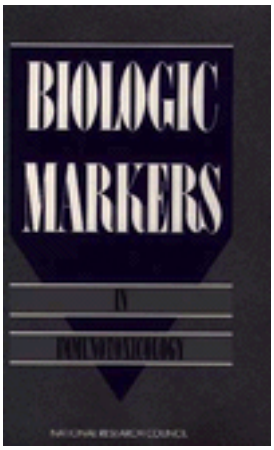

Biologic Markers in Immunotoxicology

Subcommittee on Immunotoxicology, Committee on Biologic Markers, Board on Environmental Studies and Toxicology, National Research Council

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Subcommittee on Immunotoxicology
Committee on Biologic Markers
Board on Environmental Studies and Toxicology
Commission on Life Sciences
National Research Council

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Preface

The American people have become increasingly aware of the potential for exposure to toxic material in our environment and of a need for accurate, objective information on the health effects of pollutants. In keeping with that need, the Agency for Toxic Substances and Disease Registry of the U.S. Public Health Service, the Office of Health Research of the U.S. Environmental Protection Agency, the National Institute of Environmental Health Sciences, and the National Institute of Allergy and Infectious Disease requested the Board on Environmental Studies and Toxicology in the National Research Council's Commission on Life Sciences to examine the potential for use of biologic markers in environmental health research. The term "biologic markers" has been used by the National Research Council's Committee on Biologic Markers to refer to indicators of events in biologic systems or samples. It is useful to classify biologic markers into three types—markers of exposure, of effect, and of susceptibility—and to describe the events peculiar to each type.

The Committee on Biologic Markers was organized to consider the areas of environmental research in which the use of biologic markers offered the greatest potential for major contributions. Four biologic systems were chosen: the reproductive system, the respiratory system, the immune system, and the urinary system. A companion report, *Environmental Neurotoxicology*, emphasizes biologic markers for the nervous system. This report is the product of the Subcommittee on Immunotoxicology, which included clinicians, epidemiologists, toxicologists, pathologists, and biochemists. Our intent was to consider various kinds of basic research that might reveal markers of environmental exposure and disease, even if the original goal of the research had nothing to do with such markers. Eventually, the subcommittee decided to place major emphasis on biologic markers of three types: markers originating from the immune system, markers related to immunosuppressive toxicants of exposure, and markers of effects of environmental pollutants. Markers of susceptibility to environmental materials also were considered important and were included especially if they were of a genetic nature and could serve to identify individuals who are susceptible to autoimmune diseases.

The subcommittee decided to organize this report according to types of action on the

immune system (hypersensitivity or suppression), rather than according to specific pollutants, on the grounds that it is more important to discuss general approaches than to attempt to compile a list of pollutant-specific markers.

In the course of the subcommittee's deliberations, several additional scientists were called on to provide information. The subcommittee especially wishes to recognize the contributions of Gary Burleson of the U.S. Environmental Protection Agency.

This report could not have been produced without the untiring efforts of the National Research Council staff, especially Robert P. Beliles, the program officer; Joyce Walz, the project assistant; Danielle Corriveau, the administrative secretary; Tania Williams, who prepared the camera copy; Kate Kelly and Norman Grossblatt, the editors of the report; Devra Davis, resident scholar; and Richard D. Thomas, associate director, and James J. Reisa, director of the Board on Environmental Studies and Toxicology.

David Talmage, Chairman

Subcommittee on Immunotoxicology

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List of Abbreviations

ADCC	Antibody-dependent cell-mediated cytotoxicity
ACGIH	American Conference of Governmental Industrial Hygienists
ACTH	Adrenal cortical trophic hormone
AIDS	Acquired immune deficiency syndrome
ATSDR	Agency for Toxic Substances and Disease Registry
BALF	Bronchoalveolar lavage fluid
B-cell system	Humoral immune system
B16F10	Mouse tumor cell model (melanoma)
C_1-C_9	Complement system components
cAMP	Cyclic adenosine monophosphate
CD	Cluster of differentiation, e.g. CD3
CD3	T-cell surface marker associated with the T-cell receptor for antigen
CD4	T-cell surface marker identifying the helper (or inducer) subset of T cells
CD8	T-cell surface marker identifying the suppressor (or cytotoxic) subset of T cells
CD4:CD8	Helper/suppressor cell (ratio)
CD19	B-cell surface marker
CD20	B-cell surface marker
CD22	B-cell marker present on the membrane of mature B cells and in the cytoplasm of immature B cells
CD25	T-cell surface marker identifying marker for IL-2 receptor
CFU	Colony-forming unit
CFU-B	Colony-forming unit, basophils
CFU-G	Colony-forming unit, granulocytes
CFU-GM	Colony-forming unit, granulocytes and macrophages
CH_{50}	Hemolytic complement
CMI	Cell-mediated immunity
$C1_q$	Subunit of first component of complement
Con A	Concanavalin A
CsA	Cyclosporin A

CSF	Colony-stimulating factor
CTL	Cytotoxic T lymphocyte
DT	Diphtheria-tetanus (vaccine)
DPT	Diphtheria-pertussis-tetanus (vaccine)
DTH	Delayed-type hypersensitivity
EAE	Experimental allergic encephalomyelitis
EBV	Epstein-Barr virus
EI	Environmental illness
ELISA	Enzyme-linked immunosorbent assay
Fc	Fragment cystalline- The fragment of an antibody that is responsible for binding to antibody receptors on cells and the $C1_q$ component of complement
FEV-1	Forced expiratory volume in one second
Gm	Gammaglobulin
GM-CSF	Granulocyte-macrophage colony-stimulating factor
Gmfb	Gammaglobulin allotype
GVH	Graft versus host
HAH	Halogenated aromatic hydrocarbon
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HLA-B27	HLA associated with ankylosing spondylitis
HLA-Rw4	HLA associated with *Pemphigus vulgaris*
HSA	Human serum albumin
IFN	Interferon
Ia	Murine class II major histocompatibility complex antigen
Ig	Immunoglobulin class; A, D, E, G, M
IL-1 -IL-8	Interleukin, one through eight
IU	International unit
K562	Sensitive cell target for NK cell assay (leukemic cell line)
kg	Kilogram
KLH	Keyhole limpet hemocyanin
LAK	Lymphokine-activated killer (cells)
LALN	Lung-associated lymph nodes
LPS	Lipopolysaccharide, a B-cell-specific mitogen
MCMV	Murine cytomegalovirus
MCS	Multiple chemical sensitivity
MDI	Methylene diphenyl diisocyanate
mg	Milligram
MHC	Major histocompatibility complex
MLC	Mixed-lymphocyte culture
MLR	Mixed-leukocyte response
mm^3	Cubic millimeter
NK	Natural killer cell
PAF	Platelet activating factor
PAH	Polycylic aromatic hydrocarbon
PBB	Polybrominated biphenyl
PCB	Polychlorinated biphenyl
PFC	Plaque-forming cell

PHA	Phytohemagglutinin, T-cell mitogen
PHSC	Pluripotent hematopoietic stem cell
PPD	Purified protein derivative
ppm	Parts per million
PRP	Polyribose phosphate
PWM	Pokeweed mitogen, a T-and B-cell mitogen
PYB6	Mouse tumor cell model (fibrosarcoma)
RBC	Red blood cells
SAC	*Staphylococcus aureus* Cowen strain activator
SBS	Sick building syndrome
SCID	Severe combined immunodeficiency
SLE	Systemic lupus erythematosus
SRBC	Sheep red blood cell
T-cell system	Cellular immune system
TEAM	Total Exposure Assessment Methodology study
Thy-1	T-cell marker related to thymic maturation
VOC	Volatile organic compound
YAC-1	Mouse tumor cell model (lymphoma) used to test NK activity

Summary

The field of immunology has progressed rapidly over the past decade, as demonstrated by the rapid elucidation of the mechanisms of acquired immune deficiency syndrome (AIDS). In the general population, increasing numbers of people suffer from disorders of the immune system, such as allergies, asthma, and AIDS. The incidence of asthma has increased 58% since 1970, and it is well known that nitrogen dioxide and ozone, common air pollutants, interact with allergens to increase the frequency and severity of asthma attacks. Some prolonged periods of air pollution have caused deaths among asthmatics in the United States and abroad. Some drugs have been linked with the induction of autoimmune diseases, in which the body's immune system destroys its other tissues. People living near chemical waste sites have complained of symptoms that might be related to immune dysregulation. Experimental animal studies suggest that mixtures of chemical pollutants found in groundwater can produce suppression of the immune system. Recently, the U.S. Office of Technology Assessment issued a background paper on the identification and control of immunotoxic substances.

With the sponsorship of the U.S. Environmental Protection Agency (EPA), the National Institute of Environmental Health Sciences (NIEHS), and the Agency for Toxic Substances and Disease Registry (ATSDR), the National Research Council's Committee on Biologic Markers undertook to study the interrelationship of toxic exposure and immune-system response. The Subcommittee on Immunotoxicology, comprising scientists with diverse backgrounds in and knowledge of immunology, toxicology, immunotoxicology, risk analysis, and other disciplines, prepared this document. As two previous subcommittees on reproductive and developmental toxicity and pulmonary toxicity had done, the Subcommittee on Immunotoxicology reviewed research on currently known markers, and it identified and evaluated promising new technologies to find new markers, important research opportunities in the field, and areas where interdisciplinary research in environmental health is needed. Although the discipline of immunotoxicology is relatively young, considerable progress has been made in demonstrating that some xenobiotic substances can modulate immunity and that, in some instances, the immune system is a primary target of these materials. Progress has been slow, however, and more

information is needed about how xenobiotics affect immune-system function and human health.

The immune system is a complex intra-and interregulated mechanism. Immunocytes, directed by their receptors and secretory products, act as a network. Although the immune system often is considered autonomous in regulation and action, there is strong evidence that a significant reciprocal interaction among the nervous, endocrine, and immune systems maintains homeostasis. Interregulatory patterns confirm the existence of a nervous-endocrine-immune axis, which complicates attempts to study and model whole-body responses in vitro. Although sophisticated in vitro systems have specific applications, the use of intact animal systems is essential for accurate investigation of the immunotoxic potential of xenobiotics.

This document presents a brief history and review of immunology, immunotoxicology, and biologic markers (Chapters 1 and 2). The effects of toxicants on the immune system can be expressed in two ways. Excessive stimulation can result in hypersensitivity or autoimmunity; suppression can result in the increased susceptibility of the host to infectious and neoplastic agents. In addition, the immune system is affected by genetics, age, and life style, factors that often make it difficult to interpret research results. Tests for an agent's potential to induce hypersensitivity are widely used in the cosmetics industry and in development of some other consumer products.

There has been only limited application of immunotoxicology in the management of risks to human health from xenobiotic substances and in occupational medical surveillance to determine increased risk as a result of reduced immune-system competence. Knowledge of immunotoxicity is not often applied in the management of risk from dietary or environmental exposures.

The committee recommends that educational programs be developed to inform the public of risks from exposure to immunotoxic agents and to develop expertise among environmental health researchers in immunotoxicology.

HYPERSENSITIVITY

Hypersensitivity (Chapter 3) has become an important human health problem in industrialized societies. Inhalation of a variety of chemicals can cause asthma, rhinitis, pneumonitis, or chronic granulomatous pulmonary disorders. Hypersensitivity is an immunologically based host response to a compound or its metabolic products. This is distinguished from multiple chemical sensitivity (MCS) syndrome, which has not been shown to have an immunologic basis. Hypersensitivity reactions are frequently influenced by heredity. Immunoglobulin E is an important mediator and biologic marker of hypersensitivity. Procedures are available to assess hypersensitivity in animals and humans.

The committee recommends that research be devoted to testing the analytic accuracy of biologic markers discussed in this report to facilitate their use in predicting adverse health effects and in identifying causative agents. Markers of immunotoxicity could be useful in epidemiology to confirm causal relationships between environmental exposures and prevalence of hypersensitivity.

AUTOIMMUNITY

Autoimmune disease occurs when an immune system attacks the body's own tissues or organs, resulting in functional impairment, inflammation, and occasionally, permanent tissue damage (Chapter 4). Some xenobiotics are known to induce autoimmunity, but there is little information about the relationship of autoimmunity to environmental exposure, and only a few

animal models have been developed to study autoimmune diseases. This lack of information and the strong genetic factors of autoimmunity make it difficult to decipher the relationship between exposure and autoimmune diseases.

The committee recommends the establishment of a national registry for autoimmune diseases to determine the prevalence of these diseases. The use of genetic and immune-system markers to identify persons who are susceptible to autoimmune diseases should be explored. Animal models of autoimmunity suitable for testing the induction of these diseases by chemicals are required for further research. The surveillance of pharmaceutical workers who are exposed to drugs that induce autoimmunity offers a good opportunity to advance knowledge about the possible induction of autoimmune diseases by chemicals.

IMMUNE SUPPRESSION

The immune system provides protection against invasion by pathogens and the growth of neoplastic cells. Exposure to some drugs and chemicals can impair this natural host defense mechanism, and this can lead to an increased incidence of infectious disease or cancer (Chapter 5). Several xenobiotics have been identified as causing immune-system dysfunction. In some cases, the immune system has been identified as the most sensitive target for the minimum toxic dose of a xenobiotic. Although one or more of the many compartments of the immune system can be suppressed significantly, this suppression might not be expressed as an immune-mediated disease. Rather, suppression can be viewed as a potential risk because of the reduced ability of the host to resist natural and acquired diseases. There is limited information to suggest that humans exposed to environmental pollutants are immunologically compromised. However, it has been well established that treatment of humans with immunosuppressive therapeutic agents can result in an increased incidence of infectious disease and neoplasia. It is universally accepted that the immune systems of many animals and humans are comparable; that animal models are available to assess immune dysfunction objectively; that positive immunosuppressants, such as cyclophosphamide and cyclosporin A, are used to validate assays; and that data obtained from animal studies can sometimes be verified in humans. For immunosuppressants, the plasma concentration of an agent is an adequate marker of exposure that also serves as the effective biologic dose. Markers of effect suggesting changes in the immune system are indicated by alterations in subpopulations of cell type, such as the helper-to-suppressor cell ratio. Although the principles and phenomena in humans and animals are basically similar and comparable, it is recognized that different responses can occur.

The committee recommends that occupational exposures and accidental exposures to high concentrations of immunotoxicants be studied by research teams familiar with techniques used in immunotoxicology. Research that uses animal models should quantify the effects of factors such as age, stress, malnutrition, and pregnancy on the induction of immune-system suppression. The degree of suppression necessary to produce an increase in disease needs to be determined. Mechanistic and metabolic information on immunosuppressants require further exploration to reduce the uncertainties in the assessment of risk of immunosuppressive chemicals.

BIOASSAYS OF IMMUNOTOXICITY

Animal bioassays for toxicity (Chapter 6) are useful for identifying possible hazards that could attend human exposure to xenobiotics. Researchers have used animal models

to identify immunotoxic agents, to develop immune-system profiles, to identify mechanisms of action, and to identify potential health risks associated with exposure to specific xenobiotics, either consumed as drugs or through environmental exposure. The results of animal studies are useful for determining chemical hazards, managing risk, and determining relatively safe conditions of exposure. A series of animal bioassays has been developed to detect changes in the immune system caused by low oral doses of immunosuppressants. These bioassays give consistent results in different laboratories. Assays for pulmonary immunocompetence have been developed but require broader use. There is a need for additional mechanistic studies, particularly those that relate the immune system to the development of cancer.

The committee recommends that biologic markers be identified in humans and animals to allow detection of potentially dangerous changes in local immune function in lung and skin tissues, because these tissues are often the tissues of first contact. Studies of the interaction of the immune system and ultraviolet light might be particularly rewarding.

