes. Together, this system of cords and sinuses serves as an effective filter for removing particulate material from lymphatic fluid. Following antigenic stimulation a major portion of the antibody is produced by plasma cells found within these medullary cords.

Spleen. Lymph nodes serve as a major filter for lymph, while the spleen serves a similar function for blood. Since the spleen is the major filter of bloodborne antigens, it is also the major site of immunologic responses to these antigens. In addition, the spleen is a site of extramedullary erythropoiesis and removal of damaged blood cells. There are two major histologic regions within the spleen: the red and the white pulp (Figure 9–4). These areas have been named for their color in a freshly cut spleen. The white pulp consists of numerous white blood cell aggregates and lymphoid follicles. The red pulp contains cords and venous sinuses analogous to the medullary region of lymph nodes. The spleen has no afferent lymphatic vessels; thus, all antigenic material or cells enter the spleen through the blood vasculature. The marginal sinus in the spleen is structurally and functionally similar to the subcapsular sinus of the lymph node.

Gut-Associated Lymphoid Tissue (GALT). The lamina propria of the intestinal tract represents another secondary lymphoid tissue and, on a volume basis, is a major source of lymphoid tissue. Lymphocytes within the GALT are scattered in loose connective tissue or organized into lymphoid follicles (i.e., Peyer's patches) which contain germinal centers and diffuse concentrations of T lymphocytes analogous to the cortex and paracortex of the lymph node. As in all other lymphoid tissue, the lymphocyte cuff of the germinal center in the GALT is located nearest the source of antigenic stimulation (i.e., lumen of the intestine).

Antigen Recognition and Induction of Immunity

In 1959, Burnet proposed the clonal selection theory to describe the recognition of foreign antigens by lymphocytes, the induction of the

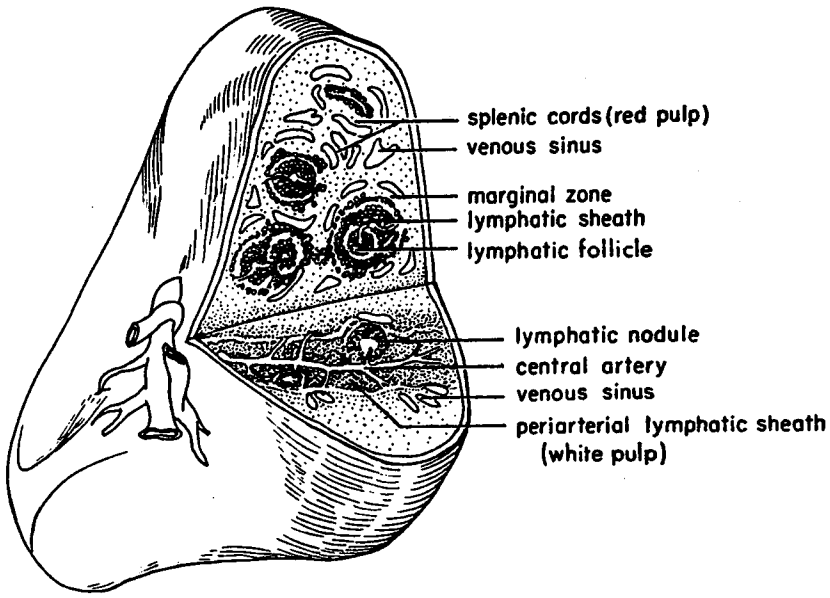

Figure 9–4. Diagrammatic cross-section of spleen.

immune response that followed, and the discrimination by the immune system of between self and nonself. In this theory, a specific antigen was believed to be nonstimulatory to all but a few lymphocytes possessing receptors with a surface structure complementary to the configuration of the antigen. Following interaction with specific antigen, the receptor-bearing cell was stimulated to undergo proliferation and differentiation, producing a clone of progeny cells that were derived from a single ancestral cell. There is convincing evidence in support of Burnet's hypothesis. Immunoglobulin (Ig) molecules are thought to represent the primary cell membrane receptor on B lymphocytes. However, it is unclear what type of receptors are involved with T-lymphocyte antigen recognition and subsequent differentiation.

Whether or not an antigen induces CMI, antibody production, or both presumably depends on a multitude of factors, including the physical and chemical nature of the antigen, the mode of presentation of the antigen to lymphocytes, the localization pattern of the antigen within lymphoid tissue, and the molecular configuration of the antigen. Those antigens generally found to elicit CMI include tissue antigens present on cells; chemical agents and drugs that conjugate with autologous proteins; and antigenic determinants on persistent intracellular microorganisms. In contrast, some antigens, for example the pneumococcal polysaccharides, predominantly elicit antibodies. The route of exposure also plays a role in the type of response generated. Sheep erythrocytes, for example, will elicit antibodies when injected intravenously or will elicit both antibodies and CMI if injected intracutaneously. It is now established that intradermal presentation of antigen favors the development of CMI.

The induction of CMI proceeds by small lymphocytes differentiating into large pyroninophilic cells, that do not contain rough endoplasmic reticulum and are thus distinct from plasma cells. These large lymphocytes ultimately divide, giving rise to cells responsible for immunologic memory and effector function. T cells can further differentiate into effector cells endowed with cytotoxic potential (i.e., cytotoxic T-cells); helper cells (T_H), which facilitate antibody responses by B lymphocytes and aid in some T-lymphocyte responses; or T-suppressor cells (T_S) capable of inhibiting both T- and B-cell responses. The steps involved in T-cell activation and the several humoral factors elaborated by T-cells are shown in Figure 9–5. These factors, termed lymphokines, include interferon (IFN), chemotactic factor (CF), and macrophage activation factor (MAF), which represent nonspecific effectors of cell-mediated immunity and are responsible for amplification and regulation of the CMI response.

The main function of B lymphocytes is production of antibody molecules in response to

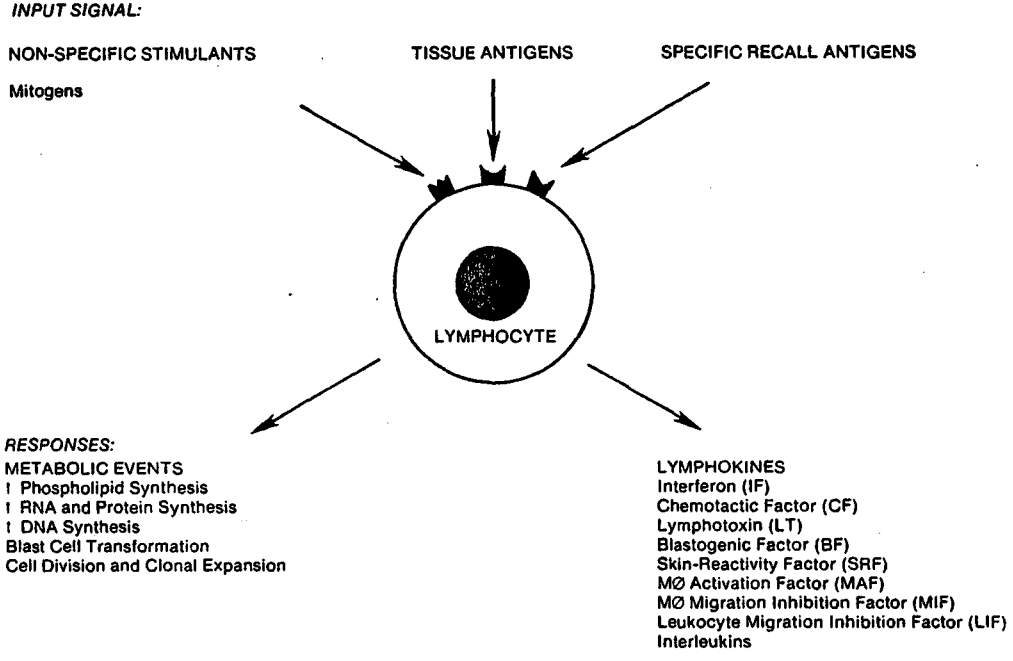

Figure 9–5. Cellular events and lymphokine production following antigen induction of CMI.

Table 9–4. BIOLOGIC PROPERTIES OF IMMUNOGLOBULIN CLASSES

CLASS	MOLECULAR WEIGHT	HALF-LIFE (DAYS)	BIOLOGIC FUNCTION
IgG	150,000	23	Fix complement Cross placenta Heterocytotropic antibody
IgA	170,000	6	Secretory antibody Properdin pathway
IgM	890,000	5	Fix complement Efficient agglutination
IgD	150,000	2.8	Lymphocyte receptor?
IgE	196,000	1.5	Reaginic antibody Homocytotropic antibody

antigenic stimulation. Antibody molecules are serum proteins synthesized in response to an antigen, which react specifically with that antigen. Based on chemical structure and biologic function, there are five classes of antibody molecules: IgM, IgG, IgA, IgD, and IgE. Table 9–4 lists the principal physical and biologic characteristics of each of the classes.

Over a period of three to five days following the introduction of antigens into an immunocompetent host, B lymphocytes differentiate into lymphoblasts, immature plasma cells, and finally antibody-secreting plasma cells. There is an early rise in IgM antibody titer in the serum, followed several days later by the appearance of IgG antibodies. The production of IgM antibodies precedes that of IgG antibodies. Figure 9–6 depicts the time course of detectable serum antibody following immunization. During this differentiation process, lymphocytes are committed to immunologic memory so that when the same antigen is encountered a second time, an enhanced response is observed, characterized by a shorter latency to the appearance of serum IgG, increased production of Ig, and sustained production of IgG antibodies.

Figure 9–7 is a diagrammatic representation of an immunoglobulin molecule. It consists of four peptide chains, two light chains, and two heavy chains, held together by disulfide bonds. Furthermore, each heavy and light chain is subdivided into a variable and constant region. It is the variable region that determines the molecular specificity for antigen while the constant region of the heavy chains is responsible for the biologic activities of the molecule. For example, the constant region contains the sites that allow IgE to bind to mast cells or allow IgG to bind complement. All antibody molecules are variations of this basic structure and may occur as monomers, or in some instances as dimers (some IgA molecules) or pentamers (IgM).

Antibody molecules have four basic functions in protecting the host from infectious agents. The first is virus neutralization. If antibodies are made to viral antigens, they may bind to the virus particles and prevent them from infecting target cells. Antibodies also aid in the elimination of foreign agents by opsonization. Antibody molecules can coat an infectious agent (i.e., bacteria or virus), and the antibody-antigen complex can bind to PMNs or macrophages via their Fc receptors, resulting in enhanced phagocytosis of the antibody-coated agent. The third way in which antibody molecules may function is via antibody-dependent cellular cytotoxicity (ADCC). Some leukocytes have receptors for the constant portion (Fc) of the molecule. Following interaction of the antibody with antigens

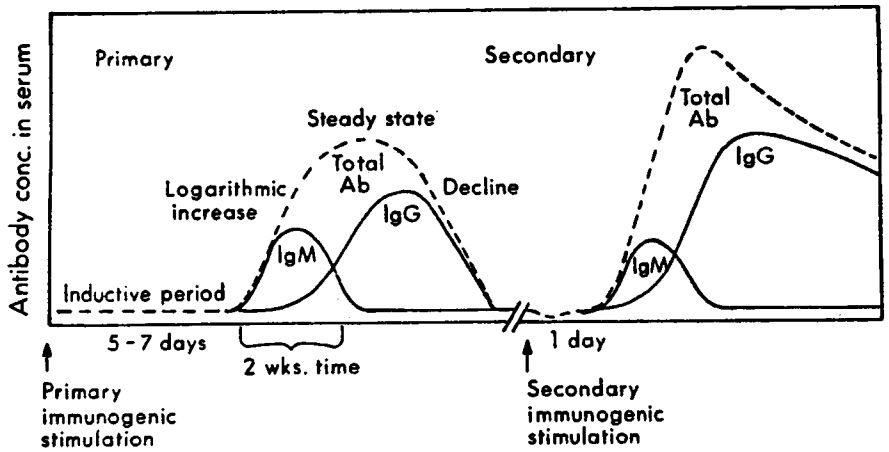

Figure 9–6. Kinetics of the antibody response.

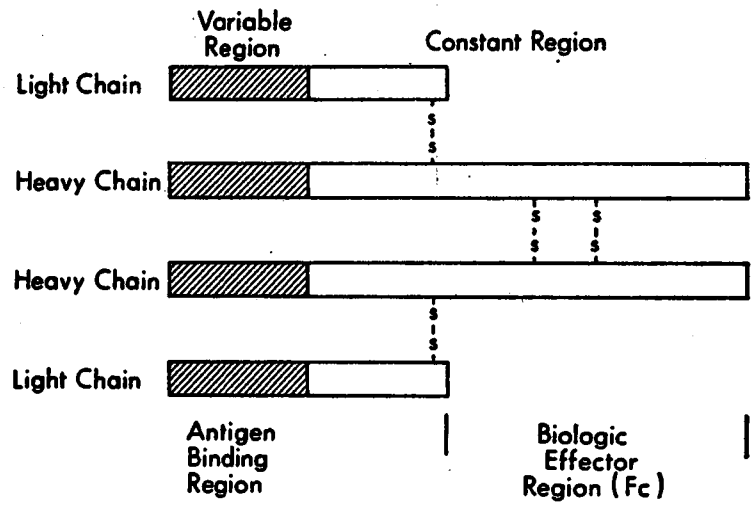

Figure 9–7. Structure of an immunoglobulin molecule.

on target cells, the Fc portion binds to the leukocyte, which can then lyse the target cell. In this way, the antibody molecule provides the specificity for the action of the effector cell. The last way in which antibody molecules can protect the host from infectious agents is through cell lysis mediated by the complement (C′) system. This cascading system consists of 20 chemically and immunologically distinct serum proteins (see review by Muller-Eberhard, 1975). The initial protein in this cascade can combine with antibody following its interaction with antigen. Subsequently C′ components interact with other proteins in a sequential fashion to generate biologic activities, that result in lysis of red blood cells, foreign or transplanted cells, lymphocytes, platelets, bacteria, and certain enveloped viruses (Figure 9–8). Many of the products of complement activation mediate inflammatory reactions (e.g., the C5 cleavage products). Evidence for the biologic importance of this system of proteins comes from the markedly increased susceptibility to infections in individuals with congenital or acquired deficiencies in complement components.

Cellular Regulation of Immune Responses

Macrophages are required for activation of some antigen-specific T cells, in particular T helper cells and T cells involved in delayed cutaneous type hypersensitivity (DTH), but not for activation of T suppressor cells. The physical interaction between lymphocytes and macrophages has been well documented. In addition, T-helper cells are required for the induction of B cells to synthesize antibodies (Ab) to certain T-dependent antigens such as foreign red blood cells or serum proteins. In contrast, a variety of antigens do not require T-helper cells for induction of antibody synthesis and are termed T-independent antigens. It has been postulated that T-independent antigens can trigger B cells in the absence of T cells because their structure allows them to bind multivalently to the immunoglobulin receptor on the B-cell surface. T-dependent antigens are believed to lack this characteristic and can only bind to individual antigen recognition sites on B cells. Macrophages are required for triggering some T and B cells because the surface of the macrophage may act as a matrix to concentrate the relevant antigenic determinants (epitopes) in a manner similar to the multivalent antigens. T cells are also responsible for switching from IgM to IgG antibody expression in B cells. There is now ample data suggesting that certain T cells may exert a suppressive influence on immune responses and that these cells belong to a distinct subset of T cells with the Lyt-2,3 phenotype in mice and OKT-5,8 phenotype in humans (i.e., suppressor T cell). In contrast, T-helper cells have the Lyt-2,3 phenotype in mice and the OKT-4 phenotype in humans. Helper and suppressor T cells exist in the circulation of humans and mice in a ratio of approximately 2:1 helper to suppressor cells. An imbalance in the ratio of helper to suppressor cells is observed in the newly recognized acquired immune deficiency syndrome (AIDS).