CLINICAL APPLICATION OF EXISTING IMMUNOTOXICOLOGIC BIOLOGIC MARKERS

The use of animal data, coupled with information gained from limited human clinical and epidemiologic studies, has been valuable in human risk assessment and hazard evaluation. Several tests assess humoral and cellular immunity, as well as nonspecific resistance in humans (Chapter 7). Some of these procedures parallel those used in animal studies and require prospective evaluation in exposed populations and in control groups to ascertain the tests' usefulness.

The committee recommends a series of tests to assess immune-system competence in persons who have been exposed to known or suspected immunotoxicants. An aggressive approach will permit use of sensitive procedures for detection of chemically induced modulation of the human immune system. The use of case studies arising from such a program could be useful in identifying xenobiotics that are likely to cause damage to human health and a reduced ability to function normally.

ROLE OF BIOLOGIC MARKERS OF IMMUNOTOXICITY IN EPIDEMIOLOGY

The limits on experimentation in humans restrict the use of epidemiologic methods to obtain health information after accidental or occupational exposure to toxic substances. Epidemiologic research (Chapter 8) can involve experimental studies in which conditions are controlled and effects are subsequently observed in a test population, or it can use cohorts or cases in which the test population is observed without the circumstances being altered. Epidemiologic procedures frequently permit long-term monitoring of health effects in large numbers of persons exposed to undefined quantities of a given environmental xenobiotic. Data obtained in such investigations, which cannot be obtained otherwise for normal human populations, can provide information about immunotoxic effects. However, a review of the literature reveals no epidemiologic studies that have made full use of markers of exposure, markers of adverse immunologic effect, or markers indicating susceptibility because of variation in the capacity of the immune system.

The committee recommends that greater use be made of markers of exposure, effect, and susceptibility in epidemiologic studies to identify the influence of the immune system on diseases.

INDOOR AIR POLLUTION AND MULTIPLE CHEMICAL SENSITIVITY

Considerable public concern has arisen regarding sick building syndrome (SBS), a condition that causes discomfort brought on by mucous membrane irritation in a substantial number of occupants of air-tight buildings (Chapter 9). The resulting symptoms could be associated with exposure to irritants, chemical or biologic allergens, or mixtures of chemical pollutants, including volatile organic compounds.

The committee recommends that studies be conducted on potential methods and guidelines for reducing harmful exposures and adverse consequences of indoor air pollution in homes, schools, and the workplace.

It is well known that some individuals experience skin or pulmonary disorders in reaction to contact allergens. However, although it has been suggested, it is not known whether the immune system in a given individual is uniquely sensitive to modulation by several chemicals. Current evidence does not indicate that multiple chemical sensitivity (MCS) originates in or involves the immune system. Many confounding factors could be involved in the etiology of such conditions and, should the immune system be involved, it could be through secondary or indirect responses to conditions rather than as a directly contributing factor in the etiology of MCS.

A workshop held in the spring of 1991 aided the committee in its deliberations on the problems associated with MCS; the committee considered the proceedings of the workshop in completing this report. The proceedings of the workshop will be published under separate cover.

The committee recommends that epidemiologic research focus on the prevalence of MCS. Patients with MCS could be studied under controlled environmental conditions. A multidisciplinary team of experts in lung physiology, immunotoxicology, clinical immunology, psychiatry, toxicology, occupational medicine, and industrial hygiene is required in the evaluation of these cases. A standard comprehensive panel of clinical procedures should be applied to aid in diagnosis. Biologic markers could be useful in confirming or eliminating immune-system dysfunction as a cause of MCS.

Biologic Makers in Immunotoxicology

1

Introduction

At the request of the U.S. Environmental Protection Agency (EPA), the National Institute of Environmental Health Sciences (NIEHS), and the Agency of Toxic Substances and Disease Registry (ATSDR), the Board on Environmental Studies and Toxicology in the National Research Council's Commission on Life Sciences convened the Committee on Biologic Markers to examine the use of biologic markers in environmental health research. Biologic markers are broadly defined as indicators of events in biologic systems; they can be variations in the number, structure, or function of cellular or biochemical components. Biologic markers are of interest as a means to identify early stages of disease and to understand the basic mechanisms of the effects of exposure and the biologic responses to substances found in the environment (Committee on Biological Markers of the National Research Council, 1987). Four specific biologic systems were chosen for study: the reproductive system (NRC, 1989a), the respiratory system (NRC, 1989b), the immune system, and the urinary system. This is the report of the Subcommittee on Immunotoxicology.

The immune system recognizes and defends against infectious micro-organisms and neoplastic cells. Many foreign materials are prevented from entering the body or are rapidly eliminated by nonspecific, nonimmune mechanisms (e.g., mucous secretions and phagocytosis by macrophages) and by immune mechanisms. With some substances, individuals may develop an immune response that is specific to the substance so that the body is able to react more quickly and effectively to a future attack by the substance. This adaptive immune system may be considered in simple terms to consist of three specific elements: the foreign substance, which is called the *antigen; lymphocytes*, which are cells of the blood and lymphoid system; and *antibodies*, the immunoglobulin (Ig) proteins formed by the immune system. Interactions among these three specific elements and other nonspecific cells (e.g., antigen-presenting cells) or other biologic systems (e.g., the immune-complement system) form the basis of the activity of the immune system. A response against an antigen that requires the local accumulation of lymphocytes is termed cell-mediated immunity and the lymphocytes involved are called T cells. Responses involving antibodies made at a distant site are referred to as humoral immunity and the lymphocytes producing the antibodies are called B cells. A generalized reduction in the capacity for

either type of response is known as immunosuppression and may result in an increased susceptibility to infection by micro-organisms or to the development of tumors, as seen, for example, in acquired immune deficiency syndrome (AIDS). A generalized increase in immune responsiveness is known as immunopotentiation. One manifestation is hypersensitivity (allergy). When the immune system responds to and attacks the proteins of its own tissue, autoimmune disease may occur. In Chapter 2, the function of the immune system is given with greater detail along with an explanation for how disease may evolve from disregulation of the immune system.

Immunology is primarily a science that began in the late nineteenth century. Special interest in chemicals from nonbiologic sources—xenobiotics—is of recent origin. Immunotoxicology formally emerged as a distinct discipline within toxicology during the 1970s (Descotes, 1988), prompted by animal studies that demonstrated the researcher's ability to measure the effects of chemicals on the immune system (Koller, 1980; Vos, 1980; Dean et al., 1982; Luster et al., 1982). Landsteiner in the mid-1930's demonstrated that the guinea pig could be used to determine the sensitization potential of chemicals (Landsteiner and Jacobs, 1935, 1936), and these techniques have been used over the years in the determination of the potential to produce hypersensitivity responses, particularly with cosmetics ingredients, consumer products, and drugs. The "new" field was fully recognized in 1978 when an immunotoxicology program was established within the National Toxicology Program. The goal of the immunotoxicology program was to select, develop, and validate animal models for use in assessing immunomodulation induced by xenobiotic substances. The initial focus of investigations was the potential of nonbiologic chemicals to cause immunosuppression. More recently, active sensitization and hypersensitivity reactions to xenobiotics have received attention.

Immune responses are many and varied, and they produce markers that can indicate environmental exposure. Such markers include increases in specific antibodies, increases or decreases in total immunoglobulin, changes in the absolute or relative numbers of lymphocytes, and changes in the in vitro or in vivo reaction to antigens or mitogens. It can be difficult to distinguish responses that indicate toxicity, and are thus markers of effect, from those that are normal physiologic adaptations to the environment.

Continued integration of the work of toxicologists, pharmacologists, and other researchers has led to the use of a diversity of biologic markers. For this study, the committee, for the most part, deals with the potential of xenobiotics to produce an adverse effect on the immune system manifested by hypersensitivity, autoimmunity, or suppression of the immune system. The suppression of the immune system can lead to the development of an increased rate of infection or cancer.

Humans are often and unavoidably exposed to many hazardous environmental chemicals. The immune system functions to neutralize foreign materials and infectious agents by specifically responding to varied macromolecular factors in the environment. In addition to its response to molecules derived from bacteria and viruses, the immune system may respond to varied macromolecular components in ingested food, inhaled air, and anything that touches the skin. the responses of the immune system usually are beneficial, although some can be harmful, either because of an over-response that leads to hypersensitivity or autoimmunity or because of a suppression of the immune system that impairs the response to other environmental stimuli.

Immune responses can be specific or nonspecific. Specific responses usually involve a small fraction of highly specific lymphocytes, and the degree of response to different antigens can vary widely within an

individual. Animal experiments can demonstrate which substances are potential antigens in humans. However, animal results do not always predict the occurrence of hypersensitivity reactions in humans. For example, exposure to ragweed pollen induces a normal antibody response in mice, but it can cause severe allergy in some humans.

Many persons are afflicted with diseases, such as skin allergies and asthma, that are related to immune hypersensitivity. Immunologic (allergic) asthma is defined as variable airway obstruction that results from exposure to generally low-level concentrations of immune-reactive substances in the environment. Skin-sensitizing chemicals often are found in the industrial workplace, and 20-25% of all cases of occupational dermatitis are estimated to result from immune sensitization. Between 50,000 and 100,000 workers in the United States are regularly exposed to highly reactive compounds, such as diisocynates (Musk et al., 1988); many individuals will develop antigenspecific antibodies to toluene diisocyanate (TDI) after respiratory exposure. Five to ten percent of exposed workers have become hypersensitive to TDI. In such situations, the presence of specific antibody is a definitive marker of *exposure*, although not all workers hypersensitive to TDI have detectable TDI antibodies, nor do all workers with antibodies develop symptoms of hypersensitivity.

The Spanish toxic oil syndrome developed in approximately 20,000 people in Madrid in 1981 after they had ingested adulterated rapeseed cooking oil. Affected persons had symptoms of fever, rash, dyspnea, malaise, and gastrointestinal symptoms. Most made an uneventful recovery, but approximately 15% developed scleroderma-like illness that was suggestive of autoimmune disease.

Nonspecific effects of xenobiotics on the immune system can involve a variety of cells and similar actions are observed between individual and species. For example, exposure to whole-body radiation or cyclosporin A depresses the immune systems of mice and humans; each modality affects different cells, but they act in a similar immunosuppressive manner in both species.

Organ-transplant patients are treated with drugs that suppress the immune response to prevent organ rejection, but because their immune system is deficient, these patients frequently develop infections and some types of neoplasm. The relationship between the immune system and the development of cancer has been recognized for years. In adopting standards on 13 occupational carcinogens in 1974, the Occupational Safety and Health Administration required that "in all physical examinations, the examining physician shall consider whether there exist conditions of increased risk including reduced immunological competence. …"

Recent animal immunotoxicity studies suggest that some environmental substances induce immune-system suppression. Trichloroethylene (TCE) in the drinking water of mice has been found to suppress humoral and cell-mediated immunity. Neither the period of TCE exposure nor dose-response correlations have been established in human studies, but leukemia and increased infections have developed in some populations exposed to TCE as a result of contaminants in their drinking water. The Agency for Toxic Substances and Disease Registry (ATSDR) has recently established a registry of individuals exposed to TCE, which eventually could be helpful in establishing a relationship between the animal bioassay results and presumptive human health effects of TCE (Burg, 1990). In addition, registries for benzene and dioxin have also been established. These chemicals also suppress the immune system as part of their spectrum of toxicity.

BIOLOGIC MARKERS

In the broadest sense, biologic markers are measurements on biologic specimens

that will elucidate the relationship between environmental exposures and human diseases, so that such exposures and diseases can be prevented. Early detection that leads to prevention of disease and of disability is the ultimate goal and promise of the use of biologic markers.

The term "marker" is commonly used by immunologists for membrane proteins that "mark" different kinds of cells. Interest is growing in the use of biologic markers by researchers in clinical medicine, epidemiology, toxicology, and related biomedical fields to study the health effects of exposure to environmental toxicants. Clinicians can use markers for early detection of disease. Epidemiologists can use them as indicators of exposure to determine internal dose or health effects. Toxicologists can use them to develop estimates of dose-response relationships and to facilitate assessment of risk associated with small exposures. Biologic markers also can be helpful in clarifying the underlying mechanisms of chemically induced diseases. Recently, selected immune-system markers have been measured in epidemiologic studies of exposed populations to detect associations between exposure and disease. However, the complexity of the test populations and uncertainties in the measurement of markers of immunotoxicity have prevented meaningful evaluation.

New developments in molecular biology and biochemical approaches to medicine have elucidated sensitive markers for assessing exposure (NRC, 1991). They have also increased our knowledge of disease, improved our ability to predict the outcome of disease, and helped direct courses of treatment. There has been an explosive expansion in interest and increased activity in immunologic markers because of AIDS. Many diseases are defined not only by clinical signs and symptoms but also by the assessment of biologic markers at the subcellular and molecular levels. Diseases of the liver and kidney, for example, are often detected by measurement of enzymes in blood or proteins in urine; diabetes can be suspected if glucose is found in urine; and inborn errors of metabolism, such as phenylketonuria, are found by early biochemical analysis, rather than later as a result of clinical dysfunction.

The Committee on Biologic Markers of the National Research Council (1987) defined biologic markers as indicators of events or conditions in biologic systems or samples. As such, they are indicators of *exposure, effect*, or *susceptibility*. This classification is a useful theoretical scheme by which to characterize biologic markers of any organ system; however, it must be qualified somewhat for practical application. First, classification with respect to any of the three categories will depend on the particular definition of that event. Second, the three categories often are related and can be seen as descriptors of a continuum from environmental exposure to clinical disease (Figure 1-1).

The measurement of a chemical in a biologic specimen is a marker of exposure, and it could be more useful than measurements

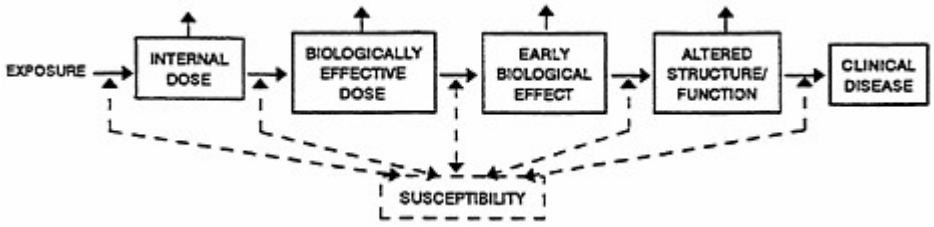

FIGURE 1-1 Simplified Flow Chart of Classes of Biologic Markers.

on air samples at a potential exposure site. Cellular or molecular changes associated with disease are biologic markers of adverse health effects. Biologic markers often are elucidated by clinical laboratory measurements or clinical tests used in the differential diagnosis of various diseases. The markers can serve as surrogates for other methods of detection in determining the molecular and cellular events in the development of health problems. If such markers could be detected before exposed persons became obviously ill, the disease process might be reversed in those affected or prevented in others. In addition, some cellular or molecular measurements can identify people who are more vulnerable to the effects of toxic exposure; these are markers of susceptibility. There also might be biologic markers that would indicate individual susceptibility to environmentally induced disease.

Markers of Exposure

A biologic marker of *exposure* is a xenobiotic chemical or its metabolite or the product of an interaction between the chemical and some target cell or biomolecule. Most commonly, the indicators of exposure are the concentration of the material in urine, blood, or other body tissue, including the hair or nails. The most definitive immune-system markers of exposure are antigen-specific antibodies or cellular responses to a particular xenobiotic. Immune-specific biologic markers of exposure include antibodies to toxicants (Pezzini et al., 1984), in vitro proliferative responses of immune cells upon exposure to toxicants or toxicants conjugated to proteins (Kapsenberg et al., 1988), and in vivo responses (e.g., as shown by skin tests) to toxicants (Baur et al., 1984).

Several problems limit the usefulness of antigen-specific markers of exposure. First, many environmental xenobiotics are small molecules that must act as haptens to elicit an immune response. Haptens alone cannot evoke an immune response; they must be covalently linked or strongly bound to "carrier" proteins in the tissues. For instance, isocyanates react chemically with tissue proteins, and nickel forms very strong tissue chelates. Antigen-specific immune markers can be detected for both of these agents. However, many environmental toxicants do not trigger antigen-recognition pathways and will not produce specific immune markers.

Even if a xenobiotic can elicit a specific immune-system response, the dose and route of exposure must be appropriate. Very small or very large doses of a substance can induce tolerance and even paralyze the immune system, ablating any indicators of exposure. The route of administration also can be critical.

A third problem with specific immune-system markers of exposure is that they decay with time after exposure ceases. This decay of responsiveness is the reason re-immunization is often required for maximum protection. A false-negative result can therefore be obtained if specific immune markers are sought long after exposure. The persistence of immune markers varies widely among the different humoral mediators and cellular components, and it can depend on conditions within the organism. Highly reactive humoral mediators that act locally (such as prostaglandin) are often inactivated within minutes of their formation, even though their inactivation products can circulate in the system much longer. Serum IgE is cleared more quickly than is serum IgG, but IgE bound to mast cells or basophils persists much longer than any serum protein does.

Markers of Effect

A marker of *effect* is a measurable cellular or biochemical alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease. A marker of

susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance.

Immunologic markers of effect include changes in the components of the immune system itself (such as shifts in the distribution of lymphocyte subpopulations) and changes in other tissues caused by immune-mediated dysfunction (such as signs of kidney failure caused by autoimmune kidney disease). As with all organ systems, the most critical aspect of defining immune-system markers of effect is to define the criteria for determining whether the effects have occurred (Radford, 1981). This point is especially important because changes in the immune system can reflect an extremely broad spectrum of biologic effects ranging from normal variation to sudden death (Table 1-1). Therefore, immune-system markers of effect must be defined in terms of the specific health effects for which they might serve as indicators.

When there are clinically apparent health effects caused by immune-system dysfunction, changes in immune-system markers are often evident and serve as helpful indicators of pathogenesis. Biologic markers of effect are usually less obvious during the inactive phases of immune-mediated disease. If immune-related effects occur below the clinical disease level, the altered state of the immune system cannot necessarily be "recognized as impairment or disease" (Committee on Biological Markers of the National Research Council, 1987). However, because of immune "memory," a silent effect can still have disease potential. Ultimately, the usefulness of immunologic markers of effect will be determined by the extent to which they indicate or predict actual disease. The greater the correlation between an immunologic marker change and an eventual problem with host resistance, hypersensitivity, or autoimmunity, the greater the value of the marker in determining public-health priorities.

Markers of Susceptibility

Susceptibility factors, also called effect modifiers, can act at any point along the exposure-disease continuum (Figure 1-1), and in some cases, these factors cause uniformly

TABLE 1-1 Examples of Health Effects Associated with Immune Dysfunction

Effect	Hypersensitivity	Immunosuppression
Death	Lethal allergic reaction	Overwhelming sepsis
Overt disease	Chronic asthma	Frequent recurring infections, some cancers
Subclinical disease	Early chronic beryllium lung disease	Increased susceptibility to infection
Subclinical dysfunction	IgE hyperproduction	Complement C5 deficiency
Nonspecific complaints	Arthritis, headache, fatigue	Recurrent minor infections, longer infection duration
Years potential life lost	Contributions from all above	Contributions from all above

exposed populations to exhibit markedly different effects (Hulka and Wilcosky, 1988). However, in other cases, it can be difficult to distinguish between susceptible and nonsusceptible individuals, either because of multiple interactive influences and genetic polymorphisms or because of difficulties with measuring susceptibility factors.

Potential sources of normal variability in markers of immunotoxicity can be the result of genetic, environmental, or biorhythmic influences other than the specified events that are the object of study. Such influences can cause differences between populations, differences between individuals, and differences within individuals over time. Three of the most important sources of variability are genetic, age-related, and neuropsychologic factors.

Some genes code for the antigen recognition portions of antibodies and T-cell receptors, while other genes code for the type of antibody produced (such as IgG or IgE). In some cases, susceptibility genes code for proteins involved with other activities not directly related to immune-system function. For instance, the probability of developing autoimmune illness from exposure to procainamide is influenced by the genetically determined rate at which it is acetylated (Reidenberg and Drayer, 1986). Women are predisposed to autoimmune disease (Bias et al., 1986). The activation of some oncogenes (Haluska et al., 1986; Nowell and Croce, 1988) also could signal an increased susceptibility, especially toward immunoproliferative disorders (lymphocytic leukemias and lymphomas). Confounding factors can be controlled in epidemiologic study designs by restriction or matching and in analyses by stratification or multivariate methods. When genetic factors confound, the choice of inheritance models is important for interpreting results.

Immune function and markers of the immune system vary normally with age, and differences are especially notable in the very young and the elderly. In general, the variation of many immunologic factors, which is relatively small in young populations, increases with age (Hausman and Weksler, 1985); hence, there can be a wide range of variability in older populations. The developing fetus is unable to recognize and react to a wide range of foreign substances, and it is more susceptible to long-term immunotoxic effects than is the adult (Lewis et al., 1978; Bick, 1985; Hausman and Weksler, 1985).

Pregnancy produces profound changes that can have specific and nonspecific effects on the immune system. The influence of pregnancy on many immune-system markers is not well established, so interpretation of results from pregnant women can be difficult. The nonspecific effects of pregnancy on the immune system include those mediated by hormones such as estrogen, which is largely immunosuppressive. For instance, women with autoimmune inflammatory diseases such as lupus often undergo a profound remission of symptoms while pregnant, although the underlying disease often reappears shortly after the birth (Jansson et al., 1987). The specific effects of pregnancy involve the maternal immune response to paternally derived antigens of the fetus and placenta. Placental antigens (mostly from the major histocompatibility complexes) can evoke antibody, cellular, and morphologic responses by the maternal immune system (Gill, 1985). Humoral responses to antigens of the human fetoplacental unit include antibody to antigens of the ABO and Rh blood groups. Immunoglobulin G levels appear to decrease during gestation and could be inversely correlated with α-fetoprotein levels (Wajner et al., 1987). Some of the results reported for peripheral blood leukocyte and lymphocyte subset counts during different stages of pregnancy have been contradictory, but a decrease in peripheral blood lymphocytes in early pregnancy has been observed consistently (Siegel and Gleicher, 1981; Valdimarsson et al., 1983; Degenne et al., 1988; Iwatani et al., 1988; Castilla et al., 1989). A

relationship between reduced lymphocyte counts and normal birthweight has been suggested (Milns and Gardner, 1989). Pregnancy-related changes in mitogenicity also have been reported (Yoshida et al., 1989). Cytotoxic antibodies and lymphocytes to human leukocyte antigen (HLA) increase in prevalence as pregnancy progresses. The antibody response is detectable in the first month of gestation during the first pregnancy, and by the second month in subsequent pregnancies. The cell-mediated response begins at about 14 weeks of gestation and increases rapidly thereafter (Gill, 1985). Transplacental sensitization of the fetus also can occur by passage of molecules and cells from the mother. This transfer would be important in studies of cord blood, fetal tissues, or neonates.

Involution of the thymus gland, the site of T-cell lymphocyte maturation, and the decline of serum thymic hormone activity are changes of immune function found in all persons as they age (Hausman and Weksler, 1985). Results of T-cell changes in the elderly are contradictory, some showing small changes in total cell counts and their distributions among subclasses and others showing no differences (Antonaci et al., 1987). The total number of B cells in peripheral blood does not appear to change significantly with age (Antonaci et al., 1987). Enzyme activity in lymphocytes can change with age (Weksler and Hütteroth, 1974). Small, but statistically significant, changes in the concentration of immunoglobulins are found in serum of older persons compared with those who are younger. The concentration of IgA and IgG in human serum increases in older blood donors; serum IgM decreases (Buckley and Dorsey, 1970).

Older people have an increased frequency of autoantibodies to nucleic acids, smooth muscle, mitochondria, lymphocytes, gastric parietal cells, immunoglobulin, and thyroglobulin (Pandey et al., 1979; Hausman and Weksler, 1985). These antibodies might contribute to the deregulation of the immune system that accompanies aging. It is important in evaluating age-related immunotoxic effects to distinguish between an alteration in the immune response that causes shortened survival and an alteration in the immune response that results from factors that lead to reduced survival.

Neurogenic and psychogenic factors also can influence susceptibility to immune-system dysfunction. Neurogenic factors (for which useful markers are difficult to measure) are probably involved in the pathogenesis of atopic reactions, IgE-mediated hypersensitivity (Greene et al., 1988). Psychoneuroimmunology is a relatively new research area, but the clinical literature suggesting that the immune system can be altered by psychological means is old. Nonspecific effects of neurogenic factors on the immune system are well established, but specific effects are difficult to explain and must be viewed with some skepticism. Ader et al. (1990) recently reviewed the interactions between the brain and the immune system. The interactions are sometimes rather remarkable. For example, Braun (1983) reported that patients with multiple personality disorders can have distinct hypersensitivity-response profiles in each of their different personalities.

Studies in humans have implicated psychological elements in the susceptibility to and recovery from infections, bacterial, allergic, autoimmune, and neoplastic diseases. Stress of various types (loss of a spouse is frequently mentioned) can affect the immune system. Stress involves the endocrine, neurobehavioral, and immune systems. Changes in glucocorticoid levels are frequently cited as a result of this interaction. The influences of stress on the immune system are generally suppressive and can lead to decreases in qualitative and quantitative immune factors (Bartrop et al., 1977; Crary et al., 1983).

Interactions between the nervous system and the immune system can also be demonstrated in experimental animals. For

example, Haloperidol, an antipsychotic drug, reduces antibody responses in normal mice, but restores humoral immunity in mice stressed by crowding (Baranic et al., 1979). The cellular and molecular events that form the basis of this interaction are only now being elucidated. Immunologic cells can have a variety of receptors for hormones, including corticosteroids, insulin, and sex hormones. Conversely, some of the cytokines that transmit signals through the immune system also can affect endocrine and nerve tissues. For instance, interleukin-1 (IL-1) can trigger adrenal cortical trophic hormone (ACTH) and glucocorticoid synthesis by way of the pituitary-adrenal axis (Bateman et al., 1989) and could have direct neurologic effects (Fibbe et al., 1989).

Simon et al. (1990) suggested that association of unpleasant job-related conditions might be the underlying basis of some apparent hypersusceptibility responses in workers. Animal models show responses can be immunosuppressive (Dantzer and Kelley, 1989) or immunoreactive (Koehler, 1985). In experimental animals, immunosuppression can be conditioned by pairing a stimulus with an immune suppressive agent, such as cyclophosphamide phosphamide (Ader and Cohen, 1991). Modification of homeostasis in immune system organs or direct interaction of neurotransmitter such as epinephrine, serotonin endorphins, enkephalins, norepinephrine, and acetylcholine with the cells of the immune system can occur. Receptor sites for some neurotransmitters among subpopulations of lymphocytes have been demonstrated (Bishopric et al., 1980), as have binding sites on other constituents of the immune system (Root-Berstein and Westfall, 1984).

Although neuropsychologic, age-related, and hereditary factors can influence the functional integrity of the immune system, susceptibility to immune disorders also can be influenced by a variety of other conditions and behavioral and environmental factors, and the markers of immunotoxicity can be influenced by a variety of physiologic or pathologic processes, some of which are completely unrelated to toxicant exposure or immune function. These factors include exposure to toxicants acting either directly or indirectly on the immune system, concurrent disease and the associated medications, dietary factors, and even daily or seasonal variations in the exposure to light. Table 1-2

TABLE 1-2 Factors Influencing the Immune System and Associated Markers

Factors	Results
Hormonal, diurnal, seasonal, age	Variability within a healthy individual
Environmental, genetic, neuropsychologic	Variability between healthy individuals
Viral, rickettsial, bacterial, parasitic	Infectious diseases
Immunoproliferative diseases	Myeloma, leukemia, lymphoma
Chronic reactive diseases	Autoimmune diseases
Acute reactive diseases	Allergies, asthma, death (anaphylaxis)
Suppressive disorders	Increased host susceptibilities, death (infection or cancer)

indicates a variety of the factors that influence the immune system and their outcome. The importance of controlling for these factors in any particular study will depend on the study design and the populations involved.

Although previous exposure to an antigen resulting in sensitization is an obvious example of an exposure resulting in an effect on the immune system, atmospheric contaminants can have an effect on such immunologic markers as serum immune complement-inactivation products (Stiller-Winkler et al., 1989). Simultaneous exposure to low levels of two or more air contaminants can result in a synergistic effect on the immune system (Holt and Keast, 1977). Because of the geographic variability of air pollutants, immunotoxic differences should be considered in field studies that compare geographically disparate groups. There is extensive evidence from human and animal studies that exposure to environmental tobacco smoke results in changes in the immune system (Holt and Keast, 1977). Peripheral blood leukocytes from smokers exhibit impaired chemotactic responsiveness, and smokers invariably have increased numbers of all major classes of peripheral lymphocytes (Corre et al., 1971; Holt and Keast, 1977). An association has been demonstrated between smoking and serum IgE levels (Warren et al., 1982). Other immunoglobulins, such as IgA, are decreased in smokers. Smoking also could decrease levels of natural-killer-cell activity (Ferson et al., 1979).

Much of the knowledge of immunotoxicology is derived from evaluations of exposure to therapeutic drugs. Any study of the effects of environmental chemicals on the immune system must entail consideration of the drugs the subjects use. Descotes (1988) has provided a comprehensive review of immunotoxicologic effects of drugs. Almost all categories of therapeutic agents can have some effect on the immune system: antimicrobial and antiparasitic agents; drugs acting on the nervous, cardiovascular, gastrointestinal, and respiratory systems; hormones and hormone antagonists; anti-inflammatory, immunosuppressive, and immunoenhancing drugs; drugs that produce blood clotting and fibrinolysis; and vitamins and miscellaneous drugs. Nonprescription drugs can be important modulators of immune-system markers. Aspirin, for example, can have a wide range of immunologic effects, including possible inhibition of lymphocyte response to mitogens and depression of neutrophil function. Vitamins also are used commonly, and their individual or combined influence on most immune-system markers is not known. Likewise, the influence of oral contraceptives on the immune system is poorly established, although the well-known influence of sex hormones on the immune response should lead to the expectation that oral contraceptives exert similar immunologic effects. Other widely used drugs are generally believed to exert effects on the immune system. Glucocorticosteroids are directly lymphocytolytic. Other anti-inflammatory drugs, such as acetaminophen and ibuprofen, can be expected to exert various immunopharmacologic effects (Descotes, 1988) and could thereby influence immune-system markers. In addition to considering the effect of a single drug on the immune system and its markers, investigators also must be concerned about the effects of multiple-drug use. This area has received little attention and remains a potential source of confounding variables.

Frank malnourishment is unquestionably immunosuppressive and causes a decrease in many immune markers (Chandra, 1987) and in host resistance to infection (Chandra and Wadhwa, 1989). The effects are so pronounced that, theoretically, certain immune-system markers could be used in nutritional assessment; skin-test anergy and decreased peripheral blood lymphocyte counts are considered the most reliable of such markers (Dominioni and Dionigi, 1987). The effects

of marginal malnutrition on immune status are not clear, but T-cell functions might be particularly sensitive (Rogers and Newberne, 1987). Protein deficiency results in decreased IL-I release and in impaired tissue responses to it (Klasing, 1988). Deficiencies of certain amino acids, vitamins, and trace elements also are associated with decreased immune competence (Chandra, 1987). These effects can be compounded by other variables, most commonly by aging (Thompson et al., 1987) and alcoholism (Watson, 1988). Conversely, the excessive intake of polyunsaturated fatty acids, iron, and vitamin E can be immunosuppressive (Chandra, 1987).

Serum concentration of IgE may vary with the time of day. Seasonal variations in light may indirectly influence immune responsiveness by altering antigen exposure—e.g., increased pollen—or indirectly by psychologic mechanisms. Photoallergy is an important component of contact hypersensitivity. Light, particularly in the wavelengths of 315-280 nm (sometimes referred to as UVB), alters immune-system function. Immunologically mediated contact sensitivity reactions to drugs, cosmetics, and soaps occurring in conjunction with sunlight have significantly increased in the past two decades (Harber and Baer, 1972). Guinea pigs are most often used in testing chemicals for their potential to produce immunologically mediated contact photosensitivity.