IMMUNE DYSFUNCTION

Hypersensitivity, and Allergy

The function of the immune system is to recognize and eliminate agents that are harmful to the host. When the immune system is functioning properly, the foreign agents are eliminated

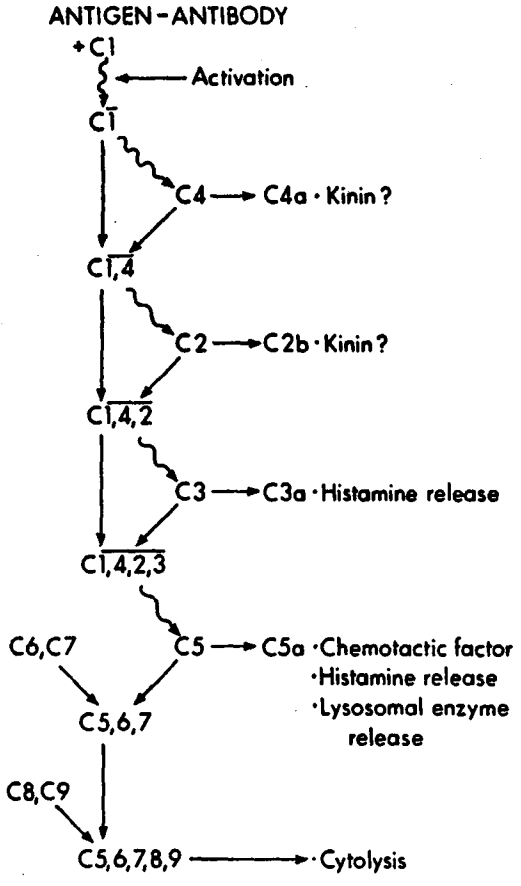

Figure 9–8. Schematic diagram of the classic complement activation cascade. (Modified from Cooper, N. R.: The complement system. In Stites, D. P.; Stobo, J. D.; Fudenberg, H. H.; and Wells, J. V. (eds.): *Basic and Clinical Immunology*, 4th ed. Lange Medical Publications, Los Altos, Calif., 1980, pp. 124–35.)

quickly and efficiently. Occasionally the immune system responds adversely to environmental agents, resulting in an allergic reaction. Coombs and Gell (1975) have divided allergic responses into four general categories based on the mechanism of immunologic involvement. These are summarized in Table 9–5 (for review, see Wells, 1982).

The type I or anaphylactic reactions are mediated by homocytotropic antibodies (IgE in man). The Fc portion of IgE antibodies can bind to receptors on mast cells and basophils. If the antibody molecule then binds antigen, pharmacologically active amines such as slow-reacting substance of anaphylaxis and histamine are released from the mediator cell (e.g., mast cell, basophil). These agents result in vasodilation, edema, and generation of an inflammatory response. The main targets of this type of reaction are the gastrointestinal tract (food allergies), the skin (urticaria and atopic dermatitis), the respiratory system (rhinitis and asthma), and the vasculature (anaphylactic shock). These responses tend to occur quickly after rechallenge with an antigen to which the individual has been sensitized and are termed immediate hypersensitivity.

The type II or cytolytic reactions are mediated by both IgG and IgM antibodies. These reactions are usually attributed to the antibody's ability to fix complement, opsonize particles, or function in an antibody-dependent cellular cytotoxicity reaction. The major target is often tissues of the circulatory system including red and white blood cells and platelets. The interaction of cytolytic antibody with these cells or their progenitors results in depletion and the production of hemolytic anemia, leukopenia, or thrombocytopenia. Additional target organs include the lungs and kidneys, as observed in Goodpasture's disease. In these type II reactions, an in-

Table 9–5. GELL AND COOMBS CLASSIFICATION SCHEME OF ALLERGY

CLASSIFICATION	EXAMPLES	MECHANISM
Type I Anaphylaxis (immediate hypersensitivity)	Asthma, urticaria rhinitis, atopic dermatitis	IgE bound to mast cell/basophil triggers release of soluble mediators (e.g., histamine).
Type II Cytolytic	Hemolytic anemia, Goodpasture's disease	IgG and/or IgM binds to cells and results in destruction via complement, opsonization, or ADCC
Type III Arthus	Systemic lupus erythematosus, glomerular nephritis, rheumatoid arthritis, serum sickness	Antigen-antibody complexes deposit in various tissues and may then fix complement
Type IV Delayed-type hypersensitivity	Contact dermatitis, tuberculosis	Sensitized T lymphocytes induce a DTH response

dividual may develop antibodies to respiratory and glomerular basement membranes, resulting in glomerulonephritis and pulmonary hemorrhaging.

The type III or Arthus reactions are mainly mediated by IgG through a mechanism involving the generation of antigen-antibody complexes that subsequently fix complement. The complexes become deposited in the vascular endothelium, where a destructive inflammatory response occurs. This is contrasted to the type II reaction, where the inflammatory response is induced by antibodies directed against the tissue antigens. The main target tissues are the skin (lupus), the joints (rheumatoid arthritis), the kidneys (glomerulonephritis), the lungs (hypersensitivity pneumonitis), and the circulatory system (serum sickness). The antigens responsible for these types of reactions may be self-antigens, as is thought to occur in lupus and rheumatoid arthritis, or foreign antigens, as in serum sickness.

The type IV or delayed-hypersensitivity response is not mediated by antibodies, but rather by macrophages and sensitized T lymphocytes. When sensitized T lymphocytes come in contact with the sensitizing antigen, an inflammatory reaction is generated: lymphokines are produced followed by an influx of granulocytes and macrophages. The target for this type of reaction can be almost any organ, the classic example being skin.

Autoimmunity

For the immune system to function properly, it must be able to distinguish self-antigens from nonself-antigens. Occasionally, the delicate balance that prevents an individual from elaborating an immune response to self-antigens becomes perturbed, resulting in an inappropriate response to self. This phenomenon is known as autoimmunity and can be manifested by the production of antibodies to self- or modified self-antigens, or by tissue destruction from T lymphocytes or macrophages. (For a review of autoimmunity, see Theofilopoulos, 1982.)

Autoimmune diseases can belong to any of the four Gell and Coombs classifications (see Table 9–5). There are several hypotheses to explain the pathogenesis of autoimmune responses. During embryonic development, it is thought that the immune system becomes tolerized to the tissues and antigens to which it is exposed either by eliminating those lymphocytes that react with self-antigens or by generating suppressor T cells that inhibit the production of an immune response to self-antigens. If effector cells arise that are specific for self-antigens, or specific suppressor T cells are lost or become nonfunctional, an immune response directed against self may occur, resulting in tissue destruction. Alternatively, during development of the immune system there are many sequestered self-antigens to which the immune system is not exposed and thus are not perceived as self-antigens. Some examples of these types of antigens are found in the tissue of the central nervous system, the lens of the eye, the thyroid gland, and the testes, as well as antigens such as DNA or RNA sequestered within cells. If these antigens become exposed, an autoimmune response may develop. Some examples of autoimmune diseases include systemic lupus erythematosus (SLE) (type III, IV), rheumatoid arthritis (type III), Goodpasture's disease (type II), serum sickness (type III), and hemolytic anemia and thrombocytopenia (type IV).

Autoimmune diseases are not necessarily the result of an immune response to self-components. Environmental agents may bind to tissue or serum proteins, and an immune response may be generated against these modified self-antigens, resulting in cell injury or death. Many drugs, chemicals, and metals have been implicated as causative agents in autoimmune diseases. For example, hydralazine and procainamide can induce an SLE-like syndrome, α-methyldopa and the pesticide Dieldrin have been shown to cause an autoimmunelike hemolytic anemia, and metals (gold, mercury) have induced a glomerular nephritis similar to that seen in Goodpasture's disease.

Immunodeficiency

Pathogenic states of decreased immunoresponsiveness can be illustrated using examples of well-characterized, naturally occurring immunodeficiency disorders. These disorders may be subdivided according to etiology into primary and secondary deficiencies. Primary immunodeficiencies are genetic or congenitally acquired and can affect either specific or nonspecific components of the immune response. Patients with these disorders are subject to characteristic alterations in resistance to various types of infections depending on the cell types or other lesions involved. In some instances the study of immunodeficient patients has helped clarify many of the mechanisms involved in resistance to infectious agents. The majority of primary immunodeficiencies involve defects in either cellular or humoral immune responses, or both, subsequent to a loss of immune cell function or absence of a particular immune cell population (Table 9–6).

Secondary or acquired immunodeficiency disorders are more common than primary immunodeficiencies and have varied etiologies (see Table 9–7). Viral infection, malnutrition, can-

Table 9–6. EXAMPLES OF CONGENITAL IMMUNODEFICIENCY

TYPE	DEFECT	TREATMENT
Thymic hypoplasia (DiGeorge's syndrome)	T lymphocytes	Fetal thymus transplant thymosin?
Infantile X-linked agammaglobulinemia	B lymphocytes	γ-Globulin
Severe combined immunodeficiency	T and B lymphocytes	Bone marrow transplant Fetal liver transplant Fetal thymus transplant
Chronic granulomatous disease	Enzyme deficiency in granulocytes	Early and prolonged antibiotic therapy
C3 deficiency	Deficiency of C3 activator	Infusion of normal plasma

cer, renal diseases, and aging are a few examples of potential causes of secondary immunodeficiency; however, in many instances the underlying cause of the condition remains obscure. Acquired as well as primary immunodeficiencies can be life-threatening. Immunosuppressive drugs may also lead to immunodeficiency and are often clinically exploited for this characteristic.

Immunosuppression is of particular clinical importance in the prolongation of allograft survival and in the treatment of autoimmune disorders. In general, primary immune responses are amenable to suppression, and secondary responses are not. Drug-induced immunosuppression depends on the characteristics of the drug and the time of its administration relative to the generation of an immune response. In this regard, the immune response can be subdivided into two phases: the inductive phase, which follows antigen exposure and is characterized by lymphoproliferation; and the productive or effector phase, characterized by antibody production and cell-mediated effector function. Most immunosuppressive agents are maximally active when administered during or just prior to the inductive phase of the immune response. An alternative classification of immunosuppressive drugs is based on their mode of action. In general, these drugs are effective because of their antiproliferative or lympholytic and lymphomodulatory actions. They usually function as general rather than specific immunosuppressants.

Table 9–7. CAUSATIVE AGENTS THAT MAY RESULT IN SECONDARY IMMUNODEFICIENCY

Drugs	Immunosuppressants, anticonvulsants, corticosteroids, chemotherapeutic agents
Infections	Acute viral, coccidioidomycosis, measles, tuberculosis, leprosy
Neoplasia	Acute leukemia, Hodgkin's disease, chronic leukemia, lymphosarcoma, thymoma, multiple myeloma, reticulum cell sarcoma
Autoimmune disease	Systemic lupus erythematosus, rheumatoid arthritis
Others	Aging, genetic disorders, malnutrition, radiation, nephrotic syndrome

Many immunosuppressive drugs were originally developed as cytoreductive cancer chemotherapeutic agents because of their ability to interfere with cell growth and proliferation. Because of the high rate of proliferation in antigen-stimulated lymphocytes, these cells are sensitive to many of the same drugs as rapidly dividing tumor cells, and the use of antiproliferative drugs in transplant patients or patients with certain autoimmune diseases has become almost routine. Azathioprine is a commonly utilized antiproliferant whose active metabolite interferes with the synthesis of compounds required for cell metabolism, growth, and division. Therefore, this drug and other antiproliferatives are most effective when administered following antigen stimulation, during the inductive portion of the immune response.

Lympholytic or lymphomodulatory agents generally act by directly destroying the lymphocyte or lethally damaging their ability to undergo mitosis; thus, as immunosuppressants these agents are most successfully used when administered just prior to the introduction of antigen. Common examples include the corticosteroids, which cause massive lympholysis in some species and act primarily through modulation of lymphocyte trafficking and effector functions in other species, including man. In contrast, alkylating agents such as cyclophosphamide cross-link DNA, causing immediate cell death or cytolysis during mitosis. Since these effects are similar to radiation-induced cell injury, alkylating agents are often referred to as radiomimetic drugs. Cyclosporin A is a relatively new immu-

nosuppressant that appears to act by mechanisms dissimilar to those previously discussed (for review see White, 1982). Although its mode of action is not completely understood, immunosuppression by Cyclosporin A may involve altered lymphokine release or blocking of lymphocyte membrane receptors for an interleukin (IL2) required to stimulate lymphocyte proliferation. Its major benefit is its apparent specificity for T-helper cells and its minimal effects on other immunoresponsive cells.

Immunosuppression may also be achieved through methods other than drug administration. Common approaches involve the use of radiation, antilymphocyte serum, and antigen (e.g., allergic desensitization). Immunosuppression, as evidenced by depressed antibody-mediated immunity and/or cell-mediated immunity, has also been observed in rodents exposed to sublethal levels of several chemicals of environmental concern. Chemicals that have produced immune alterations in rodents include: 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD); diethylstilbestrol (DES); polychlorinated biphenyls (PCB); polybrominated biphenyls (PBB); dimethyl vinyl chloride (DMVC); gallic acid; hexachlorobenzene (HCB); orthophenylphenol; organometals; and heavy metals. The observation that residents of Michigan, Japan, and China accidentally exposed to polybrominated biphenyl or polychlorinated biphenyl exhibited immune alterations similar to those observed in rodent studies has increased concern over the effects of xenobiotic agents on suppression of the immune system.

IMMUNOLOGIC MECHANISMS OF HOST RESISTANCE

The eradication and control of most bacterial agents that produce acute infections (e.g., *Staphylococcus, Streptococcus*) is facilitated by the production of specific antibodies. These antibodies enhance phagocytosis and killing of pathogenic microorganisms by granulocytes and macrophages through opsonization (Figure 9–9).

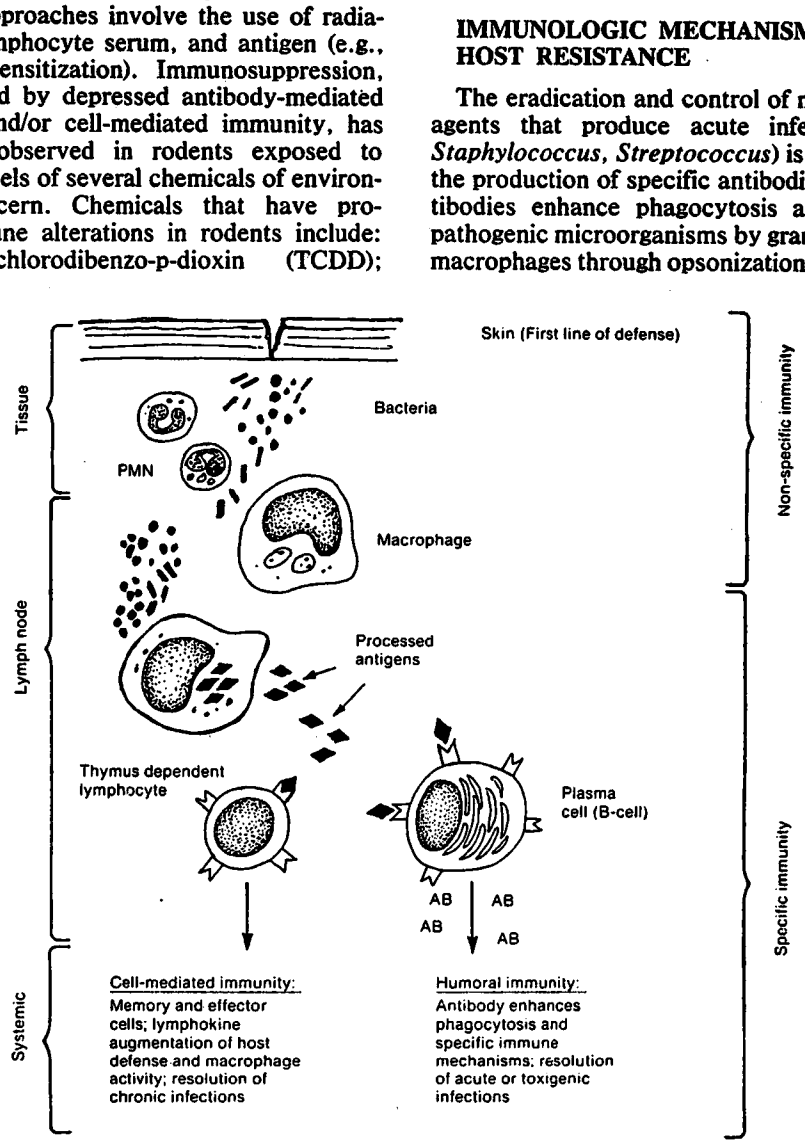

Figure 9–9. Diagrammatic representation of host resistance to bacteria indicating the roles of cell-mediated and humoral immunity. (Modified from Dean, J. H.; Luster, M. I.; Boorman, G. A.; and Laver, L. D.: Procedures available to examine the immunotoxicity of chemicals and drugs. *Pharmacol. Rev.*, 34:137–48, 1982.)

In contrast, chronic bacterial infections are usually caused by organisms such as *Listeria monocytogenes* or *Mycobacterium tuberculosis*, which are facultative intracellular pathogens that can multiply within the phagocytic cell and thus escape antibody-mediated reactions. CMI enhances granulocyte phagocytosis or macrophage-mediated killing of such intracellular pathogens through the production of lymphokine (see Figure 9–9). Toxigenic infections, which result from the production of toxins by certain bacteria, require the production of specific antibody for toxin neutralization (e.g., tetanus toxin). CMI plays little, if any, role in managing toxigenic infections. Antibodies responsible for neutralization of bacterial toxins often prevent binding of the toxin to specific receptors, thus preventing their harmful effects.

Viral antigens expressed on the surface of infected cells may also serve as targets for cytotoxic T lymphocyte-mediated cytolysis. CMI is also instrumental in eliminating viral infections through the production and release of the lymphokine interferon by lymphocytes. Interferon signals adjacent cells to produce an antiviral protein that blocks virus replication. Interferon does not have direct antiviral activity, but causes adjacent cells to manufacture antiviral proteins.

An increased incidence of infectious disease and neoplasia has been frequently associated with primary immunodeficiency diseases and immunosuppressive therapy. Gatti and Good (1971) observed a significant frequency of lymphoreticular neoplasia in patients with primary immunodeficiency diseases (Table 9–8) and suggested that the incidence would have been higher had not most of the patients died of bacterial or fungal infections before they developed neoplasia. Immunosuppressive therapy has been widely used to prevent rejection of transplanted organs and to treat certain autoimmune diseases, collagen-vascular diseases, and chronic inflammatory disorders. Nonspecific therapeutic depression of immunity has frequently caused serious complications with bacterial, viral, fungal, and parasitic infections, and less frequently has been associated with an increased incidence of certain malignancies. One important complication in transplant patients on immunosuppressive therapy is the inadvertent transplantation of malignant cells in an organ obtained from a cadaver or living donor suffering from cancer. Of 89 patients who received organs from donors who had been diagnosed for neoplasia within five years of donation, 42 percent of the recipients developed the transplanted neoplasia (Penn, 1978). Currently, transplantation of cancer is a rare event as surgeons avoid using organs from donors with cancer.

A second and more important complication in immunosuppressed transplant patients has been the high frequency of *de novo* cancer. In a sampling of renal transplant patients (Table 9–8) who survived one year, 26 percent had developed cancer, while at ten years 47 percent were so affected (*see* Penn, 1985). The types of tumors observed included skin and lip cancer (21-fold increase over general population), non-Hodgkin's lymphomas (28- to 49-fold increase), Kaposi's sarcoma (400 to 500-fold increase), and carcinomas of the cervix (14-fold increase) (Penn, 1985).

Cell-mediated immune responses are believed to be important in controlling spontaneously arising tumors and limiting the growth of estab-

Table 9–8. EVIDENCE OF INCREASED CANCER INCIDENCE IN CONGENITALLY IMMUNODEFICIENT AND RENAL TRANSPLANT PATIENTS*

DISEASE	IMMUNE ALTERATION	% CANCER INCIDENCE	TUMOR TYPE
Congenital (Bruton's) agammaglobulinemia	B cells†	6 (10,000 × normal)	Acute lymphatic leukemia
Severe combined immunodeficiency	T and B cells	2	Lymphoreticular
Common variable immunodeficiency	B cells†	10	Lymphoreticular and carcinomas
Ataxia-telangiectasia	T cells	10	Lymphoreticular, sarcoma, and carcinomas
Renal transplant patients	T and B cells	26 (by 1 year) 47 (by 10 years)	Skin and lip cancer, non-Hodgkin's lymphoma, Kaposi's sarcoma, carcinoma of cervix

* Data from Gatti and Good (1971) and Penn (1985).
† Recent evidence of excessive suppressor cell activity.

lished neoplasms. In this regard, an imbalance or transient dysfunction of the immune surveillance mechanism is thought to facilitate development of neoplastic disease. Most tumor cells have unique cell-surface antigens that clearly distinguish them from normal cells, although immune responses to these antigens may vary considerably. In rodents, for example, chemically and virally induced tumors evoke strong antitumor immune responses, which may result in regression or elimination of the developing tumor, while spontaneously arising tumors are generally less immunogenic. The weight of the evidence, however, suggests that cell-mediated immune responses are important in recognition and destruction of arising neoplasms. Indeed, this hypothesis is the basis for the concept of immune surveillance, which views CMI as the effector mechanism for eliminating spontaneously arising neoplastic clones.

The principal methods of tumor cytolysis have been elucidated through *in vitro* studies and involve direct T cell–mediated cytotoxicity, antibody-dependent cell-mediated cytotoxicity (ADCC), and natural killer cell cytolysis of tumor cells. Cytotoxic T lymphocytes (CTL) can be generated in response to specific membrane-associated antigens on tumor cells or foreign grafts. CTLs are capable of lysing the sensitizing tumor cells through direct cellular contact. *In vitro* studies with rodents have clearly shown the effector cell to be the T lymphocyte and have also demonstrated the tumor specificity of cytolysis.

In contrast, the macrophage can be considered as both an antigen-specific and nonspecific cellular mediator of tumor cytolysis. Macrophages may specifically lyse tumor targets following interactions with lymphokines, or by serving as the effector cell in antibody-dependent cellular cytotoxicity (ADCC). In ADCC, specific antibodies to tumor membrane antigens serve to focus the effector cell on the tumor cell. Several cell types may participate as effectors of ADCC including killer (K) cells, which are lymphoid in origin but are devoid of the usual B- and T-cell surface markers, macrophages, and granulocytes. In addition, fully activated macrophages are capable of nonspecific tumoricidal activity without the requirement of an interposed antibody, through a nonphagocytic mechanism of cytolysis.

An additional subpopulation of lymphocytes with tumoricidal activity, called natural killer or NK cells, has been functionally characterized in humans and rodents (see reviews by Herberman and Holden, 1978; Herberman and Ortaldo, 1981). These blood cells have unique cell surface markers distinguishing them from other cytolytic effectors and are constitutively present in nonimmune animals. They are capable of spontaneously lysing tumor cells *in vitro* and respond to different immunomodulators than either T cells, B cells or macrophages. NK cells are circulating lymphocytes whose activity may be potentiated by a variety of chemical and biologic agents including interferon and interferon inducers.

THE TIER APPROACH TO ASSESSMENT OF IMMUNOLOGIC FUNCTION

Since a single immune function assay cannot be used to comprehensively evaluate deleterious effects on the immune system following exposure to chemicals or drugs, a flexible tier of sensitive *in vivo* and *in vitro* assays has been proposed to assess immunotoxicity in rodents (Dean *et al.*, 1982a) and is currently being further refined and evaluated. The tier approach to immunotoxicity assessment consists of a screening panel assessing three parameters (TIER I), which enables the identification of compounds that may produce immune alterations. Agents testing positive in TIER I assays can be further evaluated with assays selected from a more comprehensive panel (TIER II). TIER II assays allow an in-depth evaluation of the underlying mechanism(s) of immunotoxicity. Since most immune function assays require a working knowledge of the complex interactions and functions of the immune system, it is recommended that a competent immunologist assist with any evaluation of immunotoxicity.

Immunocompetency assessment should include the evaluation of immunopathology, CMI function, humoral immunity, MØ function, and host resistance. These parameters form the basis for the assays used in the TIER I screening panel (Table 9–9). If warranted by data obtained in the preliminary TIER I screen, additional immunologic tests can be selected from TIER II (Table 9–10) to examine the underlying mechanism(s) of a particular chemical or drug-induced immune alteration. If the pathophysiologic mechanism responsible for the deleterious effect on the target cell can be defined, it may be possible to synthesize new analogs of the compound that produce the desirable effects, but lack the immunoalterative effects.

The number of immunologic tests available or under development to study altered immune function is extensive. The procedures in general use to evaluate immune function following exposure to chemicals and drugs have been described in detail (Dean *et al.*, 1979b; Luster *et al.*,

Table 9–9. SCREENING PANEL (TIER I) FOR DETECTING IMMUNE ALTERATION FOLLOWING CHEMICAL AND DRUG EXPOSURE IN RODENTS

PARAMETER	PROCEDURES PERFORMED
Immunopathology	Hematology—complete blood count and white blood cell differential
	Weights—body, spleen, thymus, kidney, adrenal
	Histology—spleen, thymus, bone marrow, lymph node, adrenal
	Spleen and bone marrow cellularity
Cell-mediated immunity	Lymphocyte blastogenesis in response to mitogens (PHA or Con A and LPS) and allogeneic leukocytes (mixed leukocyte response)
	Natural killer cell activity
Humoral immunity	Antibody plaque forming cell response to sheep erythrocytes (IgM) or specific antibody level

1982b). Assays in TIER I represent a general immunologic screen and offer a means of assessing compounds for their immunotoxic potential. These methods in TIER I are well defined for both mice and rats, and are frequently used in the clinical immunologic evaluation of humans. If the immune function data obtained from the TIER I panel are negative, there can be reasonable confidence regarding the safety of the drug or chemical for the immune system under the conditions and dosages defined in the screen. If conservative extrapolations are made using data from appropriate and clinically relevant immune function and host challenge assays, the most accurate estimate possible of chemical safety relative to the immune system will be obtained.

The following is a brief description of the tests currently utilized to assess the immunomodulatory potential of a suspect agent.

Immunopathology

Lymphoid organ weight, cellularity, and histopathology are useful in initially screening the immunomodulatory potential of an environmental agent or drug in rodents. Thymic and splenic weights, which are best expressed as organ-to-body-weight ratios, may be indicators of immune dysfunction. Thymic atrophy occurs following exposure to many chemicals and appears to be a useful indicator of chemical insult to the immune system; however, thymic atrophy alone is not necessarily a specific indicator of immu-

Table 9–10. COMPREHENSIVE PANEL (TIER II) FOR FURTHER CHARACTERIZING IMMUNE ALTERATIONS FOLLOWING CHEMICAL OR DRUG EXPOSURE

PARAMETER	PROCEDURES PERFORMED
Immunopathology	Cell surface marker profile (% T, B, MØ; T-cell subsets)
Host resistance	*Listeria monocytogenes* challenge
	Susceptibility to transplantable syngeneic tumor (TD 10–20 of PYB6 sarcoma or B16F10 melanoma)
	Streptococcus challenge
	Influenza challenge
Cell-mediated immunity	Lymphokine quantitation
	Cytotoxic T lymphocyte function
	Antibody-dependent cellular cytotoxicity
Humoral immunity	B-cell progenitor-cell quantitation
	Primary antibody response (IgM) to T-independent (LPS) antigen and secondary antibody response (IgG) to T-dependent antigen (SRBC)
	Mishell-Dutton assay
Macrophage function*	Quantitation of peritoneal macrophase cell number and phagocytosis ability
	Macrophage ectoenzyme levels
	Cytolysis of tumor target cells
	Bactericidal activity
Granulocyte function	NBT reduction
Bone marrow	Pluripotent stem cell quantitation
	Granulocyte/macrophage progenitor quantitation

* Utilizes both resident and activated peritoneal macrophages.

nosuppression since stress, severe weight loss, or general toxicity can also induce similar thymic lesions. Cellularity and histologic studies of bone marrow, spleen, and lymph nodes are also recommended. Splenic weights and cellularity may be decreased as a result of lymphoid depletion or markedly increased by extramedullary hematopoiesis since the spleen retains its hematopoietic potential during adult life. Histologic evaluation of the spleen is often helpful in determining the nature of a weight change. For example, following the introduction of an antibody-inducing antigen, there is a rapid increase in both the size and number of germinal centers. Lack of a germinal center reaction is a common finding following the administration of certain immunosuppressive drugs, chemicals and radiation, and is accompanied by a decreased ability of the animal to produce IgG antibodies. In contrast, the induction of cell-mediated immunity by antigen results in a massive proliferation (i.e., 50- to 100-fold increase) in the cells within the paracortex of lymph nodes. Both proliferation and recruitment of new cells to the paracortex occur. The use of drugs that suppress CMI depresses this massive cellular expansion within the paracortical region.

Another potentially useful procedure in immunopathology is quantitation of lymphocyte subpopulations. Specific surface markers and receptors on macrophages and lymphocytes are well characterized in rodents and humans (see Table 9–3). One method for quantitating splenic leukocyte subpopulations in mice uses fluorochrome-conjugated antisera against specific cell surface antigens identifying B cells (Ig), T cells (Thy-1 and Lyt-1,2,3), and MØs (MAC-1).

Cell-Mediated Immunity

While the previously mentioned procedures allow quantitation of leukocyte populations, they do not allow an assessment of the functional capacity of these cells. Several assays are available to examine cell-mediated immunologic functions (for detailed methods see Luster *et al.*, 1982b). These include both *in vivo* (e.g., delayed hypersensitivity, graft-versus-host reactions, or skin graft rejection) and *in vitro* techniques (e.g., lymphoproliferation and lymphokine production). Historically, CMI has been examined using delayed-type cutaneous hypersensitivity (DTH) responses. Current approaches include measuring the *in vitro* lymphoproliferative responses to mitogens and allogeneic leukocytes in mixed leukocyte cultures (MLC). Values obtained from animals exposed to several doses of chemical are analyzed and compared with values from vehicle-treated controls.

Lymphoproliferative responses are widely used in assessing CMI. The lymphocyte proliferation assay utilizes mitogens (e.g., stimulants such as plant lectins and bacterial products) or antigens (e.g., tissue or soluble antigens) to stimulate proliferation in selective lymphocyte populations. Proliferation is quantitated by incorporation of tritiated thymidine into lymphoblast DNA. In some instances animals treated with immunosuppressants will exhibit depressed proliferative responses can be seen when normal numbers of lymphocytes are present, thus indicating a failure of cell function. Recent studies have demonstrated that altered responsiveness may also occur through suppression by regulatory subpopulations of macrophages and T lymphocytes (Katz, 1977). Other factors may also cause depressed proliferative responses. These include chemically induced cytotoxicity of lymphocytes or accessory cells; redistribution of lymphocyte subpopulations (i.e., T, B, or null cells), and maturational defects in lymphocyte development. The lymphoproliferative assays are reliable predictors of immune alteration and are widely used in clinical medicine.