Leenutaphong et al. (1989) propose two types of solar-induced skin response. The first is IgE-mediated hypersensitivity to specific photoallergens generated only in patients with solar urticaria. The second type is the result of IgE-mediated induction of hypersensitivity to a nonspecific allergen and is generated in patients with solar urticaria and in normal persons.

Schwarz (1988) has demonstrated that UVB exposure leads to local and systemic immunosuppression. Epidermal Langerhans cells are the main target of the local action, and they lose their antigen-presenting capacity after UVB exposure.

Photohypersensitivity is a major concern in immunotoxicology but was beyond the scope of this report. Only a few chemicals have been tested for photoactivation to produce a structure that elicits a contact hypersensitivity response. The role and mechanisms of chemical-induced photosensitization also require further investigation. In addition, because light might have a role as an immunosuppressant, gathering additional information about the role that the immune system might have on development of skin tumors seems warranted.

VALIDITY OF BIOLOGIC MARKERS

In the course of developing this document, the subcommittee noted that various disciplines used validity and sensitivity in different ways. The oversight committee discussed the validation of biologic markers for use in assessments of human health, particularly in epidemiologic studies. To validate the use of a biologic measurement as a marker, it is necessary to understand the relationship between the marker and the event or condition of interest, for example, the potential for health impairment or susceptibility to disease. Sensitivity and specificity are critical components in the process of validation (MacMahon and Pugh, 1970). Sensitivity is the quality of an epidemiologic test method that confers the ability to identify correctly persons who have the disease or condition of interest. A test is specific if it correctly identifies persons who do not have the disease or condition of interest. By extension, the ability of markers of exposure or effect to indicate true exposure or disease (sensitivity) and their ability to indicate lack of exposure or disease (specificity) must be validated. Analytical sensitivity refers to the ability to detect small amounts of the marker. The terms *validated* and *sensitivity* are

used in describing the usefulness of certain bioassays by immunotoxicologists, who speak of a sensitive assay as one that produces the end points indicating an adverse effect on the immune system at low doses. Low doses are those that do not otherwise overwhelm other systems of the body involved with the maintenance of homeostasis. Immunotoxicologists also speak of validated bioassays to indicate those that have been used in several laboratories and provide similar results for compounds that are known to alter immune function. These terms are used in this way in chapters 3 and 6 with regard to the animal tests discussed therein.

Specificity of markers is important in the consideration of analytic validity. For example, urinary concentrations of trichoroethanol and trichloroacetic acid, metabolites of TCE, are used as indicators of exposure, but they are not specific for TCE because they are also formed by the metabolism of perchloroethylene and chloral hydrate. In addition, because of their relatively short half-lives, they must be measured within short times after exposure. In a similar manner, immune system biologic markers of isocynate hypersensitivity may lack some of the specificity that one would normally expect because of cross-reactivity (Thorne et al., 1987a).

The variability between individuals and the influence of the factors affecting sensitivity greatly influence the ability of the markers to detect those individuals exposed. Some markers that are validated generally have been established only for obvious clinical events. For instance, serum levels of immunoglobulin isotypes (IgG, IgA, IgM, IgD, and IgE) can be measured with precision and accuracy, mainly because of standard reference ranges published by the International Union of Immunological Societies and the World Health Organization (Bentwich et al., 1988). Reference ranges for adult and pediatric populations have been established, so it is known that these levels show wide normal variation between individuals. Results that fall well outside of the ranges are highly predictive of obvious health effects, such as immunodeficiency, infection, or neoplasm. However, the significance of variability within the normal range is not well known. Some correlations have been observed between shifts in values within the normal range and toxicant exposures (e.g., to lead and cadmium). Correlations in susceptibility or health effects within the normal range have not been established.

Serum immunoglobulins are proteins for which reference standards can be developed and measurements validated. Many immune-system markers are cellular, and these are generally much more difficult to measure in a standard fashion. Functional markers, for example, those found from in vitro cell stimulation or in vivo skin testing, present even more difficulties in standardized measurement. And although cellular and functional measurements offer the greatest opportunities for more sensitive markers, until such assays are made standard and their predictive values determined, they cannot provide useful information in epidemiologic studies.

These analytic considerations are complicated by the intrinsic variability of the immune system within and between individuals, making the sensitivity and specificity of many tests for immune-system markers modest to poor. Optimizing the analytic specificity and sensitivity of current assays and developing new assays with better specificity and sensitivity also should be given the greatest possible attention.

A major purpose of markers in environmental health research is to identify exposed persons, so that risk can be predicted and disease prevented. Validation involves both forward and backward processes of association: from the marker back to exposure and from the marker forward to effect. Validation of markers as applied to their use in epidemiologic studies is discussed in Chapter 9.

UNCERTAINTY AND RISK

Because so many factors can affect the immune system, a review of uncertainty factors is necessary for a comprehensive assessment of immune function in the assessment of risk. When genetic factors confound, the choice of inheritance models is important for interpreting results. The variation in age groups is particularly important and can often be controlled for epidemiologic studies. The neuropsychologic-immune relationship must be considered an important variable in analyzing immune-system markers, especially in situations where the threat or effect of environmental exposure can subject study groups to greater stress than control groups experience. Under such conditions, assessment of stress should be an essential component of study design. The importance of controlling for these factors and others known to influence the immune system in any particular study will depend on the study design and the populations involved. However, confounding factors can influence the distribution of markers significantly.

Restricting analysis to a single factor would make it more easily subject to intervening or confounding influences that could be attributed mistakenly to a xenobiotic exposure. Conversely, such factors could mask a true effect. An important question is how such factors should be analyzed statistically. Choosing among multiple markers requires attention to the appropriate statistical approaches that assist in the determination of whether the markers are statistically independent of one another. The complex interactions of the immune system require that a judgment be made of whether immunologic markers are biologically independent and should be treated as statistically independent or statistically correlated. Confounding factors can be controlled in epidemiologic study designs by restriction or matching and in analyses by stratification or multivariate methods.

ETHICAL AND PRACTICAL ISSUES

The availability of highly sensitive assays that can identify dose and effects resulting from the interplay between low-level exposures and genetic or acquired susceptibility raises several thorny ethical and moral questions. The reader is referred to Ashford and Miller (1989), Committee on Biologic Markers of the National Research Council (1987), Schulte (1987), and Weiss (1989) for discussion of those issues. Primary among them is the use that is to be made of biologic marker information, Take, for example, the theoretical case of a biologic marker known to reflect susceptibility. Should a worker who tests positive or has an increased measurement be removed from the workplace? If so, should he or she be offered an equivalent job in the same industry? Or should the workplace be cleaned up to protect the most sensitive worker?

In reality, few, if any, biologic markers are established as predictors of individual risk associated with inborn traits, exposure, or a combination of the two. On the other hand, in regard to immunotoxicity, some markers might have this predictive potential. Therefore, it is important to inform research-study participants in advance that the results are interpretable only on the group level. Participants in such studies should be provided test results that are presented and discussed in context with available information (or lack thereof) on the variability within and between people in the normal (nonexposed) population, as well as that observed in the research-study group. Participants in epidemiologic studies may resist invasive techniques for obtaining markers, or they may resist providing markers obtained by techniques perceived to be not fully safe. For example, the use of urinary concentration as a marker may be more universally accepted than a marker from a blood sample, from bronchiolar lavage, or from imaging technique. In addition, if the marker technology is to be applied to relatively large populations, it must be economically feasible.

STRUCTURE OF THE REPORT

Because of the multidisciplinary nature of this report, certain concessions have been made in application of particular terms. In this report, the discussion of the impact of materials on the immune system is largely limited to the effects of xenobiotics. This is the major drive in immunotoxicology, although antigens from biologic sources are certainly the major cause of immune modulation. In this report, the term marker is used for indicators of exposure, effect, or susceptibility, not as an identifying feature of subpopulations of lymphocytes. The reader should, however, be alerted to the special use of *validity, sensitivity*, and *specificity* as these terms apply to analytic capabilities, immunotoxicologic bioassays, or epidemiologic investigations.

Chapter 2 provides information on the structure of the immune system and on the mechanisms of immunotoxicity. Chapter 3 deals with excessive responses of the immune system that result in hypersensitivity. Chapter 4 examines how autoimmune reactions might result from chemical exposure. Chapter 5 reviews the toxicity and disease that result from factors that suppress the immune system. Chapter 6 discusses the potential role of animal models in immunotoxicology, and Chapter 7 assesses strategies for applying biologic markers of immunotoxicity to humans. Chapter 8 gives an approach to the design of field studies to evaluate the effects of immunotoxic chemicals. Chapter 9 addresses the need for updating research on the use of biologic markers in controversial areas of environmental health. Chapter 10 is a summary of conclusions and recommendations.

2

The Structure and Function Of the Immune System And Mechanisms of Immunotoxicity

This chapter provides an overview of the structure and function of the immune system and the mechanisms of immunotoxicity.

Immunotoxicology is the study of injury to the immune system that can result from occupational, inadvertent, or therapeutic exposure to a variety of environmental chemicals or biologic materials. The field has two broad research areas: One involves studies of the suppression of immunity; the other concerns studies of enhanced or excessive immune response, such as allergy or autoimmunity. The immune system acts as a passive target for suppressive xenobiotics, and exposure to them can result in an increased incidence of infectious disease or neoplasia because of the host's inability to respond. When the immune system responds to other xenobiotics, a marked increase in a specific immune response can occur and adverse health consequences, such as respiratory tract allergies (e.g., asthma and rhinitis), allergic contact dermatitis, or autoimmune disease, can develop. Therefore, although other tissues can be affected after alterations in an immune response, the immune system is the primary site of action.

The field of immunotoxicology has grown considerably since the early 1970s (Luster et al., 1988). The discipline developed through studies that combined knowledge of immunology and toxicology to determine the effects of xenobiotics on the functioning of the immune system. The field now includes initial identification of suspected immunotoxic chemicals and the development of sensitive, quantitative animal assays to assess any chemically induced immunologic effects and determinations of the mechanisms by which xenobiotics can alter immune function. Various assays have been developed to characterize the immunotoxic properties of xenobiotics in animals (Dean et al., 1982). These immunotoxicologic assays measure effects on humoral immunity, cell-mediated immunity, macrophage function, natural-killer-cell cytotoxicity, and cytokine activity in animals. Recent developments in monoclonal antibody technology and the advances in cell-culture techniques and molecular biology allow immunotoxicologists to examine the molecular mechanisms of action of drugs and chemicals.

Certain chemical exposures and doses that do not affect particular organs can result in immune dysfunction. For example, lead, polychlorinated biphenyls, toxaphene, pentachlorophenol, and some organophosphate pesticides have been shown to affect significantly the immune response of mice and

rats at exposures that did not alter other commonly tested measures of toxicity, such as body weight and some blood chemistry measurements. The conclusion is that the immune system could be more sensitive to certain xenobiotics than other major organ systems are.

The immune system can be affected by a variety of conditions, substances, and agents. Those most commonly encountered are radiation, immunosuppressive drugs, bacteria, viruses, protozoa, other parasites, and some forms of cancer. There are a number of hereditary syndromes of immune deficiency. Occasionally, immune deficiencies result from other environmental causes. Stress, for example, from loud noise, electric shock, infant-mother separation, overcrowding, the death of a spouse, or severe emotional or mental dysfunction could be associated with immunologic abnormality. Hereditary and environmental factors must be evaluated carefully in the assessment of suspected chemical-induced immune dysfunction. The putative causes of immune modulation, particularly in humans, are diverse, and therefore, they must at least include emotional distress as a contributing factor.

DEVELOPMENT AND FUNCTION OF THE IMMUNE SYSTEM

The immune system is the body's main defense against foreign materials and biologic agents such as bacteria, viruses, chemicals, and foreign cells and tissues. The immune response includes specific action of lymphocytes (one type of white blood cell) and is facilitated by other white blood cells, including neutrophils, monocytes, macrophages, eosinophils, and basophils. The immune system can be viewed as a system controlled by negative feedback. The central component of the system is the lymphatic tissues, which include mature T (thymic) lymphocytes that have matured through development in the thymus and mature B lymphocytes that have matured in the bone marrow. Each of these cell groups is composed of subpopulations with varied functions, especially the T cells (Twomey, 1982; Golub, 1987; Mangan, 1987). Some T-cell populations act to enhance or suppress immune function; the B cells differentiate to varied immunoglobulin-secreting plasma cells after the immune system's exposure to the antigen (Paul, 1984; Miedema and Melief, 1985; Young and Geha, 1986).

Lymphocytes circulate throughout the body, moving in and out of tissues via the circulatory and lymphatic systems, where they meet foreign antigens. The immune response is called specific because each mature T or B lymphocyte has a specific antigen receptor on its surface. After an antigen is bound to the receptor, stimulation of a B cell produces antibodies (immunoglobulins) that specifically react with the stimulating antigen. The binding of antibodies to antigens can lead to the inactivation or removal of the foreign antigen (Twomey, 1982). Various T cells, including the T-helper and T-suppressor cells, regulate the immune response. There also are cytotoxic (killer) T cells, which destroy target cells. Target cells are virally infected or transformed cells whose elimination will prevent the progression of a virus infection or tumor development, respectively. Target cells have surface antigens that bind to antigen-specific receptors on the surfaces of killer T cells. About 5% of the circulating lymphocytes are called "null" lymphocytes because they lack the specific cell-surface molecules characteristic of B cells and T cells. The cell-to-cell regulation of the immune system is mediated by locally acting hormones called cytokines, which are secreted by activated cells. Lymphokines are cytokines secreted by lymphocytes. Interleukin-2 (IL-2) is a lymphokine produced by helper T cells that acts as a growth factor for all T cells including the cells that make it. Helper T cells make other lymphokines, e.g., gamma interferon and IL-4.

The development of the human immune system begins late in the fetal period, is functioning at birth, and reaches maximum capacity around the time of puberty (Paul, 1984; Claman, 1987). In the human adult, the majority of circulating lymphocytes are T cells, and the remainder are B cells and NK cells. However, the production of B cells and T cells continues, albeit at a reduced rate, throughout life. The total lymphocyte count in the peripheral blood can be up to 3,000/mm ^{3} during childhood; in the adult, the average is about 2,500/mm^3 with a low below 1,000/mm^3 (Golub, 1987).

The sequence of events in the immune response is shown in Figure 2-1 and Figure 2-2. After exposure to an antigen (a molecule that stimulates a specific immune response), there is phagocytosis (ingestion) of the antigen by macrophages, during which the antigen undergoes intracellular breakdown through enzymatic hydrolysis. After hydrolysis, the fragments of the antigen move to the surface of the macrophage for reaction with specific T lymphocytes, called helper-inducer T cells. Activation of these T lymphocytes occurs only if the interacting lymphocyte has specific receptors that bind to a complex of antigen fragment and a special protein derived from the major histocompatibility complex (MHC) (Paul, 1984; Claman, 1987; Golub, 1987). The Class I MHC protein defines transplantation antigens normally recognized during graft rejection and present on all nucleated cells. The Class II MHC is a cluster of genes that encodes specific cell-surface molecules that are normally restricted to phagocytic cells (e.g., macrophages) and some lymphocytes (e.g., B cells). When a large number of the specific receptors on the individual T cell binds to the complementary complex of an antigen fragment and the MHC Class II protein on a macrophage, the T cell is stimulated into a proliferative response that leads to clonal expansion and secretion of lymphokines. If an activated helper T cell then binds to the antigen-MHC complex on a B cell, that B cell is stimulated to proliferate and differentiate into an antibody-secreting plasma cell. An activated killer T cell will bind and kill any target cell (e.g., an infected cell) if that cell carries on its surface the appropriate antigen-MHC complex. T cells that bear the CD8 or CD4 molecule will interact with target cells that bear Class I or Class II MHC protein, respectively.