Assessment of Humoral Immunity

There are a variety of methods to quantitate immunoglobulins (Igs) and specific antibodies. A disadvantage of merely quantitating Ig levels is that the half-life of some immunoglobulins may exceed the subchronic (14-day) exposure period, thus rendering the method insensitive to change in short-term studies. However, quantitating immunoglobulin levels is an acceptable procedure for chronic dosing studies, although it may lack the predictive ability of methods that measure specific antibody responses following antigenic challenge.

Assessment of a specific immune response following challenge with a novel antigen such as sheep erythrocytes (SRBC) or bovine gamma globulin has more commonly been used in immunotoxicity assessment. Antigenic challenge is usually performed following exposure to the chemical or drug. The immune response to the antigen can be quantitated either by measuring serum antibody titers or by determining the number of splenic lymphocytes producing antibody to the specific eliciting antigen. Both methods are acceptable, and the former methodology can be quantitated by hemagglutination, complement lysis, or antibody precipitin procedures. Enzyme-linked immunosorbent assays (ELISA) and radioimmunoassay methodology provide even more sensitive methods for quantitating specific serum antibodies and, in addition, lend themselves to automation.

We prefer the method of quantitating numbers of splenic lymphocytes producing antibody to a specific eliciting antigen. In this procedure, single cell suspensions of splenocytes from animals previously exposed to sheep erythrocytes (SRBC) are mixed with complement and SRBC in a specialized hemocytometer. After incubation, plaques (clear areas) indicative of sheep erythrocyte lysis occur circumscribing the cells producing the specific antibody. If one adds an anti-IgG antisera to the mixtures described above, the number of cells producing IgG type antibodies can be quantitated as well. These assays are sensitive, simple, and useful in characterizing a variety of chemical- and drug-induced lesions in the humoral immune response.

Macrophage Function Assays

Macrophages not only provide nonspecific phagocytic functions but are also specifically directed and regulated by lymphocytes through lymphokines. In addition, they are involved in cellular interactions and the elaboration of products (i.e., prostaglandins and monokines) that have feedback and regulatory roles in immune responses. Macrophage functions include phagocytosis, intracellular killing of infectious agents, antigen processing and presentation, interferon production, ecotoenzyme production, and cytostasis and cytolysis of virally infected or neoplastically transformed cells. Obviously, an evaluation of MØ function is essential in immune assessment. Chemical exposure may alter basal activity or the ability of MØ to respond to activation stimulants. Thus, macrophage function in chemical- or drug-exposed mice should be assessed in resident and activated macrophages. Assays quantitating phagocytosis and inhibition of tumor cell growth are preferred for TIER I analysis of MØ function. Assays defining MØ activation can be utilized to further characterize impairment of MØ function. Most of these functional tests can be readily quantitated and are described in a recent review (Adams and Dean, 1982).

Granulocyte Function

Granulocyte function can be assessed by measuring physiologic activities such as phagocytosis, chemotactic activity, bactericidal activity, or nitro blue tetrazolium (NBT) dye reduction. Perhaps the best single assay is the NBT dye reduction procedure, which has been extensively employed in the diagnosis of persons with chronic granulomatous disease. Failure of granulocytes to reduce NBT was found to correlate with an impaired enzymatic ability to kill phagocytosed bacteria. The number of granulocytes reducing dye can be easily quantitated histochemically. This procedure is included in the TIER II panel and can be utilized if altered bacterial resistance is observed in the presence of normal CMI, HI, and macrophage function.

Bone Marrow Progenitors

Bone marrow hypoplasia is a significant complication of cancer chemotherapy and has also been implicated as a result of exposure to numerous drugs and environmental agents. The bone marrow contains pluripotent stem cells capable of differentiating along hematopoietic lines giving rise to lymphocytes, macrophages, or granulocytes. Chemical toxicity to progenitor cells, which ordinarily possess impressive proliferative capacity, can result in a magnification of chemically induced lesions, which may ultimately be expressed as altered host resistance. During the past decade, a variety of *in vitro* culture techniques have been developed for quantitating precursors for all the hematopoietic cell lines. Examination of colony formation by hematopoietic progenitor cells following exposure to various agents has proven to be a sensitive indicator of toxicity; therefore, bone marrow cellularity (TIER I) and progenitor cell assays (TIER II) are included as an integral part of the panels.

One method for quantitating murine pluripotent stem cells is by injecting bone marrow cells into irradiated recipients and subsequently counting the number of colonies forming in the spleen. *In vitro* assays quantitating progenitor cells are also available. Committed progenitor cells can be stimulated to form colonies in semisolid media by adding appropriate growth factors to the culture medium. Currently, clonal progenitor assays exist for quantitating B lymphocyte, T lymphocyte, macrophage-granulocyte, megakaryocyte, eosinophil, and erythroid precursors.

Challenge Models

A simple method for detecting immunomodulatory chemicals or drugs is to challenge the chemically exposed animal with an infectious agent. This procedure provides a general approach to determine whether the chemical interferes with host resistance to pathogens. Analysis of host susceptibility to carefully selected pathogens constitutes a holistic approach that can aid in characterizing immune dysfunctions.

Challenge with *Salmonella typhimurium, Klebsiella pneumoniae, Escherichia coli, Streptococcus pneumoniae* or *pyogenes*, and *Staphylococcus aureus;* with facultative intracellular organisms such as *Listeria monocytogenes* and *Candida albicans;* or with influenza or herpes virus, allows assessment of humoral or cell-

mediated immune resistance. Immune defense to extracellular organisms requires the interaction of T lymphocytes, B lymphocytes, and macrophages for the production of specific antibodies that may activate the complement system to aid in phagocytosis and/or lysis. Antibodies also can directly neutralize some bacteria and viruses. Resistance to intracellular organisms requires induction of CMI through T-lymphocyte and macrophage interactions, which results in the production of lymphokines and further facilitates the bactericidal activities of macrophages. A chemical- or drug-induced lesion in any of these cells or a disruption of their activation or ability to interact with each other could result in an enhanced susceptibility.

Resistance to transplantable syngeneic or semisyngeneic tumor cells is also a sensitive parameter for detecting altered host resistance following chemical exposure (Dean *et al.*, 1982a). The tumor models used include PYB6 fibrosarcoma and B16F10 melanoma. Almost any tumor model in which resistance is dependent on T-cell immunity or natural cytotoxicity can be employed.

APPROACHES TO HYPERSENSITIVITY ASSESSMENT

Another consideration in immunotoxicity assessment involves evaluating the potential of drugs and chemicals to induce allergic responses. There are three principal experimental models for evaluating the allergenic potential of an agent. The first is the Draize test (described in Klecak, 1983). In this assay, guinea pigs are exposed to the agent in question intradermally, rested, and then rechallenged by intradermal injection of the agent at a virgin site. Allergic responses, indicated by erythema and edema, are subsequently quantitated. The Buehler occluded-patch test (Ritz and Buehler, 1980) is similar, except the test agent is applied by an occluded patch rather than by an intradermal injection.

A more rigorous method for experimentally determining the allergenic potential of a compound is the Magnusson and Kligman guinea pig maximization test (Magnusson and Kligman, 1969). Animals are sensitized by subcutaneous injection with and without complete Freund's adjuvant followed by further sensitization by occluded patch. Rechallenge is by topical application with an occluded patch two weeks following the last challenge. The site is evaluated for a response 24 hours after removal of the patch. For a more complete discussion of the various testing procedures see Klecak (1983).

Two principal clinical tests are utilized for detecting immediate hypersensitivity in humans. The most common is skin testing (reviewed by Norman, 1976) in which a patch of skin is scratched or pricked followed by topical application of the suspect agent. Alternatively, the agent can be administered intradermally or by an occluded patch and the individual monitored for the development of an allergic reaction. By monitoring of both the time course and appearance of the elicited reactions, these assays can be used to distinguish between type I, II, or IV allergic reactions.

In humans, immediate hypersensitivity responses are mediated by IgE; thus, as an alternative to actual challenge with a suspected allergen, the individual can be evaluated for the presence of specific IgE antibodies in a radioallergosorbent test (RAST). Briefly, suspected allergens are immobilized on filter paper disks and the disks incubated with serum from the individual. This is followed by addition of radiolabeled anti-IgE antiserum. If an individual has IgE antibodies directed against the test agent, they will bind to the allergin-impregnated disk, and these bound antibodies will subsequently bind the radiolabeled anti-IgE antibodies. By determination of the amount of radioactivity bound to the disk, the level of specific IgE antibody to the test allergen in the patients serum can be determined.

AGENTS THAT ALTER THE IMMUNE RESPONSE

Allergy Induced by Chemicals and Metals

The problem of occupational and environmental hypersensitivity is now widely recognized. Industrial workers and consumers are exposed to many materials capable of inducing asthma, hypersensitivity, and contact dermatitis. This section will deal with the problems of sensitization to environmental contaminants followed by a detailed discussion of some of the classes of compounds of most concern.

Immunologic Lung Disease. One of the major types of hypersensitivity observed in an industrial setting is asthma. In the United States, the exact proportion of asthma cases with an occupational or environmental link is unknown. However, estimates from other industrialized nations suggest that 2 percent of asthma cases are of industrial origin. The Japanese have determined that 15 percent of asthma in men may be directly attributable to industrial exposure. Since 3 to 5 percent of the U.S. population suffers from asthma, the question of occupational-induced asthma is of concern.

Table 9–

COMPOUND
Formaldehyde
Phthalic anhydrides
B. subtilis
Pesticides
Ethylenediamine
Food additives (azodyes, BHT, BH
Antimicrobials (e.g., Parabene, ED mercurials)
Resins and plasticizers (toluene diisocyanat trimellitic anhydride)
Platinum compounds
Nickel
Chromium
Gold, mercury
Beryllium
Drugs (penicillin, quinidine, tetracycli

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United States, some
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Table 9–11. EXAMPLES OF AGENTS THAT INDUCE ALLERGIC REACTIONS

COMPOUND	EXPOSURE	TYPE OF REACTION	REFERENCE
Formaldehyde	Disinfectants, cosmetics, deodorants, paper, dyes, photography, textiles, inks, wood products, resins	Type IV	Maibach, 1983
Phthalic anhydrides	Saccharin production	Type I	Bernstein *et al.*, 1982
B. subtilis	Detergents	Type I	Luster and Dean, 1982
Pesticides	Food, exterminators	Type I, IV	Ercegovich, 1973
Ethylenediamine	Plastic industry	Type I	Popa *et al.*, 1969
Food additives (azodyes, BHT, BHA)	Ingestion of processed foods	Type I	Juhlin, 1980
Antimicrobials (e.g., Parabene, EDTA, mercurials)	Cosmetics, shampoos, creams, lotions	Type IV	Schorr, 1971; Baer *et al.*, 1973
Resins and plasticizers (toluene diisocyanate, trimellitic anhydride)	Plastics, glues, nail lacquers, wood products, resins	Type I, IV	Patterson *et al.*, 1982 Bernstein, 1982
Platinum compounds	Metal refining	Type I	Luster and Dean, 1982
Nickel	Jewelry, garment fastners	Type I, IV	Baer *et al.*, 1973 Wahlberg, 1976
Chromium	Leather products, printing	Type IV	Peltonen and Fräki, 1983
Gold, mercury	Medicinal treatments, photography	Type II, III, IV	Druet *et al.*, 1982 Baer *et al.*, 1973
Beryllium	Manufacture of alloys	Type I, IV	Reeves and Preuss, 1984
Drugs (penicillin, quinidine, tetracycline)	Medicinal treatments	Type I, II, III, or IV	DeWeck, 1978 Parker, 1982 Van Arsdel, 1981

While there are no overall figures for the United States, some data are available concerning asthma induction by industrial exposure to specific compounds (for review, *see* Luster and Dean, 1982). For example, 5 percent of workers exposed to the chemical toluene diisocyanate (TDI) develop asthma. Studies in the detergent industry indicate that 2 percent of workers exposed to enzymes from *Bacillus subtilis*, which is used in the manufacture of enzyme-containing detergents, develop asthma. In addition, it has been found that direct industrial exposure in the workplace is not necessary for development of this condition. Living near a plant utilizing these agents may result in sufficient exposure to develop allergy (e.g., TDI). Table 9–11 lists several agents that have been shown to induce allergic reactions along with the most common sources of exposure.

Allergic Contact Dermatitis. Another major type of allergic reaction to environmental agents is allergic contact dermatitis, a type IV reaction (see discussion of Gell and Coombs classification) mediated by sensitized T lymphocytes. Symptoms include a red rash, swelling, itching, and possibly blisters. A variety of substances may evoke this type of reaction, including poison ivy, drugs, cosmetics, certain metals (i.e., nickel, chromates), and other chemicals (see Table 9–11). In allergic contact dermatitis, symptoms may appear seven to ten days following the initial exposure to the allergen, but more often the reaction develops after several years of continued low-level exposure. Once sensitized, contact with the offending agent will produce symptoms within 24 to 48 hours. Patch tests (see above) are the preferred assay for diagnosing the specific causative agent(s).

A common criterion of most agents inducing contact hypersensitivity is a low molecular weight, usually less than 500 to 1000 MW. In most cases, the offending agent is not sufficient to induce the allergic reaction itself, but must be conjugated to a protein to induce sensitization. This process is known as haptenization, and the small-molecular-weight compound is known as a *hapten*, while the protein to which it is conjugated is known as a *carrier*. The plasticizer, TDI, sensitizes in this manner. Presumably, the sensitizing agent acts *in vivo* to haptenate self-proteins, and this haptenated self-protein serves as the stimulus for generation of the allergic immune response. Some chemicals may induce contact hypersensitivity only after interacting with sunlight (i.e., photoallergy). This topic has been recently reviewed by Morison and

Kochevar (1983). In photo-induced allergies, the immune response is thought to be directed against antigens that arise after the chemical, one of its metabolites, or an altered host molecule absorbs light energy. The prototype photoallergic chemical is tetrachlorosalicylanilide, an antibacterial agent in soaps.

Chemicals That Are Allergenic. *Plastics and Resins.* Toluene diisocyanate (TDI) is representative of a class of compounds used in the manufacture of plastics and resins. TDI is highly reactive with amino groups and can readily haptenate self-proteins and produce allergic reactions. Indeed, both asthma and contact dermatitis have been demonstrated in workers exposed to TDI. Recently, an animal model for TDI sensitization using both dermal and inhalation exposure was developed in guinea pigs (Karol *et al.*, 1980). In this model, sensitization was dependent on exposure to a threshold concentration of TDI vapor for the induction of pulmonary sensitivity and reaginic antibody production. When animals were exposed to the same total amount of TDI as those developing hypersensitivity, but at a lower level for a longer period of time, no allergic responses were noted (Karol, personal communication). This correlated well with human findings where workers exposed to high levels of TDI (i.e., spills or splashes) developed a pulmonary response, while workers exposed to continuous low levels of TDI remained free from TDI-induced allergic reactions. Inhalation exposure did not appear to be mandatory for the development of pulmonary hypersensitivity, since dermal exposure sensitized guinea pigs such that subsequent inhalation of TDI resulted in a pulmonary response (Karol *et al.*, 1981).

With most allergens, removal of the offending agent abrogates the allergic response. Interestingly, patients with TDI-induced asthma continue to be symptomatic for months and even years after cessation of TDI exposure. The reason for this is unknown, but it is thought that TDI may cause the airways to become hyperreactive to many agents such as smoke and other air pollutants. In addition, some individuals susceptible to TDI-induced asthma develop cross-reactivity to other diisocyanates (e.g., diphenylmethane diisocyanate) to which they have never been exposed.

Textile Finishes. Another class of chemicals that has been demonstrated to induce allergy is the resin finishes used in the textile industry to improve the wrinkle resistance and durability of fabrics. Probably the most prevalent and best studied compound in this field has been formaldehyde. This highly reactive, low-molecular-weight compound is extremely soluble in water and haptenates human proteins quite easily (Maibach, 1983). When formaldehyde resins were first used in the garment industry to provide wrinkle-resistant finishes, many workers developed allergic reactions due to the free formaldehyde. Fabrics are now allowed time to gas-off the formaldehyde or are washed prior to being used.