The generation of antibody-producing

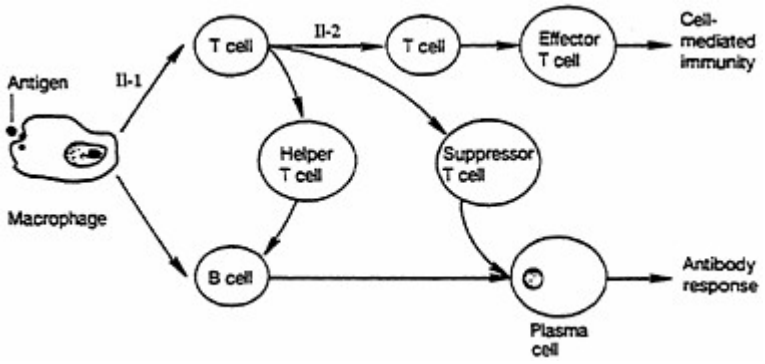

FIGURE 2-1 Cellular interactions involved in generation of immune response.
Antigen presentation leads to stimulation of T-cell or B-cell systems. Factors involved in T-cell system include interleukin-1, which stimulates T cells to acquire receptor for T-cell growth factor called interleukin-2 (Il-2); same subpopulation T cells can also secrete Il-2. Source: NRC, (1989b).

plasma cells (B cells) and cytotoxic T cells requires the presence of biochemical factors (lymphokines and cytokines) secreted by T cells and macrophages. As a result of the clonal expansion, the number of these specifically reactive T and B cells increases so that subsequent exposure to the same antigen leads to a rapid, specific immune response, the secondary response, which is characterized by increased secretion of antibody or proliferation of specific populations of effector T cells (Katz, 1977; Paul, 1984; Claman, 1987; Golub, 1987). As an immune response occurs, a decrease of the T cells is likely and negative feedback into the earlier phases prevents the excessive reaction. Thus, the specific antibody reacts with the offending antigen to cause neutralization or inactivation while effector T cells inactivate or destroy cellular targets.

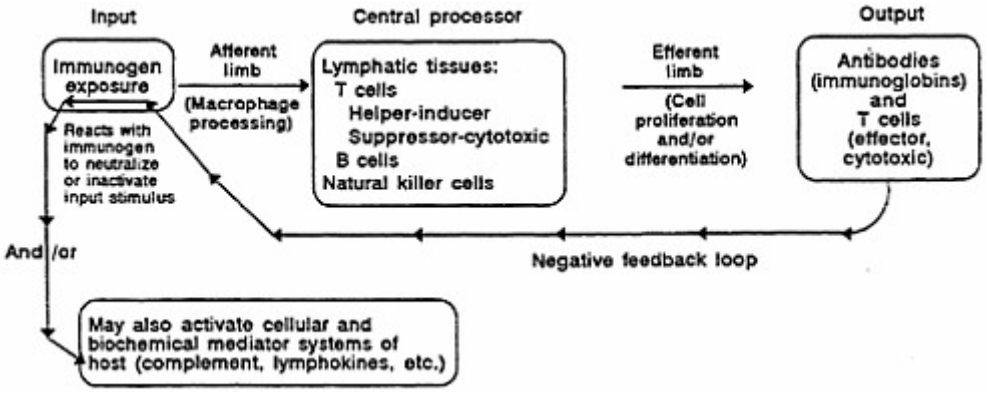

FIGURE 2-2 A model of the competent immune system depicting normal interrelations of the major components.

The normal function of the immune system involves a complex sequence of cellular and biochemical events. Opportunities for dysfunction can occur at any point in the system and can result in a variety of immunologic effects from hypersensitivity to immunodeficiency (Twomey, 1982).

MECHANISMS OF CHEMICALLY INDUCED IMMUNE DISEASE

Exposure to immunotoxicants can cause immunologic suppression, resulting in altered host resistance. The outcome of immune suppression is influenced by the dose and mechanism of action of the immunotoxicant along with concomitant exposure to other agents, such as bacteria, viruses, parasites, or chemicals at levels so low they might normally be innocuous. In its suppressed condition, the immune system does not respond adequately to the hazardous agent. Adverse health consequences are those of severe disseminated infectious disease caused by a variety of agents that are not usually pathogenic. Age, poor nutrition, and stress (physiologic and psychologic) can exacerbate the development of such immunologic diseases.

Xenobiotics also can act as sensitizers to stimulate the immune system as antigens by provoking a substantial immune response that leads to hypersensitivity. Immunologic tissue damage can result from activation of cellular and biochemical systems in the host. The major immunologic tissue reactions are summarized in Table 2-1. The interaction of an antigen with a specific antibody or with

effector lymphocytes triggers the sequence of humoral and cellular events to produce the pathophysiologic effects that lead to tissue injury and disease (Samuelsson, 1983; Frank, 1987). Diseases that are immune-reaction mediated include rheumatoid arthritis, some types of diabetes, and myasthenia gravis. Each immune reaction is associated with different principal cells and biochemical mediator systems (Lachmann and Peters, 1982). This classification by the cells and immune function involved is used in Table 2-1. An older classification of immune reactions, developed by Gell and Coombs (Gell et al., 1975), is noted in Table 2-1 for comparison.

IgE-Mediated Hypersensitivity

IgE-mediated hypersensitivity involves antibodies that are produced in response to exposure to a wide variety of antigens, such as grass pollens, animal danders, foods, and commonly encountered environmental substances (Sheffer and Pennoyer, 1986). This is the most common type of hypersensitivity disorder, and it has been known variously as atopic, reaginic hypersensitivity; Prausnitz-Kustner (P-K) reactivity; or as the Type I reaction in the Gell-Coombs classification. The name used here is preferred.

A susceptible individual is initially sensitized by exposure to an antigen and subsequently develops a significant IgE response. The IgE then disseminates through the circulation and binds via specific receptors to the cell membranes of basophils in the circulation and to mast cells in the tissues. Those cells are then "sensitized," and the individual is susceptible to reacting on later exposure to the antigen.

Exposure of a sensitive individual leads to the activation of mast cells by binding of specific antigen with the IgE on the cell membranes. This interaction leads to the release of cytoplasmic granules and histamine, leukotrienes, and other vasoactive substances (Ishizaka, 1984; Sheffer and Pennoyer, 1986). These substances cause vascular dilatation and increased blood flow, increased permeability, and tissue edema along with smooth muscle contraction and hypersecretion of mucus. The local tissue reaction depends on the site of antigen exposure. Inhaled antigens cross the mucous membranes of the nose, sinuses, and lower respiratory tract to cause an increase in mucus secretion, congestion, and edema of the mucous membranes. Constriction of bronchi and bronchiolar smooth muscles limits pulmonary air exchange and causes wheezing. Sinusitis, rhinitis, and bronchial asthma can all result from IgE-mediated hypersensitivity.

Antigens ingested in food or medications can cause symptoms of intestinal hypermotility, dyspepsia, colicky pain, or a sensation of fullness and bloating. Those symptoms result from the local release of mediators in the mucosa of the gastrointestinal tract. Antigens that are absorbed in the tract enter the circulation and disseminate throughout the body, causing systemic reactions (generalized anaphylaxis) or local reactions in the skin and viscera. Skin reactions include acute edematous papules and large urticarial lesions. Chronic skin reactions, such as maculopapular rashes and eczema, also can occur. The injection of an antigen, such as a drug or vaccine, can cause disseminated systemic anaphylactic reactions, which can lead to vascular collapse, respiratory insufficiency, and death unless there is immediate treatment (Delage and Irey, 1972). Such systemic reactions result from the massive release of biochemical factors from circulating basophils.

Complement-Mediated and Immune-Complex-Mediated Injury

The complement system is made up of a series of serum proteins (C1 through C9) that can be activated by immune and nonimmune

TABLE 2-1 Immunologic Hypersensitivity Reaction—Types I-IV

Types of Reaction[a]	Onset	Antibody	Principal Cell	Site of Reaction	Biochemical Reaction
Antibody dependent: Anaphylactic (Atopic reaginic) (Type I[a])	Rapid (seconds to minutes)	IgE (homocytotropic)	Basophil/mast cell	Varies with antigen portal of entry	Histamine, serotonin, platelet activating factor (PAF), leukotrienes (SRS-A), prostaglandins, thromboxanes
Complement-mediated immune adherence (Phagocytic reaction) (Type II[a])	Intermediate (minutes to hours)	IgM/IgG	R-E cells	Vascular sinuses reticuloendothelial system	Complement activation C3 to C3b
Immuno-complex (or tissue reactive antibody) (Type III[a])	Intermediate (30 minutes to 2 hours)	IgM/IgG	Neutrophil	Varies with tissue localization of complexes or site of reaction with tissue constituents	Complementchemotactic factors Neutrophil lysosomal hydrolytic enzymes
T-cell reactions: Granulomatous (Type IV[a])	Prolonged (delayed 18-24 hours)	None	Responder T cell and monocyte/macrophage	Varies with tissue localization of antigen	Soluble lymphokines from antigen stimulated T cell

[a] Gell and Coombs (Gell et al., 1975) classification of Types I-IV.

factors. Activation of complement causes chemotaxis, cell adherence, cell lysis, phagocytosis, and mast cell activation. Immune activation of complement occurs only with antigen-antibody reactions that involve IgG or IgM antibodies (Wiggins and Cochrane, 1981; Frank, 1987). Alternative pathway activation of complement can occur nonspecifically but still yield effects similar to those of immune activation. Complement-dependent immune reactions are usually localized to the tissue sites where antigen-antibody complexes form or lodge. They constitute the principal types of immunologic tissue injury.

The antigens in complement-mediated immune reactions arise from varied sources. Those from infections are derived from bacteria, fungi, or viruses. Others are from injections or inoculations (in a course of immunization), blood transfusions, or drug therapy. Sensitization occurs in an individual who was exposed to an antigen previously and has developed a specific IgG or IgM antibody response. Subsequent exposure to the specific antigen can result in immune-complex formation with complement activation, which yields molecular fragments that exhibit a variety of biologic activities. Some are potent chemoattractants for neutrophils and monocytes; others facilitate immune adherence and phagocytosis or cause release of mast cell mediators.

The sequence of events in immune-complex injury is as follows (Wiggins and Cochrane, 1981): soluble immune complexes form in the circulation of a sensitized individual. The complexes can become localized in tissue, via permeability of small venules, and produce tissue lesions. Fragments of activated complement promote chemotaxis of monocytes and neutrophils to the specific tissue site, where they release destructive hydrolytic enzymes that cause necrotic lesions. Examples of disseminated immune-complement disease in humans include systemic lupus erythematosus (SLE), various forms of glomerulonephritis, hypersensitivity pneumonitis, and polyarteritis nodosa.

Another form of complement-dependent cell destruction (Table 2-1) results from immune activation of complement on the surface of an antibody-coated target cell that generates abundant fragments of C3b complement (Fearon, 1984; Schifferli et al., 1986). The C3b molecules promote phagocytosis by attachment to the target cells and to cell-surface receptors on macrophages and reticuloendothelial cells and thus speed removal of the target cells from the circulation. The C3b-coated cells removed by phagocytosis are destroyed within the phagocyte. If erythrocytes are the target cells, chronic destruction leads to severe anemia. These reactions often occur when IgM or IgG antibody reacts with native cell-surface antigens, with drugs, or with infectious agents that have become bound to the cell surface. Autoimmune hemolytic anemias, neutropenias, and immune thrombocyto-penias are examples of this type of immune injury in humans.

T-Cell Reactions

Immune-system reactions that result in specific T-lymphocyte activation and response can cause T cells to act as autoimmune effector cells (Turk, 1980). Alternatively, some T-cell-dependent reactions might result in the formation of granulomas (Turk, 1980; Springer et al., 1987) (Table 2-1). Activated T cells produce and release lymphokines, biochemical factors that mediate T-cell reactions. Some lymphokines activate blood monocytes to cause their transformation to macrophages; others are chemotactic for monocytes and attract them into tissue sites. If the process is chronic (lasts for weeks or months), the attracted macrophages cluster and fuse to form giant cells in the tissue at the site of lymphocyte activation. Those cellular events produce a typical lesion called a granuloma. The

multinucleate giant cells (macrophages) are mixed with clusters of mononuclear cells (macrophages and lymphocytes). Granulomatous reactions are characteristic of chronic T-cell-dependent reactions (Table 2-1). They are found in infectious diseases in which the antigen persists with continued T-cell activation. Granulomatous reactions are common in tuberculosis and in fungal infections. They also can occur with exposure to particulate chemicals such as beryllium and talc.

Another T-cell-dependent tissue injury results from the binding of specific T-effector cells to a cell-bound antigen on target cells. Such effector cells are called killer T cells because they release chemicals that kill the target cell shortly after contact. Examples are found in a variety of viral infections, chemical contact sensitivity, and drug reactions. In chemical contact sensitivity, the chemical becomes adsorbed to a cell surface and becomes the target antigen for specific effector T cells that kill the cell linked to the chemical.

EFFECTS OF XENOBIOTICS ON THE IMMUNE SYSTEM

Some drugs and chemotherapeutic agents can suppress the immune system. These agents are used clinically to prevent rejection of transplanted organs and in the treatment of some autoimmune diseases. When the drugs are administered at doses that prevent organ rejection, patients are at increased risk of infection and neoplasia as a consequence of their reduced immune function. Other xenobiotics are suspected of causing immunosuppression in humans through environmental exposure, but definitive evidence is lacking.

Toxic agents might cause abnormalities in immune function at several points. These points are illustrated in Figure 2-3 superimposed on the model in Figure 2-2. There are four major sites at which toxic agents might affect immune function (Gibson et al., 1983; Luster et al., 1987). At point 1, a toxic material could lead to a specific immune response that produces tissue injury through

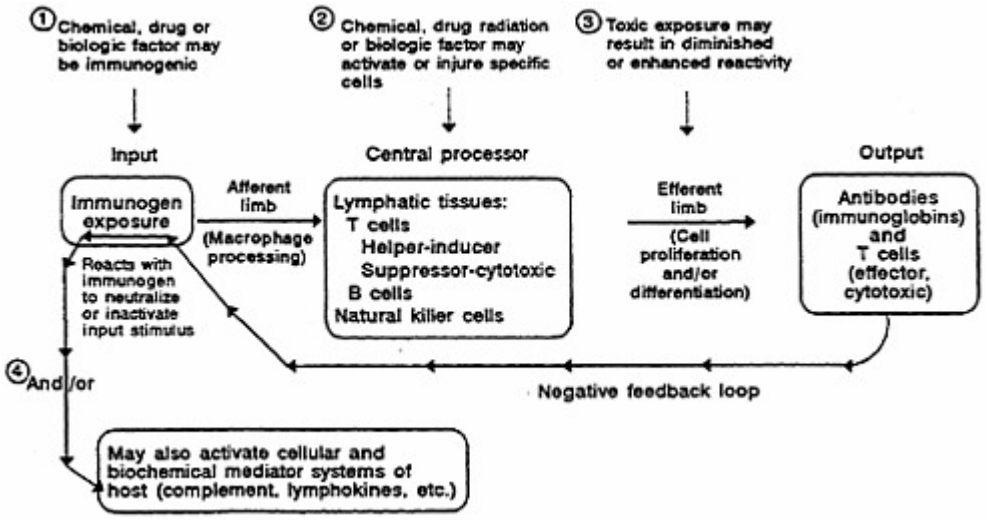

FIGURE 2-3 A model of the competent immune system depicting sites of potential effects on the major components by toxic factors.

any of the different types of immunologic injury (Twomey, 1982). At point 2, a toxic agent could alter the activity of specific lymphocytes. The result can be either a regulatory defect in the immune function, which could lead to excessive immune reactivity, or an autoimmune reaction (Twomey, 1982). A third point of potential toxic effect might be on lymphocyte proliferation or differentiation that could lead either to a defect or to an enhancement of specific reactivity. Finally, at point 4, immune reactivity could be excessive in widespread activation of other cells (macrophages, granulocytes, platelets, and mast cells), activation of potent plasma biochemical systems (complement, fibrinolytic, coagulation, and kinin), or secretion of biochemicals from stimulated lymphocytes.

Chemicals that suppress bone marrow function can affect the reserves of stem cells that are needed for cell replacement. Blood-cell lines are derived from pluripotent stem cells, which in adult humans are primarily in the bone marrow. Within the marrow microenvironment, these self-renewing cells mature into committed progenitor cells, which are in peripheral blood and tissues. The continued development of these cells is under the control of various growth factors, many of which originate in bone marrow stromal cells. Bone marrow stromal cells also provide a supporting matrix for development of hematopoietic cells. Various studies, including those on the use of long-term bone marrow cultures, have demonstrated the importance of the microenvironment in regulating myeloid and lymphoid development. Stem cells often appear to be sensitive targets for therapeutic and environmental toxicants, most likely because of their rapid proliferation. Myelotoxicity or bone marrow toxicity caused by xenobiotics or various drugs can result in profound immunosuppression due to loss of stem cells.

Xenobiotics can act as immunogens to stimulate the production of specific immunoglobulin as a part of an immune response. Specific immunoglobulins might be used as markers of exposure to specific xenobiotics.

Other biologic markers that could be applied to the human immune system are discussed in subsequent chapters. They are derived principally from or related to the varied biochemical and cellular factors discussed in the foregoing sections. They include the total and relative numbers of circulating lymphocytes and their subpopulations; the different classes of immunoglobulins (in addition to the specific antibodies already mentioned); lymphocyte proliferation stimulated by mitogens and specific antigens (xenobiotics); complement activation by specific xenobiotics; skin test response patterns to xenobiotics; in vitro lymphocyte and monocyte activation by xenobiotics, with measurement of lymphokine secretion; and other markers found by the techniques of cellular and molecular biology that can sensitively assess the structure, function, and complex interactions of the many components of the immune system. Animal models could prove useful in defining appropriate immune-system markers for xenobiotics in humans. The study and application of immune-system markers in humans to the assessment of toxic environmental exposures are now in the initial stages. Many questions require experimental and epidemiologic answers to ascertain the usefulness of particular markers. It is expected that this report will help stimulate the major research needed.

3

Biologic Markers For Immune-Mediated Disease

This chapter defines immune-mediated disease and the extent to which some chemicals in our environment cause hypersensitivity or immunopotentiation. The discussion focuses on three principal questions:

- To what extent do chemicals in our environment have the potential to cause immune-mediated disease and thus pose a health threat in the United States today?
- How can conventional and novel biologic markers be used to identify exposure and susceptibility to these agents and provide diagnostic information that is useful in the management of hypersensitivity disorders?
- What are the critical issues, important questions, and kinds of studies that need to be considered to adequately define and manage this health problem?

This chapter is not a comprehensive treatise or catalogue of immune-mediated disorders and xenobiotics known to cause them. Rather, examples of the various disorders, chemicals that are suspected to cause them, and markers that indicate their presence are presented to review the scientific issues and suggest future directions.

DEFINITION OF THE PROBLEM

Hypersensitivity disorders are by far the most widely recognized manifestations of immunotoxicity. These disorders can result from exposure to environmental contaminants or chemicals in the workplace and have been widely reported and amply documented (Trizio et al., 1988). Hypersensitivity reactions are also the most common type of immunotoxicity associated with chemicals in the environment. Despite the relatively high morbidity and long history of these disorders (particularly in the workplace), much of the pathophysiology has only recently been established, and many questions remain to be answered. Progress in addressing this important health problem has been impaired by the slow development of appropriate animal models. Consequently, we have depended to a large extent on clinical research to establish mechanisms and effective modes of treatment.

It must be emphasized that hypersensitivity responses and susceptibility to autoimmune diseases are strongly influenced by genetics. In addition, variation from what is normal in the neurologic-hormonal balance

can significantly influence hypersensitivity responses.

Several features distinguish hypersensitivity from immunosuppression. First, hypersensitivity disorders are far more common in the general population than is immunosuppression, and hypersensitivity disorders are more readily defined and studied. Second, they usually entail a specific antibody, receptor, cell population, or target tissue. Third, because of this specificity, diagnostic and epidemiologic approaches tend to be problem oriented. Last, biologic markers of susceptibility, exposure, and effect are more often defined by suspected mechanisms, such as the presence of a specific antibody or the release of a particular mediator, rather than by the consequences of suppression of the immune system (by the presence of infection or tumors).

Consequently, the appropriateness, predictive value, and utility of laboratory tests tend to be more obvious for immune-mediated disease than for immunosuppression.

Although clinical signs of hypersensitivity generally have provided adequate markers of effect (the presence of a rash and bronchoconstriction), linkage to the offending chemical has presented a special challenge. The natural history, epidemiology, and clinicopathologic aspects pertinent to the consideration of biologic markers of these disorders are discussed in this chapter. Because exposure to environmental chemicals occurs principally through contact with the skin, the respiratory tract, and the gastrointestinal system, the discussion is organized around these routes of exposure to potentially sensitizing agents.

EXPOSURE THROUGH INHALATION (PULMONARY HYPERSENSITIVITY)

Pulmonary diseases attributed to hypersensitivity responses in the respiratory tract have been recognized for centuries. Three general categories of hypersensitivity can cause pulmonary disease. The most common category includes asthma or rhinitis that results from the production of specific IgE to inhaled allergens. The second category is hypersensitivity pneumonitis. Far fewer persons are affected than are those who have asthma or rhinitis, but because some individuals develop severe alveolitis after repeated exposure to inhaled organic dusts, this is a particularly serious liability. Hypersensitivity pneumonitis is primarily a disorder of nonatopic subjects and is associated with high levels of antibody. Both antibody and cellular immunity could be important in responses that lead to lung damage. The final category of hypersensitive lung disease includes disorders associated with cellular immunity, one example of which is chronic beryllium lung disease.

Occupational Asthma and Rhinitis

Asthma and rhinitis are common reactions to inhaled environmental plant and animal allergens. Inhalation of chemicals also can cause asthma. Asthma induced by inhaled chemicals is characterized by variable airway obstruction and hyperactivity of the airways caused by exposure to sensitizing concentrations of substances present in the environment (Chan-Yeung and Lam, 1986; Cotes and Steel, 1987), or workplace. It is estimated that 50,000 to 100,000 workers in the United States are potentially exposed to one such group of chemicals, diisocyanates (Musk et al., 1988).

Reactions to inhaled chemicals can be produced by a variety of mechanisms. The agent can act as a nonspecific irritant, it can act as an allergen that induces specific antibody production, or it can nonspecifically stimulate release of biologically active mediators. In many examples of occupational asthma, the actual mechanisms have not

been determined. Clinically, asthmatic responses to chemical haptens that induce IgE are difficult to distinguish from airway obstruction caused by irritants.

It is not clear how irritants cause asthmatic responses in the absence of an immune response. However, inflammation is an important component of asthmatic responses, and the induction of pulmonary inflammation by inhaled chemicals could be important in airway responses after inhalation of irritants. In late-phase asthmatic responses, eosinophils and neutrophils are increased in bronchoalveolar lavage fluids after antigen challenges (Metzger et al., 1985; Diaz et al., 1986). Although the exact role of inflammation in asthmatic responses is not clear, it is possible that inflammation induced by inhaled chemicals could be important in the induction of nonimmunologic asthmatic responses. The exposure of dogs to ozone results in increased airway responsiveness, suggesting that the neutrophil plays an important role in airway hyperreactivity (Holtzman et al., 1983). The production of a variety of mediators by inflammatory cells could play an important role in increasing nonspecific airway responses (Henderson et al., 1984; Cuss et al., 1986).

Many agents associated with occupational asthma are low-molecular-weight chemicals, such as nickel, platinum, palladium, toluene diisocyanate (TDI), and trimellitic anhydride (Pepys et al., 1972; McConnell et al., 1973; Zeiss et al., 1977). However, complex organic molecules, including bacterial enzymes used in detergents, also can cause this reaction (Newhouse et al., 1970). Thus with complex aeroallergens, there is an association between the presence of the disease and the atopic status of the workers.

Low-molecular-weight chemicals may act as haptens, binding to body macromolecules to form antigens that can induce haptenspecific antibodies. In some cases, antibodies also recognize a portion of the carrier molecule. Thus, immune-system responses to inhaled chemicals can be directed against a range of antigen specificities.

The initial sensitization, after exposure of the mucosa to a chemical, is thought to occur in lymph nodes that drain the lung or airways. After immunization, B cells that produce specific IgE can be found in mucosal tissues. The production of IgE in mucosal tissues and in lymph nodes that drain mucosal tissues results in the sensitization of circulating basophils and tissue-fixed mast cells throughout the body. Binding of IgE on these cells by allergens causes them to release mediators that contribute to allergic symptoms.

The incidence of occupational asthma has been estimated to be 2-4% (Salvaggio et al., 1986). Particularly potent sensitizing chemicals, including platinum salts, have been observed to sensitize more than 50% of exposed subjects (Roberts, 1951). Up to 20% of workers exposed to acid anhydrides and 5% exposed to diisocyanates have become sensitized to these agents (NIOSH, 1978; Bernstein, 1982).

Atopy is a predisposing factor for occupational asthma induced by high-molecular-weight sensitizers, such as enzymes, flour, and animal dander or venom (Newhouse et al., 1970). However, there has been no demonstrable correlation between atopy and response to low-molecular-weight sensitizers, such as TDI and trimellitic anhydrides. Other factors that could be important in the initiation of disease include altered adrenergic tone (Szentivanyi, 1968), recent or concurrent respiratory viral infection (Empey et al., 1976), and alterations in the integrity of the tight junctions of basal membranes (McFadden, 1984). Smoking does not appear to be a predisposing factor (Chan-Yeung and Lam, 1986; Cotes and Steel, 1987).

Although IgE specific for high-molecular-weight sensitizers usually can be demonstrated, they are less frequently detected for low-molecular-weight chemicals. Many individuals

diagnosed with clinical asthma associated with TDI do not demonstrate isocyanate-specific IgE in their sera (Salvaggio et al., 1986). Bronchial hyperresponsiveness to nonspecific provoking agents (histamine and methacholine) is present in most, but not all, subjects with occupational asthma. This appears to be an effect rather than a factor predisposing to sensitization (Chan-Yeung and Lam, 1986; Mapp et al., 1986a,b; Cotes and Steel, 1987). Also, some reports suggest that cell-mediated immunity may play a role in hypersensitivity reactions (Baur, 1983; Patterson et al., 1982).

Hypersensitivity Pneumonitis

Hypersensitivity pneumonitis is characterized by a spectrum of lymphocytic and granulomatous interstitial and alveolar-filling pulmonary disorders associated with intense and often prolonged exposure to any of a wide range of inhaled organic dusts and related occupational antigens. The list of organic materials that can induce hypersensitivity pneumonitis continues to grow, with disease classification based on the offending inhaled material.

The limited data available show that hypersensitivity pneumonitis can be induced by inhalation of chemicals. Diphenylmethane diisocyanate, TDI, trimellitic anhydride, and heated epoxy resin are examples of chemicals that can induce hypersensitivity pneumonitis (Blake et al., 1965; Charles et al., 1976; Salvaggio, 1987; Musk et al., 1988), but the frequency of this disorder is considerably less than the asthmatic type response to these chemicals.

Inhaled organic particulates must be deposited in the terminal airways and alveoli to induce an immune response leading to hypersensitivity pneumonitis (Salvaggio, 1987). Some of these antigens have adjuvant properties that could be important in the induction of these reactions (Bice et al., 1977). The induction of immunity to these antigens is not understood, but it seems likely that antigens deposited in the lower respiratory tract are phagocytized by neutrophils or alveolar macrophages and carried via the lymphatics to the lung-associated lymph nodes (Harmsen et al., 1985, 1987). Immune cells produced in these tissues are released into the circulatory system and recruited from the blood into the lung (Bice and Shopp, 1988). Inflammatory reactions to antigens deposited in the lung could be responsible for the recruitment of immune cells (Bice and Shopp, 1988).

Although antibody produced to inhaled antigens can be identified in the blood of most exposed persons, the levels do not correlate well with disease (Pepys, 1986). In most cases of hypersensitivity pneumonitis, pulmonary lesions are substantially different from immune-complex-mediated vasculitic lesions (Salvaggio, 1987). Nevertheless, some data suggest that antibody and immune complement in the lung could be important in the production of lung lesions (Warren et al., 1977; Pepys, 1986).

In provocation exposures, pulmonary reactions in individuals with hypersensitivity pneumonitis appear several hours after exposure. Moreover, many materials that induce hypersensitivity pneumonitis also activate immune complement by the alternative pathway. This mechanism could contribute to hypersensitivity pneumonitis (Warren et al., 1977). Other reports suggest that cellular immunity plays a role in the pathogenesis of pneumonitis (Salvaggio, 1987). The histologic features of lungs from such patients suggest cellular immune reactions. Sensitivity to a pulmonary challenge with some agents can be passively transferred using lymphocytes from immune animals (Bice et al., 1976; Schuyler et al., 1987, 1988).

An increased number of cells and lymphocytes appears in lungs of individuals with hypersensitivity pneumonitis when assayed by bronchoalveolar lavage. However, as observed with specific antibody and cellular

immunity, an increase in total cells and lymphocytes in the lung is not always directly associated with disease. In longitudinal studies, clinically normal individuals can have significantly elevated numbers of lymphocytes for extended periods (Cormier et al., 1984, 1986). Disease and the presence of antibody, the level of cellular immunity, and numbers of lymphocytes in the lung are poorly correlated. It should be considered that disregulation of immunity may be important in the pathogenesis of hypersensitivity pneumonitis.

EXPOSURE THROUGH INGESTION

Adverse reactions to the ingestion of foods have been documented (Metcalfe, 1989). When the pathogenesis of these reactions involves an immunologic response to a component of food, the reaction is called food hypersensitivity (Anderson, 1986). A nonimmunologic response to ingested food is called food intolerance, which can be pharmacologic, toxic, or metabolic. Food intolerance includes illnesses caused by foods that contain noxious substances, such as histamine, tyramine, and possibly other biogenic amines or lectins. Some foods also elicit the nonspecific release of chemical mediators (histamine, prostaglandins, leukotrienes, and lymphokines) from mast cells and perhaps from macro-phages and lymphocytes (Moneret-Vautrin, 1983). These nonspecific responses or a lack of tolerance to food additives or other chemicals can lead to responses that mimic allergic reactions (Schulz, 1983).

Food hypersensitivity includes several clinicopathologic entities: anaphylaxis and other immediate reactions, eosinophilic gastroenteritis, eczema, and protein-induced gastroenteropathy in infants and children (Metcalfe, 1989). Although antibody and cellular immunity have been associated with immunologic reactions to ingested materials, most reactions are IgE dependent; cellular immune reactions appear to account for few of these disorders (Moneret-Vautrin and André, 1983). As for all responses that appear to have an allergic cause, it is important to remember that a variety of mechanisms can lead to symptoms that resemble allergy symptoms. The prevalence of food hypersensitivity in the general population is unknown (Metcalfe, 1989). An important problem in estimating the number of persons with food hypersensitivity is the difficulty in distinguishing true allergic reactions from nonimmunologic adverse responses. One study of 480 children followed from birth to the age of three revealed that approximately 8% had adverse reactions to particular foods. One to two percent of all children have an immunologic basis for their reactions (Bock, 1987).