Sensitization with formaldehyde can induce type IV (contact dermatitis) reactions. Sensitive individuals may have difficulty avoiding formaldehyde exposure. There have been reports of individuals so sensitive that they will react to formaldehyde found in the newsprint dyes (free formaldehyde of 0.02 percent) and photographic films and papers. When one considers the ubiquitous nature of formaldehyde and its increasing usage in everyday products (furniture, auto upholstery, cosmetics, resins), the magnitude of the problem becomes apparent (for review, *see* Cronin, 1980).

Cosmetics. Another group of chemicals that induces hypersensitivity is the antimicrobials used in cosmetics. These include paraben esters, sorbic acid, phenolics (e.g., hexachlorophene), organic mercurials, quaternary ammonia compounds, ethylenediamine tetraacetate (EDTA), and formaldehyde (for review, *see* Schorr, 1971). Cosmetics are applied topically; thus, the major type of reaction is a contact dermatitis (type IV) reaction.

Metals. Metals have also been implicated in many hypersensitivity responses. Some individuals exposed to nickel in costume jewelry and metal garment fasteners have developed contact hypersensitivity. It has been estimated that 5 percent of all eczema can be linked to contact with nickel-containing compounds. Occupational hypersensitivity to beryllium has also been noted. Beryllium was previously used to coat fluorescent lamps, which led to skin sensitization when shards of broken lamps became embedded under the skin. This use of beryllium has since been discontinued. Another reaction associated with beryllium exposure thought to involve immune hypersensitivity is the chronic pulmonary syndrome berylliosis. This disease is frequently fatal and involves cough, chest pain, and a chronic progressive pneumonitis. Upon biopsy, interstitial granulomas are found. Other metals known to cause hypersensitivity responses include platinum, chromium (from the tanning of the leather products and the printing industry), and mercury and gold (usually from the medicinal use of gold salts). This subject has been extensively reviewed by IARC (1981).

Autoimmunity Induced by Chemicals and Metals

Haptenization of proteins is the most probable mechanism by which chemicals and metals cause allergic sensitization. It is interesting to

note that many drugs may also haptenate self-proteins and result in autoimmune reactions rather than hypersensitivity. This difference is probably attributed to the different routes of exposure, since individuals are exposed to metals and chemicals mainly through contact or inhalation. This type of exposure results in haptenization of cells in the skin and mucous membranes and leads to the development of hypersensitivity or dermatitis. The main exposure to drugs, however, is systemically, which may predispose to development of an autoimmune response. This is not to imply that chemicals and metals are devoid of autoimmune-inducing potential. For example, an individual who had been exposed to the pesticide Dieldrin developed immune hemolytic anemia. When blood from this person was analyzed, it was found to contain anti-Dieldrin antibodies bound to the red blood cells (RBCs). Presumably, the Dieldrin was binding to the RBCs, and this subsequently led to the autoimmune destruction of the RBCs (Hamilton *et al.*, 1978).

Heavy metals have also been implicated in autoimmune processes that may be classified as type II or type IV reactions. For example, gold salts and mercury-containing compounds can induce an immune complex glomerulonephritis (Druet *et al.*, 1982), or they may induce antiglomerular basement membrane antibodies resulting in glomerulonephritis similar to that seen in Goodpasture's disease. The mechanism by which heavy metals produce autoimmunity is unknown. One hypothesis views metals as haptens, while the second hypothesis suggests that metals alter the antigenicity of cellular proteins, rendering them "foreign" to the host. However, in mercury-induced glomerulonephritis in rabbits and gold salt-induced glomerulonephritis in humans, metals have not been observed localized at the site of the lesion (Druet *et al.*, 1982). These observations have led to a third hypothesis, which perceives metals as interfering with immune regulatory cells, resulting in the generation of an anti-self response. There is experimental evidence in support of the latter hypothesis. Weening *et al.* (1981) found that mercury was able to significantly inhibit the generation of suppressor T lymphocytes in PVG/c rats. It is plausible that metals may decrease the suppressor T lymphocyte balance necessary for preventing the formation of anti-self antibodies, thus leading to autoimmunity.

Many people are being exposed to chemicals, metals, and drugs, some of which are capable of inducing allergic reactions and autoimmunity. Therefore, an increasing amount of research emphasis should be placed on developing predictive rodent models, on a better understanding of types of agents that haptenate self-proteins, and on mechanistic studies of how these compounds exhibit their effects.

Allergy and Autoimmunity Induced by Drugs

Clinically it is difficult to distinguish between immunologic and nonimmunologic reactions to drugs. In clinical diagnosis of drug or chemical allergy, certain guidelines strongly suggest an immunologic basis for an adverse drug reaction. These have been summarized by de Weck (1978) as follows: The reaction should (1) not resemble the pharmacologic reaction of the drug; (2) be elicited by minute amounts of the drug; (3) occur only after an induction period of at least five to seven days following primary exposure to the drug; (4) include symptoms classic for allergic reactions to natural macromolecular antigens (e.g., anaphylaxis, urticaria, serum sickness syndrome, asthma); (5) reappear promptly on readministration of the drug in small amounts; and (6) be reproduced by drugs possessing similar and cross-reacting chemical structure. Immunologic testing can in some instances verify the existence of drug hypersensitivity through the detection of antibodies or sensitized lymphocytes specific for the suspected allergen. More commonly, however, the immunologic basis for the reactivity is difficult to establish since an appropriate test antigen or reactive metabolite may be difficult to identify.

Penicillin. The β-lactam antibiotics (penicillin, semisynthetic penicillin, and cephalosporins) share a common molecular structure and are responsible for the majority of allergic reactions to drugs (for reviews, *see* de Weck, 1978; Ahlstedt *et al.*, 1980). Penicillin allergy has been studied in detail and much of our current knowledge on the induction and elicitation of drug hypersensitivity has been based on results obtained from studies of penicillin hypersensitivity.

There is a high frequency of anaphylactic reactions in patients demonstrating adverse reactions to penicillin, although these individuals do not usually have detectable serum antibody titers to penicillin itself. Instead, it appears that the biotransformation product of penicillin (e.g., the penicilloyl group) is capable of combining with self-proteins, which then act as effective inducers of an antibody response (Parker, 1982). Penicillin, itself, does not appear to be sufficiently immunogenic to elicit a response. Alternatively, commercially prepared penicillin solutions may contain high-molecular-weight contaminants that could serve as carriers for penicillin antigens, thereby increasing the immunogenicity of penicillin (Ahlstedt *et al.*, 1980). Additional sources of carrier molecules might include the gastrointestinal contents, bacteria or bacterial products, and autologous proteins.

Route of administration also appears to be an important consideration in development of penicillin allergy. There is, for example, a higher frequency of allergic reactions following intramuscular compared to oral administration (Ahlstedt *et al.*, 1980; Van Arsdel, 1981).

Penicillin allergy can be of either the immediate or delayed type. Of these, the immediate type, particularly those involving anaphylaxis, can be life-threatening. Penicillin hypersensitivity is the most frequent cause of anaphylaxis in man. Therefore, it is of clinical importance to be able to identify those patients at risk for possible adverse reactions to penicillin. Usually a patient's history concerning drug allergy provides the major basis for this assessment; however, skin testing and, more recently, radioallergosorbent tests (RAST) and enzyme-linked immunosorbent assays (ELISA) measuring serum IgE to penicilloyl-polylysine, penicillin, and penicilloic acid have been used to identify individuals at risk (Ahlstedt *et al.*, 1980; Parker, 1982). In addition to anaphylaxis, penicillin and other β-lactams have been implicated in the clinical incidence of several types of hypersensitivity reactions including serum sickness, urticaria, allergic fever, hemolytic anemia, rashes, allergic contact dermatitis, and possible renal disease following the administration of antibiotics containing β-lactam rings. Penicillin may, in fact, produce nephropathy in renal tubules suggestive of a drug-induced autoimmune reaction in which autoantibodies to tubular epithelial basement membranes can be demonstrated (Border *et al.*, 1974; Parker, 1982).

Methyldopa. Methyldopa is extensively used in the treatment of essential hypertension. Allergic reactions may occur in patients receiving methyldopa over extended periods. Perhaps the most serious of these are a number of reported cases of hemolytic anemia. This drug-induced autoimmune reaction usually regresses upon discontinuation of the drug (Parker, 1982; Van Arsdel, 1981). In contrast to penicillin-induced hemolytic anemia, where penicillin acts as a hapten, methyldopa is not haptenic (Parker, 1982), but appears instead to modify erythrocyte surface antigens. IgG against modified erythrocyte surface antigens can be demonstrated in the blood of these patients. Although this autoantibody response is present in only about 1 percent of patients receiving chronic high dosages of methyldopa, other indications of immune reactivity have been more prevalent, in particular, the development of positive direct antiglobulin Coombs reactivity. There have also been reports of positive tests for lupus and rheumatoid factor (Parker, 1982) in patients receiving methyldopa.

Salicylates. Aspirin (acetylsalicylic acid) is one of the most widely used drugs in the world. It is utilized extensively for its analgesic, antipyretic, and antiinflammatory properties. Aspirin may occasionally produce symptoms such as urticaria, rhinitis, and bronchospasm, which mimic drug allergy (e.g., pseudoallergy), although the immunologic bases for these symptoms remain doubtful. Even though commercial aspirin contains potentially immunogenic compounds to which patients could become sensitized, and despite the eosinophilia usually observed in aspirin-intolerant patients, no distinct immunologic mechanisms for this reactivity have been demonstrated (de Weck, 1978). The weight of the evidence indicates a nonimmunologic basis for aspirin intolerance. This includes the observation that molecularly unrelated drugs produce responses similar to aspirin in aspirin-sensitive individuals while molecularly similar drugs (e.g., sodium salicylate) do not (Settipane, 1981). Many of the symptoms of aspirin intolerance appear to be related to an inhibition of the cyclooxygenase oxidative pathway for prostaglandin synthesis, which results in alterations in the relative amounts of prostaglandins and leukotrienes formed (Flower *et al.*, 1980).

Immunosuppression

Benzene. Benzene exposure has frequently been associated with myelotoxicity expressed as leukopenia, pancytopenia, anemia, aplastic or hypoplastic bone marrow, lymphocytopenia, granulocytopenia, and thrombocytopenia (*see* review, IARC Monograph, 1982). In workers occupationally exposed to benzene, a strong correlation was noted between the most frequently cited symptom, lymphocytopenia, and abnormal immunologic parameters. Benzene exposure in rabbits, rats, and mice resulted in anemia, hypoplastic bone marrow, and dose-related lymphocytopenia. Myelotoxicity was also correlated with the appearance of benzene metabolites in the bone marrow, and it is now evident that bone marrow can metabolize benzene (*see* review, IARC Monograph, 1982).

Studies in benzene-exposed rabbits have described increased susceptibility to tuberculosis and pneumonia as well as a reduced antibody response to bacterial antigens (IARC Monograph, 1982). Wierda *et al.* (1981) observed that exposure of C57B16 mice to benzene inhibited both antibody production and the mitogenic response of lymphocytes. Thus, the altered immune parameters reported in experimental animals may explain why the terminal event in severe benzene toxicity is often an acute, overwhelming infection.

Evaluation of a large number of workers exposed to benzene revealed depressed levels of

Table 9–12. EFFECT OF POLYHALOGENATED AROMATIC HYDROCARBONS ON HOST RESISTANCE AND IMMUNE FUNCTIONS IN RODENTS*

PARAMETER	CHEMICAL			
	PCB	*PBB*	*TCDD*	*TCDF*
Host resistance to challenge with				
Bacteria	D	NE	D	—
Endotoxin	D	NE	D	—
Virus	D	—	D	—
Parasite	D	NE	—	—
Tumor cells	D	—	D	—
Cell-mediated immunity				
DTH	D	—	D	D
Lymphocyte proliferation	I	D	D	D
Humoral immunity				
PFCs (T-dependent antigen)	D	D	D	—
Antibody titer or Ig levels	D	D	D	—
Macrophage function	—	NE	NE	—

* Modified from Dean, J. H.; Luster, M. I.; Boorman, G. A.; Leubke, R. W.; and Laver, L. D.: Application of tumor, bacterial, and parasite susceptibility assays to study immune alterations induced by environmental chemicals. *Environ. Health Perspect.*, 43:81–88, 1982.

I = increased; D = decreased; NE = no effect; — = not done; PBB = polybrominated biphenyls; PCB = polychlorinated biphenyls; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDF = tetrachlorodibenzofuran.

serum complement, IgG, and IgA, but not IgM. Thus, benzene appears to be an immunotoxicant for humans, although the magnitude of this effect and the exposure threshold for immunotoxicity remain to be established.

Halogenated Aromatic Hydrocarbons. There is substantial evidence that a number of isomers of polyhalogenated aromatics are carcinogenic, teratogenic, neurotoxic, and immunotoxic (*see* review, Kimbrough, 1980). Both mixtures and individual isomers of halogenated aromatic hydrocarbons have been studied, and their immunologic effects are summarized in Table 9–12 and described in the following sections.

Polychlorinated Biphenyls. Polychlorinated biphenyls (PCBs) have been used for over a half-century in plasticizers and other industrial applications and as a heat transfer medium in transformers. PCB mixtures have been reported to suppress immune responses and alter host defense mechanisms. The most common findings in laboratory animals exposed orally or cutaneously to sublethal levels of various PCB mixtures (e.g., Aroclors) have been severe atrophy of primary and secondary lymphoid organs, lower circulating immunoglobulin levels, and decreased specific antibody responses following immunization with antigens (Loose *et al.*, 1978; Thomas and Hinsdill, 1978; Vos *et al.*, 1980).

Effects of PCBs on CMI are inconclusive; both augmentation and suppression have been reported. Prenatal and adult exposure to PCBs have been found to depress delayed-type cutaneous hypersensitivity (DTH) (Thomas and Hinsdill, 1980). However, graft-versus-host reactivity, T lymphocyte responses to mitogens, and proliferation of leukocytes in mixed leukocyte cultures (Silkworth and Loose, 1978, 1979) have been enhanced after PCB exposure. The augmentation of selected CMI assays may reflect a relative increase in T-cell numbers due to selective depletion of B cells or, alternatively, alterations in immunoregulation through alteration of the helper/suppressor cell balance.

Studies in which PCB-exposed animals were challenged with infectious agents have indicated decreased resistance in ducks to hepatitis virus and in mice to challenge with herpes simplex virus, ectromelia virus, *Plasmodium berghei*, *Listeria monocytogenes*, or *Salmonella typhimurium* (*see* review, Dean *et al.*, 1982c). The effect of PCB on tumor resistance in rodents is unclear since both augmentation and suppression have been reported (Koller, 1975; Kerkvliet and Kimeldorf, 1977).

Human exposure to PCB has been reported in Japan and China where PCB-contaminated rice oil was consumed. In Japan (Yusho accident), PCB-exposed individuals exhibited chloracne and were more susceptible to respiratory infections (Shigematsu *et al.*, 1978). Decreased serum Ig levels were also observed. In a clinical study of individuals exposed to PCB-contaminated rice oil in China (Chang *et al.*, 1980), a decreased

DTH response to *Streptococcus* antigens was observed as well as altered T-cell numbers and function.

Polybrominated Biphenyls. Firemaster BP-6 and FF-1 are commonly used flame retardants that consist of mixtures of polybrominated biphenyls (PBBs) containing primarily 2,4,5,2',4',5'-hexabromobiphenyl and 2,3,4,5,2',4',5'-heptabromobiphenyl. In Michigan, in 1973, Firemaster BP-6 was accidentally substituted for a magnesium oxide food supplement for livestock (Dunckel, 1975) and widespread pollution of the food chain occurred over a period of several months. There was prolonged PBB contamination of meat and dairy products in the area. These contaminated products were widely consumed, and high levels of PBB were subsequently found in the serum and adipose tissues of many Michigan dairy farmers, chemical workers, and local residents (Bekesi *et al.*, 1978). A high percentage of Michigan dairy farm residents had abnormalities in a number of immune parameters that were not evident in Wisconsin control farm families. These included decreased peripheral T-cell numbers, increased numbers of lymphocytes without detectable membrane markers (i.e., so-called "null cells"), increased Ig levels, and hyperreactivity to recall antigens upon skin testing. Lymphoproliferative responses were depressed in some Michigan dairy farm residents. PBB plasma concentrations did not correlate with depressed immune responses in these individuals, although all had significantly elevated plasma PBB levels. Bekesi and associates (1986) have now confirmed their original observations in the 1976 study groups and have extended analysis to include 333 Michigan farm residents. A similar frequency of immunologic abnormalities was observed in the expanded population.

Animals experimentally exposed to PBB demonstrated depressed CMI and antibody responses to a wide variety of antigens. However, the CMI effects, which included suppression of lymphoproliferative responses and DTH, were not as severe as the suppression of antibody responses, since CMI effects occured only at near toxic dosages (Luster *et al.*, 1978; Luster *et al.*, 1980b) while antibody suppression occurred at lower concentrations. Host resistance to parasitic and bacterial challenge in PBB-exposed mice was not affected (*see* review, Dean *et al.*, 1982c).

Dibenzodioxins. Rodents exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (reviewed by McConnell, 1980, Vos *et al.*, 1980) demonstrate severe thymus atrophy. Histologic evaluation of the thymus reveals cortical lymphoid depletion similar to cortisone-induced thymus atrophy. Depressed antibody responses, DTH, graft-versus-host, and lymphoproliferative responses were observed at slightly higher dosages of TCDD (see review, Thomas and Faith, 1985). In addition, increased susceptibility to challenge with the bacteria *Salmonella bern*, but not *Listeria monocytogenes* or *Pseudorabies* virus, was noted at low dosages (Thigpen *et al.*, 1975). Depressed antibody responses and DTH were also observed in guinea pigs receiving cumulative dosages as low as 0.32 μg/kg over an eight-week period (Vos *et al.*, 1973). Clark *et al.* (1983) observed depressed T-cell function following exposure of adult mice to TCDD, which was associated with an increase in suppressor T-lymphocyte expression and loss of T-lymphocyte cytotoxicity for tumor target cells. In recent studies of adult B6C3F1 mice exposed to TCDD, we observed depressed antibody (PFC) responses and depressed lymphoproliferative responses to mitogens without alterations in cytotoxicity for tumor cells or susceptibility to bacterial or tumor cell challenge (Dean and Lauer, 1984).

Exposure to TCDD during thymic organogenesis in rodents has resulted in more severe CMI suppression than that occurring following adult exposure. In some species, *in utero* exposure (via maternal dosing) appears to be necessary to induce maximum immunosuppression (Luster *et al.*, 1981). At higher dosages, antibody responses and bone marrow stem cell numbers are depressed in most species. Administration of TCDD *in utero* also results in decreased resistance of offspring to bacterial and tumor cell challenge, which correlates with altered CMI (Luster *et al.*, 1980b) in these mice.

In a recent study of 44 schoolchildren residing in the TCDD-contaminated area of Seveso, Italy (Reggiani, 1980) it was revealed that 20 children exhibited chloracne (a classic sign of TCDD toxicity), although their serum immunoglobulin levels and circulating complement levels were normal. Lymphoproliferative responses to T- and B-cell mitogens were significantly elevated, a finding frequently reported following low-level TCDD exposure in rodents. In an earlier clinical study of British workers from a chemical manufacturing plant who were accidentally exposed to TCDD, reduced levels of serum IgD and IgA and depressed lymphocyte responses to T-lymphocyte mitogens were observed (Ward, unpublished report). A correlation was suggested between chloracne and altered immune status in this study. The Air Force has recently completed the preliminary evaluation of the health and immune status of individuals involved in the aerosol use of Agent Orange in Vietnam (Ranch/Hand II study) to establish or

refute health effects of TCDD exposure in humans (Lathrop *et al.*, 1984). Immunologic abnormalities were not apparent in these studies.

Currently it is believed that TCDD-induced immunosuppression is mediated through a cytosolic receptor for TCDD. The TCDD receptor was originally described by Poland and Glover (1976) in hepatic cytosol and subsequently in thymic cytosol (Poland and Glover, 1980). Both genetic and structure-activity data indicate that TCDD-induced thymic atrophy is mediated through the TCDD cytosolic receptor protein since thymic atrophy segregates with the *Ah* locus; and halogenated congeners of TCDD that compete with [^{3}H]-TCDD for specific binding sites in thymic cytosol fractions produce thymic atrophy *in vivo* (Poland and Glover, 1980). The target for immunotoxicity is thought to principally be the thymic epithelial cells, as suggested by Clark *et al.* (1983) and Greenlee *et al.* (1985). TCDD receptor-mediated events in the thymus may include altered T-cell maturation and differentiation and may be the molecular basis for the thymus atrophy and immunotoxicity observed. Since the endocrine influence of thymic epithelium in adult animals and humans is poorly understood, immunosuppression observed in rodents following adult exposure to TCDD may also involve toxicity to the thymic epithelium.

TCDBF (2,3,7,8-tetrachlorodibenzofuran), another dibenzodioxin, has been identified in various preparations of commercial Aroclors (Vos *et al.*, 1970) and shares the same magnitude of toxicity as TCDD. The similarity between TCDD and TCDBF in chemical structure suggests competition of these substances for the putative TCDD cytosol receptor. One might expect, therefore, that TCDBF would also be immunotoxic. In animal studies, TCDBF produced severe thymic atrophy in most species studied (Moore *et al.*, 1976) and suppressed lymphocyte responses to mitogens, DTH to novel antigens, and lymphokine (MIF) production in adult guinea pigs (Luster *et al.*, 1979a).

Polycyclic Aromatic Hydrocarbons (PAH). Polycyclic aromatic hydrocarbons are a ubiquitous class of chemicals produced during the combustion of fossil fuels. As a class, PAHs consist of three or more benzene rings fused in linear, angular, or cluster arrangements containing only carbon and hydrogen atoms. Exposure of mice to 3-methylcholanthrene (MCA), 1,2-benzanthrene, or 1,2,5,6-dibenzanthracene produces a marked depression in the serum antibody response to sheep erythrocytes (SRBC) (Malmgren *et al.*, 1952). Subsequent studies have confirmed that MCA suppresses immune responses, resulting in long-lasting reductions in antibody-producing cells. A similar long-term reduction in the response to SRBC was observed in mice exposed to 7,12-dimethylbenz[a]anthracene (DMBA) and benzo[a]pyrene (B[a]P): this depression persisted for more than 32 days after exposure (Stjernsward, 1966).

In our laboratory, B[a]P-exposed mice have been observed to have depressed responses to T- and B-cell mitogens, but not to alloantigenic stimulation (Dean *et al.*, 1983a). Exposure to the noncarcinogenic congener, B[e]P, did not alter mitogen responses. Host susceptibility following challenge with syngeneic PYB6 tumor cells and the bacterium *Listeria monocytogenes* was also unaltered in B[a]P-exposed mice, as were DTH and allograft rejection following B[a]P exposure. These data suggest that T-cell immunocompetence was minimally affected. In contrast, the primary antibody plaque-forming cell responses to both T-dependent and T-independent antigens were severely depressed. Zwilling (1977) similarly noted unaltered skin graft rejection in hamsters following inhalation exposure to B[a]P-Fe_2O_3, despite severely depressed humoral antibody responses. *In utero* exposure to B[a]P resulted in a depressed anti-SRBC response, which persisted for up to eight weeks. Urso and Gengozian (1980) also found that exposure of pregnant mice to a single dose (100 to 150 μg/g body weight) of B[a]P resulted in severe suppression of antibody responses in pups shortly after birth. This suppression persisted for at least 78 weeks and was accompanied by an increased frequency of tumors in these mice during adulthood.

There are recent data suggesting that 3-methylcholanthrene exposure in mice suppresses T-cell proliferative responses to mitogens and the generation of cytotoxic T lymphocytes (Wojdani and Alfred, 1983). Prolongation of skin graft survival, an additional measure of CMI, has also been reported following administration of MCA (DiMarco *et al.*, 1971), but was only observed if the grafting occurred 11 or more weeks after exposure, a time that corresponded to the appearance of tumors. Thus, it was not possible to ascertain whether this was a tumor or chemical-related effect.

Neonatal exposure of mice to another PAH, DMBA, suppressed both the primary (IgM) and secondary (IgG) antibody response, to SRBC, while exposure of adult mice to DMBA resulted in a kinetic shift in the IgM PFC response, although no change in magnitude of the response was observed (Ball, 1970). This observation conflicts with our recent studies in which murine exposure to DMBA suppressed the number of antibody-producing cells and CMI functions including NK and CTL cytotoxicity for up to two months (Ward *et al.*, 1986). Therefore, DMBA

exposure appears to result in long-lasting immunosuppression of CMI, HMI, and tumor resistance mechanisms.

There is a rapidly increasing body of evidence supporting the conclusion that carcinogenic PAHs produce severe, long-term immunotoxicity. This may be related to the structure of the carcinogenic PAHs, since immune alterations have not been observed following exposure to noncarcinogenic congeners.

Urethane. Urethane (ethyl carbamate) is a potent multipotential carcinogen in mice, rats, and hamsters, producing leukemia, lymphomas, lung adenomas, hepatomas, and melanomas (IARC Monograph, 1974). Exposure of mice to tumorigenic dosages of ethyl carbamate caused severe myelotoxicity, led to a marked suppression of natural killer cell activity, inhibited immune elimination of B16F10 melanoma cells, and increased metastatic tumor growth in the lungs (Luster *et al.*, 1982a). Exposure to the noncarcinogenic congener methyl carbamate did not alter immune parameters. Previous studies have demonstrated that exposure to aliphatic carcinogens, especially urethane, inhibited antibody response to SRBC (Malmgren *et al.*, 1952). Gorelik and Herberman (1981) found that exposure to urethane suppressed natural killer cell activity, which was accompanied by an increased frequency of spontaneous lung adenomas in susceptible mouse strains.

Phorbol Diesters. Phorbol diesters are a family of chemicals with potent tumor-promoting potential that produce adverse effects on lymphoid cells. 12-*O*-tetradecanoylphorbol-13-acetate (TPA) is the most active of the croton oil-derived phorbol diester tumor promoters. TPA is a potent promoter of multiple skin tumors *in vivo* and enhances transformation of fibroblast and rat embryo cell cultures *in vitro* following exposure to PAH carcinogens or oncogenic viruses. TPA produces multiple effects in leukocytes following *in vivo* (Dean *et al.*, 1982b; Murray *et al.*, 1984) and *in vitro* exposure. These effects include enhanced lymphocyte mitogenesis (Touraine *et al.*, 1977), macrophage membrane alterations, enhanced pinocytosis, increased tumor cell cytostasis (Grimm, *et al.*, 1980), and suppression of NK activity (Keller, 1979).

Depression of immunosurveillance has been suggested as an important etiologic factor in neoplasia and tumor development (Burnet, 1970). A decrease in thymus weight, T-cell numbers, lymphoproliferative responses to T- and B-cell mitogens, or allogeneic leukocytes (MLC), and decreased resistance to transplantable tumors in mice following TPA exposure was observed (Dean *et al.*, 1983b). Spontaneous NK cytotoxicity was reduced by >90 percent while the number of antibody PFCs per spleen, bone marrow cellularity, and progenitor cell numbers were unaffected. Recently, evidence has been presented for a cell surface receptor for TPA on murine T lymphocytes that may account for the selective toxicity of TPA for T cells. These effects are not observed when nonpromoting phorbols are used. It is our belief that TPA may alter lymphocyte differentiation, thus accounting for these immunologic alterations.

Insecticides. Insecticides examined for immunotoxicity in rodents can be grouped into three general classes: the organophosphates, including parathion, methylparathion, dichlorophos, and malathion; the carbamates, of which carbaryl (Sevin) has been primarily studied; and the organochlorine insecticides, which include DDT, Mirex, and representatives of the chlorinated cyclodines, aldrin, and lindane. Increasing evidence suggests that certain insecticides can alter immune function (*see* review by Street, 1981).

Organophosphate insecticides, for example, have been shown to be immunosuppressive in certain species. Street and Sharma (1974) and Street (1981) observed that a 28-day oral exposure of rabbits to methylparathion (1.5 mg/kg/day) produced a marked reduction in splenic germinal centers following antigenic stimulation, as well as thymus cortical atrophy, and a reduced DTH response to tuberculin. Similarly, Fan (*see* Street, 1981) noted a dose-related increase in mortality following challenge with *Salmonella typhimurium*, and a depressed response to mitogens following methylparathion exposure. In contrast, Wiltrout (see Street, 1981) observed that another member of this class of insecticides, parathion, produced depression of humoral immunity, but only when administered at near-lethal levels. Studies by Desi *et al.* (1978) found that exposure to malathion depressed antibody responses to *Salmonella typhi*. Along quite different lines, Vijay (*see* Street, 1981) found that rats immunized with malathion developed reaginic antibodies but not antibodies of the IgG class. Desi *et al.* (1980) found that rabbits exposed to dichlorophos had depressed humoral antibody responses and tuberculin skin test reactivity. The dosage utilized in this study was near the LD50, and no general toxicity data were provided; thus, it is difficult to separate the immunosuppression observed from general toxicity in these animals.

In general, the evidence is quite good that organophosphate insecticides can suppress the immune response. As pesticides are stable, remaining for long periods in the environment, and

can become concentrated in the food chain, it is prudent to be concerned about their potential for immunotoxicity.

The carbamate insecticide carbaryl (Sevin) has been frequently studied as an immunotoxicant. As early as 1971, Perelygin (*see* Street, 1981) observed that exposure of albino rats and rabbits to carbaryl at 20 mg/kg depressed antibody responses and phagocytosis by granulocytes. Subsequent studies in rats and chickens reported that orally administered Sevin resulted in an acute and sometimes prolonged depression of splenic germinal center formation and antibody production (*see* Street, 1981). Previously reported effects of carbaryl on granulocyte phagocytosis were confirmed and found to be prolonged for up to nine months following exposure to the chemical. In contrast to studies from Soviet or western European laboratories, which have demonstrated that carbaryl is immunosuppressive, most studies performed in this country have found no consistent indication of immunosuppression except at near-lethal doses (Street and Sharma, 1974; Street, 1981). In general, carbamate insecticides have little or no immunotoxicity.

Accumulating data suggest that the organochlorine insecticides may alter immune function (*see* review by Koller, 1979). Class representatives examined in rodents include DDT, Mirex, aldrin, and lindane. Depressed serum antibody titers against ovalbumin were observed in rats orally exposed to 200 ppm of DDT (*see* Street, 1981). In contrast, guinea pigs and rats fed DDT had normal levels of antitoxin antibody and γ-globulin, and a reduced propensity to develop anaphylaxis, which correlated with a decreased number of mast cells (Gabliks *et al.*, 1975). Likewise, chickens exposed to DDT or Mirex had significantly depressed levels of IgG and IgM, although specific antibody responses were normal (Street, 1981). In the studies of Street and Sharma (1974) and Street (1981), a four-week exposure to DDT resulted in a reduced number of germinal centers in lymph nodes, thymus cortical atrophy, and suppression of CMI. Most studies have focused on the effects of DDT on specific antibody responses. It appears that DDT produces slight to negligible immunotoxicity. However, the effects of DDT on macrophage function, CMI, and host resistance have not been intensively investigated and appear to be an open question.