Allergic reactions to ingested materials appear to be more common in individuals with other allergic disorders. As many as 10% of children and 3% of adults with other allergies exhibit allergic responses to foods (Moneret-Vautrin, 1986). Early publications suggested that colorants, including azoic dyes (tartrazine, erythrosin, and amaranth) and derivatives of triphenylmethane, might produce hypersensitivity responses. In the past, sensitivity to tartrazine was thought to affect 50,000 to 100,000 persons in the United States (Lockey, 1959). More recent results, however, provide convincing evidence that tartrazine is not responsible for the chronic urticaria observed in some patients exposed to the dye (Stevenson et al., 1986).

Preservatives (antiseptics and antioxidants) also can be sensitizers. Citric acid added as an antiseptic preservative has been blamed for triggering buccal aphthous ulcers (Pradalier et al., 1984). Implicated antioxidants include tocopherols, lecithin, propyloctyl, dodecylgallates, butylhydroxytoluene, and butylhydroxyanisol. Those most often responsible for adverse reactions are sodium nitrite, sodium benzoate, sodium metabisulfite, butylhydroxytoluene, and butylhydroxyanisol (Sogn, 1984; Moneret-Vautrin, 1986).

Medications that contain these substances are another source of exposure (Koepke et al., 1984). The mechanisms responsible for many of these adverse reactions are not known.

Ingested sulfiting agents can cause urticaria, angioedema, intestinal dysfunction, vasomotor headaches, or airway constriction in sensitive individuals (Prenner and Stevens, 1976; Clayton and Busse, 1980; Stevenson and Simon, 1981; Schwartz, 1983; Bush et al., 1986; Moneret-Vautrin, 1986). Acute rhinitis, flushing, and even anaphylactoid reactions also have been reported. In some cases, increased asthmatic symptoms can occur after inhalation of aerosols from sulfite-containing foods (Werth, 1982). Although the extent of sulfite sensitivity in the general population is unknown, it appears that asthmatics are at the highest risk (Sogn, 1984). Respiratory effects of sulfite ingestion could be the greatest health threat, although some patients have reported severe abdominal distress as the principal adverse effect (Schwartz, 1983). Benzoic acid and its salts (Michaelsson and Juhlin, 1973), sodium glutamate (Allen and Baker, 1981; Swan, 1982), and nickel (Brun, 1979) are materials found in food that cause adverse reactions. The mechanisms of adverse reactions to ingested substances are not fully understood.

DERMAL EXPOSURE

Dermatitis can be caused by immune responses to antigens or haptens that come into contact with the skin or nonspecifically by contact with a substance that chemically damages the skin (irritant dermatitis). Most chemicals that cause dermatitis (70-80%) act as primary irritants, although some substances are both irritants and allergens. Only immunologically mediated dermatitis will be considered in this discussion. It is estimated that 20-25% of all cases of occupational dermatitis result from immune sensitization (Birmingham, 1988).

There are three types of immunologically mediated dermatitis. The first, allergic contact dermatitis, is the result of prior exposure of the skin to the material eliciting the dermatitis or to a chemical closely related to the substance (e.g., nickel sensitivity). The second variety, photocontact dermatitis, is similar to the first, but requires exposure to sunlight and allergen to produce a skin reaction. Allergic photocontact dermatitis is caused by antibacterial chemicals, chlorpromazine, and fragrances. In rare cases, shortwave ultraviolet light is required for the photoallergic response. Cell-mediated immunity is responsible for allergic contact dermatitis and photocontact dermatitis. The third form of allergic dermatitis is contact urticaria, a transient wheal-and-flare response caused by IgE antibody reactions. The patient's degree of sensitivity and the amount of antigen determine whether only localized urticaria occurs or if a generalized manifestation, such as angioedema, asthma, or anaphylaxis, will develop.

Contact Dermatitis

Contact dermatitis is widespread, and the substances responsible are usually low-molecular-weight chemicals that act as haptens to induce cell-mediated immunity (Fregert, 1978; Goh, 1988). Although skin changes can be seen as early as 3 hours after exposure, maximum responses usually occur 12 hours or later following exposure. Nickel sensitivity is commonly observed (5.8-8%), and women are more frequently sensitized than men, likely because of their exposure to jewelry and clothing items (Peltonen, 1979; Prystowsky et al., 1979). Chromium and cobalt also are common sensitizers. Other substances that can cause contact dermatitis include chemicals in detergents, shampoos, soaps, and perfumes, and rubber and rubber-based adhesives. Individuals exposed to epoxy glues and coatings, some amines, chromates, formaldehyde, and some antioxidants

also are at risk of becoming sensitized (Birmingham, 1988). Although these sensitivities are not usually life threatening, the large numbers of chemicals that can cause sensitivity indicates the degree of the problems.

The ability of a chemical to induce sensitization is related to its ability to traverse the skin and react covalently with host biomolecules (Eisen et al., 1952; Goh, 1988). The chemicals are carried to regional lymph nodes via lymphatic Ia-positive Langerhans cells (Stingl et al., 1978; Silberberg-Sinakin and Thorbecke, 1980; Anderson et al., 1987; Botham et al., 1987; goh, 1988). Immune cells produced in a primary immune response are distributed to all body tissues. Further exposure to the sensitizing chemical and localized immune recognition induces a mononuclear cell infiltrate and the production of a contact sensitivity reaction. The reaction peaks at 12-24 hours (Goh, 1988). Neutrophils generally are not present with contact dermatitis, while they are in inflammatory responses, infection, or irritation.

It is not known why some individuals become sensitized and others do not, but genetic differences are thought to be important. In addition, dose plays a major role in sensitization and in elicitation of response (Karol and Thorne, 1988). Increasing the concentration of sensitizer applied to the skin, increasing the skin area exposed, applying the sensitizer to inflamed skin, and repeated exposures all increase the chance that sensitization will occur.

Photocontact Dermatitis

Photocontact dermatitis is also believed to be mediated by cell-mediated immune responses. The clinical characteristics of photocontact dermatitis closely resemble those of allergic contact dermatitis. The major difference is that photocontact dermatitis requires sunlight in addition to skin exposure to a sensitizing chemical, for initial sensitization and for subsequent elicitation of skin responses. Phototoxic reactions that can be produced in the absence of immunization are not considered here.

Sensitivity to photoallergens appears to occur by the same mechanisms as that for allergic contact sensitivity. However, ultraviolet or visible radiation participates in the induction of immunity and in contact dermatitis by converting an immunologically inactive form of the photosensitizing compound into its active form (Elmets, 1986). The exact mechanisms are not known and could vary depending on the photoallergen involved (Morison and Kochevar, 1983).

Many drugs and chemicals cause photocontact dermatitis (Elmets, 1986). They frequently share structural similarities to chemicals that cause allergic contact dermatitis (Harber and Baer, 1972). Photoallergens are usually lipid-soluble, low-moleccular-weight substances that act as haptens and include sulfanilamide, halogenated salicylanilides, phenothiazines, benzophenones, and diphenhydramine. The most common photoallergens are topical agents, although some systemically administered drugs that absorb solar energy when they reach the skin can produce photoallergic responses.

Contact Urticaria

Contact urticaria is mediated by IgE to antibody allergens. As with other IgE reactions, contact urticaria may appear from a few minutes to a few hours after the eliciting agent comes into contact with the skin. The response usually disappears within 24 hours. In cases of strong hypersensitivity or large doses of allergen, symptoms can occur in other organs, resulting in rhinitis, conjunctivitis, asthmatic attack, or even anaphylactic shock. The term contact urticaria syndrome has been used for these systemic responses (Maibach and Johnson, 1975).

The substances that cause immunologic

contact urticaria include foods, chemicals in medications, industrial chemicals, cosmetics, and many chemically undefined agents (Lahti and Maibach, 1987). Some of the allergens that cause urticaria have high molecular weight (von Krogh and Maibach, 1985).

Exposure to allergens that cause contact urticaria can be through the skin or by other routes, including the respiratory and gastrointestinal tracts. The production of IgE may occur in draining lymph nodes. The specific IgE antibody binds to mast cells in the skin and elsewhere. Upon exposure, the allergens penetrate the epidermis and cause release of mediators from IgE-bound mast cells or basophils. The cutaneous symptoms, erythema and edema, are elicited by these vasoactive substances. In addition to histamine, other mediators of inflammation are believed to be prostaglandins, leukotrienes, and kinins.

NONSPECIFIC IMMUNE ENHANCEMENT

An immunologic adjuvant is a substance that enhances the immunogenicity of another substance. Examples include mineral suspensions, such as alum, on which the antigen is adsorbed, and a water and mineral-oil mixture in which the antigen is emulsified. Adding killed mycobacteria to the emulsion further increases antigenicity (Freund's complete adjuvant).

The demonstration that xenobiotics in the environment can similarly enhance antigenicity has led to the concept of environmental adjuvants, illustrated by a study in which guinea pigs were exposed to ovalbumin with and without sulfur dioxide (Riedel et al., 1988). Compared with the control group, the group exposed to sulfur dioxide developed more bronchial reactivity on subsequent challenge with ovalbumin, and more ovalbumin-specific antibodies were found in serum and bronchoalveolar lavage fluid at a sulfur dioxide exposure of 0.1 ppm for 8 hours a day for 5 consecutive days. Hence, the presence of sulfur dioxide potentiated the development of allergy to the protein ovalbumin in this study, and sulfur dioxide can be considered an environmental adjuvant.

Similar effects have been noted in monkeys exposed to a platinum salt in the presence of ozone. Findings included increased bronchial reactivity and a higher incidence of skin reactivity to subsequent platinum salt exposure in animals, which received ozone with the platinum exposure (at a concentration of 1 ppm) relative to animals exposed only to platinum (Biagini et al., 1986).

Brief exposures to high concentrations of ozone (> 5 ppm), nitrogen dioxide (70 ppm), and sulfur dioxide (330 ppm) have been shown to enhance sensitization of ovalbumin in guinea pigs (Matsumura, 1970a,b,c). Low concentrations of ozone (0.8 or 0.5 ppm), comparable to environmental exposures administered over 3- to 4-day periods, enhanced sensitization of mice to inhaled ovalbumin (Osebold et al., 1980).

Several studies have documented an increase in the incidence of deaths from asthma in this country and abroad. For example, the prevalence of asthma among Michigan Medicaid patients increased from 2.0/100 in 1981 to 2.8/100 in 1986 (Gertsman et al., 1989). Asthma deaths increased in the United States, New Zealand, Great Britain, and other countries from the 1970s to the mid-1980s (Jackson et al., 1988). Analysis of possible artifacts, such as changes in certification and coding, supports a real increase in asthma mortality. Attempts to understand the increased death rate have centered on underestimation of the severity of asthma and inadequate use of corticosteroids (Sears, 1988). An understanding of the increased incidence has centered on air pollution in general, and sulfur dioxide in particular. Exacerbation of asthma during pollution episodes have been reported. For example, in Donora, Pennsylvania, in 1948, 88% of asthmatics experienced exacerbation of their asthma during a pollution episode (Schrenk et al., 1949). Other epidemics have

been reviewed (Sheppard, 1988), and it is pointed out that the present Occupational Safety and Health Administration (OSHA) standard of 5 ppm of sulfur dioxide exposure over an 8-hour shift is one order of magnitude higher than the concentrations (0.4 ppm) known to potentiate acute bronchospasm in asthmatics during moderate to heavy exercise (Roger et al., 1985).

In Japan, allergic rhinitis to pollen changed from 1950 to 1980 from a rare disease to the most common allergic disease. A survey of 153 Japanese school children up to age 7 (Muranaka et al., 1986, and references therein) found a high prevalence of allergic rhinitis to pollen in districts polluted by automobile exhausts. A Japanese group was able to demonstrate that diesel-exhaust particulate matter was an effective adjuvant for inducing specific IgE production to Japanese cedar pollen, the most prevalent cause of allergic rhinitis in Japan (Muranaka et al., 1986). Thus, age would not seem to be an important factor in these increasing hypersensitivity reactions. The mechanisms by which inhaled pollutants apparently enhanced the immune recognition of allergens are not understood.

BIOLOGIC MARKERS OF HYPERSENSITIVITY

History and Clinical Signs

As with the diagnoses of many diseases, a good history and physical examination are often the most useful indicators for hypersensitivity disorders and are therefore useful in supporting the establishment of biologic markers. A thorough history should provide information about the extent and duration of the exposure and, perhaps, about the immunogen. Answers to questions about the home and workplace often reveal important clues. It is sometimes difficult to distinguish clinical signs of hypersensitivity disease from those of more common illnesses. For example, patients with hypersensitivity pneumonitis usually have a history of repeated bouts of dyspnea, fever, cough, malaise, and weight loss. Frequently, a presumed diagnosis of "atypical" or "viral" pneumonia is made, and patients are treated with antibiotics, rather than removed from the environment with the offending chemical. One problem that complicates the diagnosis of chronic beryllium disease, for example, is the similarity of its clinical presentation to that of sarcoidosis, a systemic granulomatous disease of unknown etiology (Sprince et al., 1978).

Skin Tests

Results of direct skin tests (by intradermal infection, prick, or scratch) probably provide the most sensitive biologic markers of IgE-mediated hypersensitivity. They are most useful when the chemical induces antibody-or cell-mediated responses that result in skin reactions. Some antigens are also irritants or are biologically active and may produce nonspecific skin reactions that mask hypersensitivity responses.

Patch tests are frequently used to diagnose skin hypersensitivity. If a skin response is antibody mediated, vesiculation and a dermatitic reaction are observed shortly after application of the patch test. Urticaria with classic wheal-and-flare appearance tends to be relatively short lived, in contrast to cellular immune reactions, for which a positive response is frequently observed at 24-48 hours. The histologic changes of cellular immune reactions include spongiosis, vesiculation, upper dermal edema, and perivascular mononuclear infiltration. These effects are produced principally by combined actions of lymphokines released from activated lymphocytes in the skin.

Skin tests alone cannot be used to diagnose hypersensitivity (Metcalfe, 1989). Some individuals have positive skin tests but have no clinical manifestations of allergy.

Serum IgE Concentration

The antibodies responsible for immediate immune responses belong to the IgE subclass of immunoglobulin (Ishizaka et al., 1966; Johansson and Bennich, 1967), and total serum IgE is increased in young patients with asthma (Johansson and Bennich, 1967; Rowe and Wood, 1970). Based on these findings, investigators have attempted to distinguish extrinsic (allergic) from intrinsic (nonallergic) forms of asthma according to whether an elevated serum concentration of IgE can be demonstrated (Ostergaard, 1985). A large, general-population study has shown that, when test subjects are standardized for age and sex, the prevalence of asthma is closely related to serum IgE level (Burrows et al., 1989). The same study showed that allergic rhinitis, in contrast to asthma, could be indicated by skin test reactions to common aeroallergens, independently of the serum IgE level.

In Vitro Assays for Specific Antibody

In vitro assays can be used to identify antibodies (IgE or IgG) against specific antigens. Conjugates of low-molecular-weight chemical haptens with protein carriers are used most frequently to detect these antibodies. Albumin is often used as a carrier in assays for immune recognition to test chemicals conjugated with a hapten; such a conjugate could ellicit a negative response in a sensitized individual if it is not the same carrier-hapten conjugate to which the individual is sensitized. In vitro assays can be especially useful if the agent in question is toxic or an irritant.

Measurement of specific IgE antibody to low-molecular-weight chemicals is difficult. It must be remembered that the absence of specific IgE in the circulation does not mean that an individual has not been exposed to a chemical or is not sensitive to the agent. Although some investigators have implicated IgE in reactions to sulfites by passive-transfer testing, others have been unable to identify antisulfite IgE (Prenner and Stevens, 1976; Stevenson and Simon, 1981). It is possible that the clinical symptoms observed could result from an irritant effect of the sulfites rather than from production of IgE-specific antibody (Stevenson and Simon, 1981). Similar difficulties have been encountered with the measurement of isocyanate-specific IgE. Karol and Thorne (1988) discussed host-related problems associated with the measurement of isocyanate IgE.

The identification of specific antibody serves as a marker of exposure. Antibody produced to inhaled antigens can be identified in the blood of most individuals exposed to large quantities of antigens, but levels of specific antibody frequently do not correlate with disease (Pepys, 1986).