Studies by Rao and Glick (1977) of chickens exposed to DDT or Mirex revealed a marked reduction in total antibody production and serum IgG level, although serum IgM levels were elevated. The alteration in IgG levels was thought to be related to altered T-cell function. Likewise, studies of rabbits exposed to lindane demonstrated a depressed antibody response to *Salmonella typhi* antigen (Desi *et al.*, 1978). Leukopenia and impaired leukocyte phagocytosis were also observed following the oral administration of lindane (Evdokimov, 1974).

Airborne Pollutants. The lungs are a primary target organ for insult by airborne chemicals of environmental and immunologic concern and have been shown to be vulnerable to a wide range of substances producing damage to tissue involved in respiratory exchange and/or nonrespiratory functions such as host defense (see review Gardner, 1984). Since the resident alveolar (pulmonary) macrophage population is the primary cell involved in pulmonary resistance to harmful agents, compounds disrupting host defense might be suspected of altering macrophage function.

Ozone. Numerous studies demonstrate that exposure to ozone (O_3) at levels as low as 0.1 ppm alter susceptibility of mice to challenge by pathogenic bacteria (Coffin and Gardner, 1972). Further studies have shown that mice forced to exercise during O_3 exposure suffer a greater mortality on challenge with pathogenic bacterial, presumably owing to an enhanced minute volume resulting in an increased O_3 uptake. This has implications for individuals exercising in areas of high ozone levels (e.g., joggers running along roadways). Similarly, mice exposed to nitrogen dioxide (NO_2) for less than three hours and challenged with a *Streptococcus*-containing aerosol have a significantly increased mortality at doses of NO_2 above 2 ppm (Ehrlich *et al.*, 1977). The increased mortality was potentiated and occurred at lower levels upon continuous long-term exposure to NO_2. While NO_2 and O_3 have produced adverse effects on host resistance following aerosol challenge with bacteria, exposure to other gaseous pollutants such as sulfur dioxide has not altered host resistance. In cases of decreased resistance, the impairment is believed to be related to a decreased phagocytic and bactericidal activity of pulmonary macrophages following exposure to these gases (*see* review, Gardner, 1984). These experimental observations in rodents correlate with epidemiologic studies in humans that emphasize a positive correlation between an increased concentration of gaseous pollutants and a higher incidence of acute respiratory disease. For example, an increased incidence of acute respiratory disease has been observed in humans in association with exposure to NO_2, cigarette smoke, O_3, and suspended nitrates and sulfates (*see* review, Gardner, 1982).

Airborne Metals. There is also evidence suggesting that a number of trace metals that alter the physiology or function of macrophages can cause a significant increase in susceptibility to infection. Animals exposed to airborne nickel, cadmium, zinc, magnesium, and lead have modified susceptibility to aerosol challenge with bacteria (Ehrlich, 1980). Increased susceptibility to challenge with pneumonia-producing bacteria in animals exposed to airborne metals was correlated with an alteration of phagocytic and enzymatic activity in alveolar macrophages (Aranyi *et al.*, 1979). Inhalation of airborne nickel and cadmium not only altered alveolar macrophage function but also depressed primary humoral immunity (Graham *et al.*, 1979). In addition, increased mortality has been observed in animals exposed to copper smelter flyash samples, but not in mice exposed to coal flyash samples. These results correlated with the adverse effects of metals on alveolar macrophages (Aranyi *et al.*, 1981).

In summary, inhalation exposure to gaseous pollutants, airborne metals, and complex metallic mixtures has been shown to alter susceptibility to bacterial challenge in mice that correlates with impairment of phagocytic, enzymatic, and bactericidal activity in alveolar macrophages. With some agents, systemic immune depression can also be observed.

Metals. Systemic metal exposure may also adversely affect the immune response and alter host resistance to infectious agents and tumors (Koller, 1979, 1980; Dean *et al.*, 1982a; Lawrence, 1985). Immunotoxic effects of metal pollutants will be discussed using lead as the prototype, since metals may share a common mechanism and the immunotoxicity of lead has been best characterized.

Lead. Several studies assessing the influence of lead on susceptibility to infectious agents have consistently shown that lead impairs both CMI and antibody-mediated host resistance (summarized in Table 9–13). Mice injected with lead nitrate intraperitoneally (ip) for 30 days and subsequently challenged with the bacterium *Salmonella typhimurium* had significantly higher mortality than controls (Hemphill *et al.*, 1971). Similar results were observed in rats exposed intravenously to lead and challenged with *Escherichia coli* (*see* review, Cook *et al.*, 1975). In these two studies, lead may have interfered with the clearance or detoxication of endotoxin, resulting in death. In another study, mice exposed orally to lead for four weeks and challenged with *Listeria* were assayed for viable *Listeria* 48 and 72 hours following challenge (Lawrence, 1981a). The highest dose of lead caused significant inhibition of early bactericidal activity, and the medium and high doses produced 100 percent mortality within ten days.

Lead exposure also increased host susceptibility to viral infections. Gainer (1977) observed that mice administered lead in drinking water for two weeks had a significantly increased mortality to encephalomyocarditis (EMC) viral challenge. It has been speculated that the enhanced susceptibility of lead-treated mice to viral challenge might be due to a decreased capacity of these animals to develop an immune response or to produce interferon (IFN). Studies by Gainer (1977) indicated that exposure of mice to lead did not inhibit the antiviral action of IFN *in vivo* or *in vitro*, although it appeared to suppress viral IFN production *in vivo*. Recently, Blakely *et al.* (1982) found that mice exposed to lead acetate in drinking water produced similar amounts of IFN as controls when both were given the viral IFN inducer Tilorone. Similarly, the *in vitro* induction of immune IFN by the T-cell mitogens, phytohemagglutinin, concanavalin A, and staphylococcal enterotoxin in lymphocytes from lead-exposed mice was unaltered compared to controls (Blakley *et al.*, 1982). Thus, lead exposure does not appear to significantly alter the ability of lymphocytes to produce immune interferon.

There is evidence suggesting that host resistance in humans may be altered by lead exposure. It has been noted that children with persistently elevated blood lead levels and infected with *Shigella enteritis* had prolonged diarrhea

Table 9–13. LEAD EXPOSURES FOUND TO IMPAIR HOST RESISTANCE TO INFECTIOUS AGENTS

SPECIES	INFECTIOUS AGENT	LEAD DOSE AND EXPOSURE	REFERENCE
Mouse	*S. typhimurium*	200 ppm/ip/30 days	Hemphill *et al.* (1971)
Mouse	EMC virus	2000 ppm/orally/2 wk	Gainer (1977)
Mouse	Langat virus	50 mg/kg/orally/2 wk	Thind and Kahn (1978)
Mouse	EMC virus	13 ppm/orally/10 wk	Exon *et al.* (1979)
Mouse	*L. monocytogenes*	80 ppm/orally/4 wk	Lawrence (1981a)
Rat	*E. coli*	2 mg/100 g/iv/1 day	Cook *et al.* (1975)
Rat	*S. epidermidis*	2 mg/100 g/iv/1 day	Cook *et al.* (1975)

(Sachs, 1978). In addition, lead smelter workers have been reported to have more colds and influenza infections per year (Ewers *et al.*, 1982) than people not exposed to lead. Secretory IgA, a major factor in immune defense against respiratory and gastrointestinal infections, was found to be suppressed in lead workers with a median blood lead level of 52 μg/100 g or greater.

Alterations in antibody-mediated immunity have also been reported in rodents following lead exposure. Reduced antibody titers in animals exposed to lead might explain the decreased host resistance to infectious agents observed, since specific antibodies can directly neutralize viruses, activate complement, and enhance opsonic phagocytosis. Lead has little effect on the serum immunoglobulin levels in rabbits, in children with >40 μg Pb/dl of blood (Reigart and Garber, 1976), or in lead-exposed workers (Ewers *et al.*, 1982). Rats pre- and postnatally exposed to lead have significantly reduced numbers of IgM PFC (Luster *et al.*, 1978). In contrast, CBA/J mice exposed to lead for one to ten weeks had unaltered IgM PFC responses to SRBC (Lawrence, 1981a). Acute oral lead exposure produces a decreased titer of specific antibodies in rabbits immunized with typhus vaccine or with pseudorabies virus. Likewise, lead-poisoned children had reduced specific antitoxoid antibody titers following booster immunizations with tetanus toxoid (Reigart and Garber, 1976). Tetraethyl lead (organic lead) also results in reduced specific antibody titers in mice and a significant reduction in IgM and IgG PFCs against sheep red blood cells (Blakley *et al.*, 1980). Although it appears likely that lead can affect antibody production, these variable data suggest that suppression may be genetically based.

The influence of inorganic lead exposure on the development of antibody responses has been further assessed by removal of splenic lymphocytes from lead-exposed mice for *in vitro* plasma cell development (Blakley and Archer, 1981). Lead exposure consistently inhibited plasma cell development. Through *in vitro* reconstitution experiments, it was concluded that inhibition of HI by lead was caused by a macrophage defect. This finding was supported by studies where 2-mercaptoethanol (2-ME), a sulfhydryl reagent that substitutes for macrophage function, was found to reverse HI inhibition by lead. These data may explain why results of studies following *in vivo* lead exposure have been variable as some of the test systems utilized 2-ME in the *in vitro* assays of immune function.

In summary, lead exposure appears to inhibit the development of antibody-producing cells and serum antibody titers. It should be noted that the dose, route of lead exposure, and genetic constitution of the host may influence immunomodulation by lead. The adverse effects of lead on humoral immunity may be due to either interference with macrophage antigen processing or antigen presentation to lymphocytes, rather than to a direct effect on B lymphocytes.

The effect of lead exposure on CMI is less clearly characterized. In a comprehensive study in Sprague-Dawley rats (Faith *et al.*, 1979), chronic low-level pre- and postnatal exposure suppressed several CMI parameters, including DTH and lymphoproliferation in response to mitogens. Gaworski and Sharma (1978) also noted that splenic lymphocytes from mice exposed orally to lead for 30 days, but not 15 days, had significantly depressed proliferative responses to T- and B-cell mitogens. In contrast, several laboratories have reported that lead exposure does not suppress T-cell proliferation (Koller *et al.*, 1979; Lawrence, 1981b; Blakley and Archer, 1982). These differences are not easily reconciled since the lead dosages and exposure periods employed do not appear to account for the differences observed.

The mechanism of lead-induced toxicity to lymphoid cells is complex. Lead, like many metals, is a sulfhydryl alkylating agent with a high affinity for subcellular sulfhydryl groups. Thus, the immunomodulatory effects of lead on immune cells may involve its association with cellular thiols since several studies have indicated that membrane and intracellular thiols are important in lymphocyte activation, proliferation, and differentiation. The study by Blakley and Archer (1982) supports this hypothesis since the inhibitory effects of lead were overcome by the addition of an exogenous thiol reagent.

Cadmium. Cadmium, like lead, is a widespread environmental pollutant producing alterations in host resistance and immune function in rodents similar to those produced by lead. Cadmium has been found to alter host susceptibility to bacterial endotoxins, *E. coli* challenge, and EMC viral challenge in mice (*see* review, Koller, 1980). Some groups, however, have reported cadmium-exposed mice to be more resistant to tumor and EMC virus challenges. Chronic cadmium exposure can result in decreased numbers of antibody-producing cells and depressed serum antibody titers in rabbits (Koller, 1973) and mice (Koller *et al.*, 1975), which is consistent with effects of other heavy metals on humoral immunity.

Gaworski and Sharma (1978) observed depressed lymphoproliferative responses to the mitogens PHA and PWM, no effect with Con A, and an enhanced response to LPS in lymphocytes from mice exposed to cadmium. Koller

et al. (1979) confirmed that cadmium produced no effect on Con A or MLC-induced lymphoproliferation although there was enhanced proliferative responses to LPS stimulation. T-cell-mediated tumor cell cytotoxicity was found to be enhanced in cadmium-exposed mice (Kerkvliet *et al.*, 1979). The data regarding effects on T-cell and macrophage function following cadmium exposure are ambiguous owing to conflicting findings between different laboratories; however, there is a consensus that humoral immune responses are depressed following cadmium exposure, results similar to those obtained with lead.

Organic and Inorganic Mercury. Several groups have reported altered host resistance in rodents following mercury exposure. Mice exposed for 84 days to 1 or 10 ppm of methylmercury chloride in food had increased mortality following challenge with EMC virus (Koller, 1975). This observation was confirmed using inorganic mercury (Gainer, 1977).

Koller (1973) and associates (1977) examined humoral immunity in rabbits after inorganic mercury exposure and in mice following methylmercury exposure. They found significantly depressed primary antibody (IgM) PFC responses. Likewise, Ohi *et al.* (1976) observed that methylmercury suppressed both the IgM and IgG antibody PFC responses in rodents when it was administered pre- and postnatally but not when given at weaning or after. Recent studies (Blakley *et al.*, 1980) have confirmed that subchronic, low-level mercury exposure in rodents results in thymic cortex and splenic follicular atrophy with concomitant depression of IgM as well as IgG antibody PFC responses.

The effect of mercury exposure on lymphocyte function and CMI has been less clearly defined. Gaworski and Sharma (1978) found that exposure of mice for 30 days to 10 ppm mercury in drinking water produced depressed lymphocyte responses to mitogens. Likewise, Hirokawa and Hayashi (1980) reported that acute exposure to nonlethal levels of methylmercury (70 mg/kg) resulted in severely depressed lymphocyte responses to T-cell mitogens. Thus, methylmercury exposure depresses polyclonal activation of lymphocytes by T-cell mitogens and antibody responses to specific antigenic stimulation.

Organotins. The immunotoxicity of organotin compounds that are used primarily as heat stabilizers, catalytic agents, and antifungal/antimicrobial compounds has been extensively reviewed (Seinen and Penninks, 1979). In long-term subchronic feeding studies of triphenyltin acetate in guinea pigs, lymphoid depletion and antibody suppression were observed. Studies by Seinen and Penninks (1979) have demonstrated that di-*n*-octyltindichloride (DOTC) or di-*n*-butyltindichloride (DBTC) exposure can selectively depress thymus cellularity and weight as well as T-lymphocyte function in rats without causing myelotoxicity or nonlymphoid toxicity. Depressed CMI evidenced by increased skin graft rejection time, reduced DTH, reduced graft-versus-host responses, and decreased responses to T-cell mitogens (*see* review, Seinen and Penninks, 1979) was observed in rats exposed to DOTC and DBTC. Inhibition of HI was also observed, expressed as reduced PFC numbers and antibody titers to sheep erythrocytes. The antibody response to *E. coli* was not affected in DOTC- or DBTC-exposed mice, suggesting that the dialkyltins do not directly affect B-lymphocyte function, but that they may alter T-helper-cell function. As with most immunotoxic chemicals, immunosuppression following DOTC or DBTC exposure is more pronounced in animals exposed immediately after birth rather than as adults.

Immune function is not impaired in mice or guinea pigs fed dialkyltins, which correlates with the absence of lymphoid tissue atrophy observed in these species following exposure (Seinen and Penninks, 1979). No species specificity is apparent following *in vitro* treatment since DOTC or DBTC added to rat or human thymocytes causes decreases in cell survival, responses to mitogens, and E-rosette formation (Seinen *et al.*, 1979) in cell cultures from both species. The data suggest that immunotoxicity produced by organotin compounds may be through an interaction of dialkyltins with plasma membrane sulfhydryl groups essential for amino acid transport.

Other Metals. Toyama and Kolmer (1918) reported over 60 years ago that feeding animals low concentrations of arsenic enhances antibody production, while antibody suppression occurs following high-level exposure. Recently, similar observations have been reported in mice fed various arsenate compounds. While high levels of arsenicals increased susceptibility to viral infection and decreased interferon activity, low levels had the opposite effect, causing increased viral resistance and viral interferon production (Gainer, 1972; Gainer and Pry, 1972). General toxicity occuring at higher levels of arsenical exposure may be, in part, responsible for the increased viral susceptibility. It appears that further studies with arsenicals are warranted.

There is evidence in laboratory animals that nickel exposure results in altered resistance to virus and bacteria (Adkins *et al.*, 1979). A direct effect on macrophage function has also been attributed to nickel (Graham *et al.*, 1978). Zinc, in

Table 9–14. CHEMICALS AND METALS REPORTED TO ALTER IMMUNE FUNCTION AND HOST RESISTANCE IN RODENTS*

CHEMICAL	RESISTANCE TO CHALLENGE WITH			
	TUMOR	BACTERIA	VIRUS	PARASITE
Arsenic	↓	—	↓	—
Cadmium	↓	↓	↓	—
Cannabinoids	—	↓	↓	—
Cyclophosphamide	↓	↓	↓	↓
Diethylstilbestrol	↓	↓	↓	↓
Dimethylvinyl chloride	↓	NE	—	↓
DDT	—	—	↓	—
Ethyl carbamate (urethane)	↓	NE	—	↓
Lead	↓	↓	↓	—
Methylmercury	—	—	↓	—
NO_2	↓	↓	↓	—
Ozone	—	↓	—	—
Polyhalogenated biphenyls	↓	↓	↓	↓
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	↓	↓	↓	—
12-*O*-tetradecanoyl-phorbol-13-acetate	↓	NE	—	↓

* ↑ = Increased; ↓ = depressed; — = not determined; NE = no effect.

contrast, is a metal essential for maintaining the integrity of the immune response. Several laboratories have found that zinc deficiency depresses antibody responses, possibly owing to a loss of T-helper-cell function (Fernandes *et al.*, 1979). The underlying requirement of zinc in maintaining immunocompetence requires further study, but may be a result of its requirement in many enzyme systems, or its ability to stabilize biologic membranes.

Some of the chemicals and metals discussed in the previous sections that have been demonstrated to alter immune function and host resistance are summarized in Table 9–14.

Drugs. The majority of drugs clinically utilized for immunosuppressive purposes were initially developed for alternative reasons. Suppression of the immune response, in many instances, was an undesirable side effect. Recently, certain abused drugs have also been shown to cause immune alterations. A partial listing of drugs that suppress the immune response along with their proposed mechanisms of action is given in Table 9–15. A limited number of these agents will be discussed below to illustrate prototype agents having quite different mechanisms of immunosuppression.

Alkylating Agents. Alkylating agents are chemicals that form covalent linkages (alkylation) with biologically important molecules, including DNA, which result in disruption of cell functions, especially mitosis. Thus, these agents are particularly toxic to rapidly proliferating cells including neoplastic, lymphoid, bone marrow, intestinal mucosal, and germinal cells. The alkylating agents are effective at any part of the cell cycle, although cytotoxicity is usually expressed during S phase as the cell prepares to divide. Cyclophosphamide is representative of this class of drugs and is the most widely used of the nitrogen mustards. Interestingly, the feasibility of using nitrogen mustards as chemotoxic agents for neoplastic cells was based on early observations of their cytotoxicity to lymphoid tissues; their use as immunosuppressants occurred later.

As a chemotherapeutic agent, cyclophosphamide alone or in combination with other drugs has been effective in treating Hodgkin's disease, lymphosarcoma, Burkitt's lymphoma, and acute lymphoblastic leukemia (Calabresi and Parks, 1985). As an immunosuppressant, cyclophosphamide is beneficial in reducing symptoms of certain autoimmune diseases (Calabresi and Parks, 1985), although its major use has been in pretreatment of bone marrow transplant recipients in an effort to prevent subsequent graft rejection (Shand, 1979).

Several reports indicate that there may be subpopulations of lymphocytes preferentially affected by cyclophosphamide treatment, at least in certain species (Shand, 1979; Webb and Winkelstein, 1982). B cells in guinea pigs, chickens, and mice, for example, have been demonstrated to be more sensitive than T cells to cyclophosphamide-induced toxicity (Shand, 1979). In contrast, higher dosages of cyclophosphamide can also suppress T-cell function in

Table 9–15. IMMUNOSUPPRESSIVE DRUGS

Therapeutic Drugs

- Alkylating agents
  - Nitrogen mustards: Cyclophosphamide, L-phenylalanine mustard, chlorambucil
  - Alkyl sulfonates: Busulfan
  - Nitrosoureas: Carmustine (BCNU), lomustine (CCNU)
  - Triazenes: Dimethyltriazenoimidazolecarboxamide (DTIC)
- Antiinflammatory agents
  - Aspirin, indomethacin, penicillamine, gold salts
  - Adrenocorticosteroids—prednisone
- Antimetabolites
  - Purine antagonists: 6-mercaptopurine, azathioprine, 6-thioguanine
  - Pyrimidine antagonists: 5-fluorouracil, cytosine arabinoside, bromodeoxyuridine
  - Folic acid antagonists: Methotrexate (amethopterine)
- Natural products
  - Vinca alkaloids: Vinblastine, vincristine, procarbazine
  - Antibiotics: Actinomycin D, adriablastine, bleomycin, daunomycin, puromycin, mitomycin C, mithramycin
  - Antifungal agents: Griseofulvin
  - Enzymes: L-Asparaginase
  - Cyclosporin A
- Estrogens—diethylstilbestrol, ethinyl estradiol

Abused Drugs

- Ethanol
- Cannabinoids
- Cocaine
- Opiates

mice (Dean *et al.*, 1979b). Both T-helper and T-suppressor cells have at times been implicated as targets; however, recent evidence indicates that certain T-suppressor-cell populations are extremely sensitive to cyclophosphamide (Shand, 1979). Thus, cyclophosphamide-induced immunosuppression may involve alteration in lymphocyte function as well as cytoreduction.

As is observed with other conventional immunosuppressants, treatment with cyclophosphamide can increase the risk of cancer and infection, which may relate to the lymphopenia and neutropenia seen following cyclophosphamide therapy (Webb and Winkelstein, 1982). In addition, exposure of experimental animals to cyclophosphamide increases host susceptibility to transplantable tumors (Dean *et al.*, 1979a).

Corticosteroids. Corticosteroids and their synthetic analogs can suppress both inflammatory and immune responses. The synthetic corticosteroids prednisone and methylprednisolone are common adjuncts in immunosuppressive therapy in transplant recipients and individuals with extreme hypersensitivity. Although the precise basis for their immunologic effects is unknown, corticosteroids cause a transient lymphopenia (Webb and Winkelstein, 1982), alter phagocytosis, and depress T- and B-lymphocyte function (Santiago Delpin, 1979). In rodents, a dramatic lymphopenia due to lympholysis can be demonstrated following corticosteroid therapy; however, lymphocytes from humans are relatively resistant to lympholysis by corticosteroids (Webb and Winkelstein, 1982). Thus, suppression in humans may be due to other diverse corticosteroid-induced effects such as alterations in leukocyte mobility, production and/or responses to lymphokines, and immune cell interactions. Part of these effects, as well as many of the antiinflammatory properties of corticosteroids, might be attributed to their stabilization of biomembranes, including plasmalemmal and lysosomal membranes (Santiago Delpin, 1979). At a molecular level, these changes may be mediated through steroid-receptor complexes capable of interacting with DNA, thereby modifying enzyme synthesis, and ultimately resulting in the immunomodulatory properties of this group of compounds (Santiago Delpin, 1979).

Antimetabolites. The antimetabolites are frequently used clinically in transplant patients as immunosuppressive drugs and can be categorized as folate, purine, pyrimidine, and amino acid analogs. The most widely used antimetabolite is the purine antagonist azathioprine. It is a derivative of 6-mercaptopurine (6-MP) and was originally synthesized with the intent of preventing the rapid methylation and oxidation common to 6-MP, thus improving its therapeutic:toxic ratio. Azathioprine is more effective in cycling cells and is maximally active as an immunosup-

pressant when given following antigenic stimulation (Santos, 1974). Immunosuppression may result from azathioprine-induced inhibition of purine synthesis; however, other mechanisms have been suggested, including the binding of azathioprine to T lymphocytes and the subsequent inactivation of surface antigen receptors (Webb and Winkelstein, 1982).

Azathioprine is also an antiinflammatory agent and can reduce numbers of neutrophils, monocytes, and large lymphocytes. The question of specificity of this drug remains unclear. Regarding lymphocytes, there is evidence that cell-mediated immunity and T-cell functions are the main target of azathioprine-mediated suppression, which is consistent with the clinical picture (Santiago Delpin, 1979). However, recent *in vitro* studies demonstrate substantial toxicity of azathioprine for both T and B cells, although the drug concentrations used in these experiments were higher than plasma levels commonly obtained in therapeutic situations (Kazmers *et al.*, 1983).

The major clinical complication of azathioprine therapy is bone marrow toxicity and leukopenia, which may predispose to secondary infection. In addition, long-term administration of azathioprine may increase the risk of developing certin malignancies.

Natural Products. Drugs of the natural products group have a variable range of immunosuppressive actions. Cyclosporin A (CyA), a relatively new compound in this group, is isolated from fermentation products of two fungi, *Trichoderma polysporum* and *Cylindrocarpon lucidum*, and has a very narrow range of antibiotic activity against fungi and yeast. CyA was found to inhibit lymphocyte proliferation in early tests designed to detect nonspecific cellular toxicity, which further increased the doubt of its potential value as an antibiotic. Fortunately, its lymphostatic and immunologic properties were further characterized. The result has been the development of a family of cyclosporins. Cyclosporin A is the most widely known of these drugs; however, cyclosporins C and G have also been shown to be effective immunosuppressants.

An important characteristic of CyA is its relative lack of secondary toxicity at therapeutic dosages sufficient to maintain immunosuppression in transplant recipients. For example, CyA does not appear to be myelotoxic, an important consideration, particularly in bone marrow transplant recipients, although some cases of hepato- and nephrotoxicity have been reported in patients receiving CyA. The incidence of secondary infection also appears to be less frequent in transplant patients receiving CyA compared to those receiving more conventional immunosuppressants, although this point is still controversial. Perhaps the greatest concern regarding its therapeutic use is a possible increased frequency of malignancies, especially lymphomas, in transplant patients receiving CyA (*see* review, White, 1982).

Part of the success obtained with CyA undoubtedly involves its unique mechanism of immunosuppression. Unlike lympholytic or antiproliferative/antimetabolic immunosuppressants, CyA appears to act through modulation of mechanisms regulating immunoresponsiveness (*see* review, White, 1982). In addition, its effects seem to be somewhat specific for T-cell function, predominantly sparing B-cell function. This could, in part, account for the reported decreased incidence of infection in transplant recipients receiving CyA (Calne *et al.*, 1981). The specificity of action of CyA seems to be partly mediated through a decreased production of lymphokines requisite for the generation of cytotoxic T lymphocytes. CyA may also act by either masking or preventing the expression of receptors required in triggering lymphocyte proliferation, maturation, and differentiation. The specificity of such a mechanism might also favor the functional predominance of T-suppressor cells in immunoregulation, thus further inducing transplantation tolerance (*see* review, White, 1982).

Estrogens. Diethylstilbestrol (DES) is a synthetic nonsteroidal compound possessing estrogenic activity which has widespread commercial usage. Mice exposed to DES during prenatal (Luster *et al.*, 1979a) or adult (Boorman *et al.*, 1980) life exhibited severe thymic cortical lymphoid depletion along with depressed MLC responses, DTH, and mitogen-induced lymphocyte blastogenesis (Kalland *et al.*, 1979; Luster *et al.*, 1979b; Luster *et al.*, 1980a). The usual ratios of T-cell subpopulations in neonatally DES-exposed mice were altered, suggesting a defect in maturation of T cells. A subsequent report has related the reduced proportion of T-helper cells to suppressed antibody PFC responses to T-dependent antigens (Kalland, 1980). Suppressed antibody responses following immunization with T-independent antigens also occur in rodents treated with DES and are consistent with the depressed *in vitro* proliferative response to LPS, a polyclonal B-cell mitogen (Kalland *et al.*, 1979; Luster *et al.*, 1979b; Luster *et al.*, 1980a). Macrophage functions, assessed by phagocytosis and tumor growth inhibition by adherent peritoneal cells, are potentiated by DES exposure (Boorman *et al.*, 1980), while macrophage suppressor cell activity is enhanced (Luster *et al.*, 1980a).

The effects of DES on immune surveillance and host resistance to disease are well characterized. Exposure of adult mice to DES resulted in increased mortality following challenge with the bacterium *Listeria monocytogenes*, the parasite *Trichinella spiralis*, and a transplantable syngeneic tumor, suggesting a lesion in CMI and/or macrophage function (Dean *et al.*, 1980).

DES probably exerts its immunosuppressive effects via estrogen receptors on lymphoid cells and thymic epithelial cells. The immunosuppressive effects of DES may be mediated through selective depletion or functional impairment of T lymphocytes and/or the induction of suppressor macrophages. The exact relationship between the putative thymic epithelial receptor for DES, DES-induced thymic atrophy, macrophage activation, and T-cell immunosuppression has yet to be clarified.

Abused Drugs. Chronic alcohol abuse in humans has been associated with impaired T-lymphocyte function (Berenyi *et al.*, 1975), myelosuppression, and defective humoral immunity (Gluckman *et al.*, 1977) as well as with a higher and more severe incidence of infections (Tapper, 1980). In studies by Loose *et al.* (1975), the primary, but not secondary, humoral response was reduced in rats chronically dosed with ethanol. In another study, rats chronically fed ethanol exhibited suppressed DTH, thymic and splenic atrophy, and suppressed secondary HI (Tennenbaum *et al.*, 1969).

Naturally occurring cannabinoids, unique to the plant *Cannabis sativa* and constituting 15 percent of the cannabis by weight, are also implicated as immunomodulatory (see review, Holsapple and Munson, 1985). The natural cannabinoids may be subdivided into psychoactive, with Δ9-tetrahydrocannabinol (Δ-9-THC) as the major constituent, and nonpsychoactive, of which there are five known constituents. Both psychoactive and nonpsychoactive cannabinoids have been examined to characterize their immunosuppressive properties, and several studies have shown that they suppress both humoral and cell-mediated immunity in experimental animals (for review, *see* Munson and Fehr, 1983).

The effective dose for 50 percent suppression (ED50) of the antibody plaque response to SRBC in mice was 70, 14, 13, and 8 mg/kg for Δ-9-THC, Δ-8-THC, 1-methyl-Δ8-THC (nonpsychoactive), and abnormal Δ-8-THC (nonpsychoactive), respectively (Smith *et al.*, 1978). In the same studies, at a dose of 100 mg/kg the cannabinoids suppressed the DTH response 35 to 64 percent. Studies in humans have been less conclusive, although Δ-9-THC has been found to suppress CMI, but not humoral immunity (*see* Munson and Fehr, 1983). Nonpsychoactive cannabinoids have also been synthesized in attempts to develop novel immunosuppressants (Smith *et al.*, 1978).

FUTURE DIRECTIONS

The application of the discipline of immunology to the toxicologic assessment of drugs and chemicals is progressing rapidly, and developmental, methods selection, and validation stages are nearly complete. The preceding few years of research have provided new models; data on correlations of immune function and host resistance; a better understanding of the biologic relevance of certain immune function parameters; and a better standardized panel of methods for immunotoxicity assessment. Future research is needed to develop and refine relevant host resistance models; to evaluate *in vitro* methods using microsomal activation systems as screens for detecting chemically induced immunotoxicity; to determine the distribution and function of specific receptors for chemicals; to develop better methods for evaluating chemical hypersensitivity and autoimmunity; and to develop immunologic data on humans occupationally or environmentally exposed to chemicals shown to be immunotoxic in laboratory animals.

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