In Vitro Assays for Cellular Immunity

Lymphocyte stimulation and other in vitro assays for cellular immunity can be used as indicators of cellular immune-mediated reactions. Some antigens or chemicals, however, are either biologically active or are cytotoxic, making interpretation of lymphocyte stimulation assays difficult. Lymphocyte stimulation has been shown to be useful in the diagnosis of drug-induced occupational allergy (Stejskal et al., 1986). It is currently used to indicate sensitivity to beryllium and may be useful in the diagnosis of chronic beryllium disease. Antibodies to beryllium have also been detected in exposed workers (Clarke, 1991).

Provocation Challenges

Although specific inhalation challenges are not without risk and must be standardized, they provide a means to demonstrate sensitization in patients with occupational lung disease (Salvaggio et al., 1986; Salvaggio,

1987). The clinical responses vary and can be immediate or delayed in onset. Late asthmatic reactions are more frequent after exposure to low-molecular-weight sensitizers (Pepys and Hutchcroft, 1975; Mapp et al., 1986a). Late or delayed asthmatic reactions to provocation challenge are associated with a temporary increase of nonspecific bronchial hyperresponsiveness (Mapp et al., 1985, 1986a).

In addition to inhalation challenges in the laboratory, test exposures can be achieved by having patients return to the environment in question for observation of their response. Ingestion of a suspected toxic chemical with subsequent measurement of the clinical response also has been used as a diagnostic test (Bush et al., 1986; Sampson, 1986). In evaluating hypersensitivity, it is also best to use double-blind, placebo-controlled challenges (Metcalfe, 1989).

The use of environmental control units is a modification of a provocation challenge. An environmental control unit houses patients suspected of having sensitivities to the volatile organic compounds that are ubiquitous in our environment. The unit is constructed of building materials and is furnished to minimize outgassing of organic chemicals. There are no sources of combustion, such as gas or fuel oil furnaces, heaters, or appliances, inside the structure. The use of perfumes and colognes is restricted. The ventilation system is designed to reduce pollution levels in both intake and recycled air. Patients are housed in the unit and monitored for improvement. Challenges with chemicals or mixtures of chemicals are then performed to study the role these play in the patients' illnesses. Chemicals in foods and water are potential sources of sensitivity and should be considered simultaneously.

Bronchoalveolar Lavage

Bronchoalveolar lavage is often performed to provide lung cytology and biopsy samples for histopathology. The presence of increased numbers of neutrophils or eosinophils in bronchoalveolar lavage fluid is suggestive of occupational asthma (Fabbri et al., 1987; Lam et al., 1987). Bronchoalveolar lavage fluid from individuals with hypersensitivity pneumonitis generally has a large number of total cells and lymphocytes. The ratio of helper (CD4) to suppressor (CD8) T cells present in fluid from patients with hypersensitivity pneumonitis is <1, as opposed to 5:1 or more in that from patients with active sarcoidosis (Leatherman et al., 1984; Costabel et al., 1985). However, it is best to be cautious when basing diagnoses on cytologic observations. Positive findings represent exposure not disease. Individuals exposed to antigens known to cause hypersensitivity pneumonitis can have increased total cells and lymphocytes in their lungs even when there is no clinical disease.

ANIMAL MODELS FOR DETECTING CHEMICALLY MEDIATED HYPERSENSITIVITY

The development of animal models to detect and elucidate the mechanisms of chemically mediated hypersensitivity will improve our understanding of these important disorders. For animal models to be useful, the mechanisms of induction of sensitivity to chemicals and the response to exposure that leads to clinical and pathological changes should be similar to those observed in humans. Immune responses produced in target tissues (skin and lung) should have responses that are comparable to those in humans.

There are significant differences in the immunologic and inflammatory responses in various laboratory animals that have made it difficult to interpret data obtained from the variety of animals models used previously. Extrapolation of data from an animal model to humans is possible when the animal model has been carefully characterized regarding normal immune function and immunopathology.

The guinea pig has frequently been used to evaluate asthmatic responses to chemicals (Karol et al., 1981; Karol, 1983). Techniques using this species (including the monitoring of core temperature by telemetry) have been refined and validated as sensitive indicators of pulmonary hypersensitivity, and they show promise as predictors of these disorders in humans (Karol, 1988; Thorne et al., 1987b). Genetically hyperresponsive rats and dogs, sheep made hyperresponsive to ozone, guinea pigs sensitized to ovalbumin (Griffiths-Johnson and Karol, 1991) and mouse and rabbit models of late allergic reactions also have been used to study bronchial hyperresponsiveness (Tse et al., 1979; Woolcock, 1988). However, animal models usually require high doses of chemicals to induce asthmatic reactions. In addition, it is difficult to measure specific IgE in some models (Woolcock, 1988).

Animals have been used to study hypersensitivity pneumonitis induced by complex antigens or soluble proteins inhaled or instilled into the lung. Results of these studies have provided some understanding of pulmonary immunity. However, whether these reactions truly represent hypersensitivity is still in question. All test animals develop immunity after exposure of the lungs to some antigens. Rarely, however, do animals develop clinical disease comparable to that observed in the small percentage of humans with this disorder.

Rats, guinea pigs, and dogs have been used to evaluate the induction of immunity to instilled or inhaled beryllium, as well as the lung lesions induced by this agent (Schepers et al., 1957; Barna et al., 1981, 1984a,b). Dogs develop lung lesions similar to those seen in affected humans (Haley et al., 1989). Some strains of guinea pigs are sensitive and some insensitive to the induction of granulomas after instillation of beryllium. Some species require the instillation of relatively large doses of beryllium to induce immunity or lung lesions (Barna et al., 1981, 1984a,b), and in some animals, the lesions produced are histopathologically dissimilar to human lesions (Schepers et al., 1957).

Cellular responses in the lungs of various animals differ significantly after lung immunization (Bice and Shopp, 1988). Immune responses in the lungs of dogs appear to be similar to those of nonhuman primates, including chimpanzees. Dogs and nonhuman primates could prove useful as models to evaluate the effects of lung immunity on the development of pulmonary disease. Numerous species, including monkeys, mice, rabbits, dogs, and rats, have been used to investigate contact sensitivity. The guinea pig produces a reaction histologically and morphologically similar to that observed in humans (Mekori and Claman, 1986). Mice offer an advantage as a model, because there is considerable knowledge about the genetics and control of immunologic responses in this species. There are some differences in the duration of the sensitized state between mice and guinea pigs, with the response being relatively short-lived in mice (Karol, 1988). It has generally been difficult to sensitize experimental animals to orally administered chemicals reliably.

Techniques Used in Animal Studies to Determine the Potential of Chemicals to Cause Hypersensitivity

The major routes of exposure that elicit an immune reaction to a chemical are skin contact, pulmonary exposure (inhalation), and exposure by the oral route (gastrointestinal tract). The design and institution of an animal study depend on the anticipated route of exposure to the xenobiotic substance. Table 3-1 provides a summary of the tests that can be derived from toxicologic studies and special tests available for detection of chemicals that induce hypersensitivity. Animal studies used to predict the hypersensitivity responses originally described by Gell et al. (1975) are outlined below.

TABLE 3-1 Methods of Detecting Chemicals Producing Contact Hypersensitivity

Class	Name	Comments
Epicutaneous methods	Buehler Test[a]	Use of occlusive technique for both inductions and elicitation.
	Open Epicutaneous Test[b]	Screens many chemicals and finished formulations. Open epicutaneous for induction and closed patch for elicitation.
	Split Adjuvant Technique[c]	Increases sensitivity during induction phase. Epicutaneous induction with adjuvant injected i.d.; occlusive patch used for elicitation.
Intradermal methods	Draize Test[d]	Intradermal induction and elicitation.
	Optimization Test[e]	Intradermal induction and epicutaneous elicitation.
	Freund's Complete Adjuvant Test[f]	Intradermal induction with and without Freund's Complete Adjuvant and epicutaneous elicitation.
	Mouse-Ear-Swelling[g,h]	Epicutaneous induction with adjuvant on day 0 elicitation on ear and measure ear thickness.
Combinations	Guinea Pig Maximization Test[i]	Intradermal induction with and without Freund's Complete Adjuvant and epicutaneous elicitation.
	Split-Adjuvant Technique[j]	Epicutaneous induction with adjuvant injected i.d.; occlusive patch used for elicitation.

[a] Buehler (1964)
[b] Klecak (1985)
[c] Maguire (1972)
[d] Draize (1955)
[e] Maurer (1985)
[f] Klecak (1985)
[g] Gad et al. (1986)
[h] Stern et al. (1989)
[i] Magnusson and Kligman (1969)
[j] Maguire (1972)

IgE-and IgG-Mediated Immediate Reactions

An immediate hypersensitivity response usually can be identified in routine toxicologic studies. Continuous daily exposure of guinea pigs, dogs, or primates provides an opportunity for a chemical to induce an immune response, allowing for adverse effects to be identified. Urticaria, asthmatype reactions, and cardiovascular collapse are indicators of immediate hypersensitivity. Because the guinea pig is considered a sensitive animal for hypersensitivity responses, special studies often use this model, with erythema, edema, urticaria, pulmonary distress, and other clinical signs of anaphylactic shock as indicators of a hypersensitivity reaction (Karol, 1988). In some species, adjuvants are used to increase the probability

of producing an immediate hypersensitivity response, but they are unnecessary with guinea pigs.

Cytotoxic Reactions

As with the anaphylactic reactions, production of major antibody to an antigenic determinant on a cell often will be manifested during subchronic or chronic toxicologic studies. Reductions in erythrocytes or platelets, for example, can indicate such a response, and special studies can be performed to investigate this possibility. Such studies are often carried out to determine whether antibodies bind to target cells and can in turn agglutinate or lyse them in the presence of the complement. Ligand-binding and histochemical assays that have been used to measure drug-induced antibodies bound to cell membranes include the use of fluorescent antibody and radiolabeled staphylococcal protein A; monoclonal-antibody techniques also have been used. These tests, as well as more traditional assays, such as the Coombs test for detecting red-cell agglutinins, are increasingly used in toxicologic research.

Antigen-Antibody Complexes

Examples of hypersensitivity disorders in which antigen-antibody complexes are deposited on cell membranes include immune-complex glomerulonephritis, pneumonitis, and vasculitis. Such disorders would usually be detected over the course of subchronic or chronic toxicity studies. The conditions can be detected clinically during the live phase of these studies, but they are more often diagnosed by histologic and cytologic examination, employing some of the techniques listed above, to detect immune complexes deposited in tissues.

Contact Hypersensitivity

Contact-hypersensitivity response is mediated by T cells and is classically demonstrated 24-48 hours after challenge. Although this type of response has been best described and investigated in the skin, it is believed to occur in other organs, such as the lung. Contact-hypersensitivity responses to chemicals have characteristics similar to the Jones-Mote response, in that the reaction decreases after 48 hours and is transferable with T cells. Like the tuberculin response, contact hypersensitivity lasts up to 72 hours.

Many skin-sensitization procedures are available for examining xenobiotic-induced contact hypersensitivity. Guinea pigs have been used in risk assessment for more than 20 years. Table 3-1 shows approaches available to demonstrate contact hypersensitivity. Guinea pig skin is used most widely for these studies, but the mouse-ear-swelling test (MEST) and the lymphocyte proliferation assay have shown promise as methods for detecting and investigating the mechanism of hypersensitivity reactions (Gad et al., 1986). The ability of the MEST to demonstrate dose-response sensitization is an important concept for biologic monitoring (Thorne et al., 1987a).

The methods used to test for the skin contact-hypersensitivity response can be organized into three classes, and details of the guinea pig methods are described by Klecak et al. (1977), who also describe several variables that are important in using the guinea pig to detect contact sensitizers. Figure 3-1 illustrates the major features necessary for a chemical to produce an initial sensitization and the elicitation of hypersensitivity response in the skin. Two phases, an induction phase and an elicitation (challenge) phase, are illustrated. Figure 3-2 shows and compares selected methods for detecting chemical contact sensitizers.

The aims of these testing procedures are

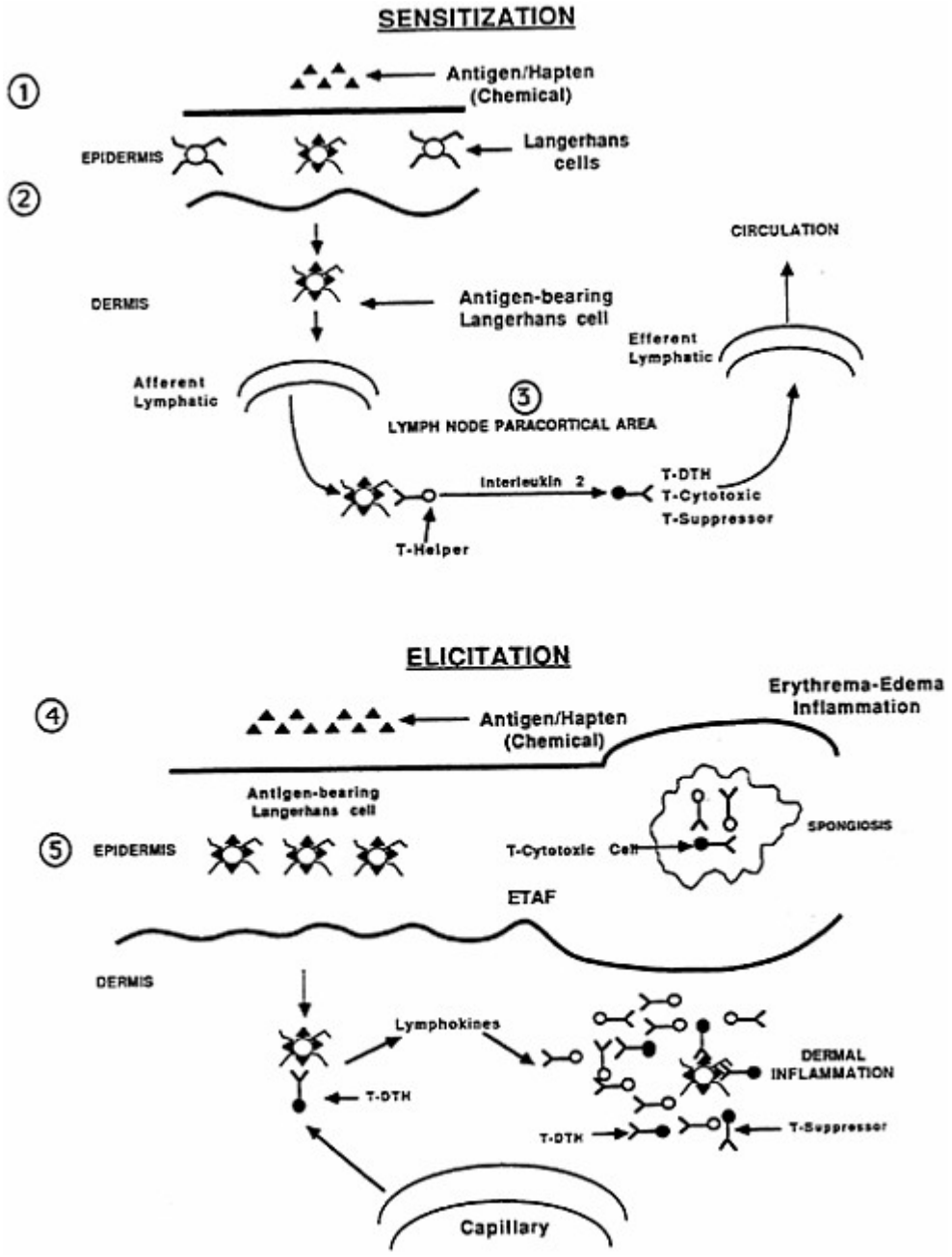

FIGURE 3-1 Schematic of the initial sensitization and the elicitation of hypersensitivity response on subsequent exposure.

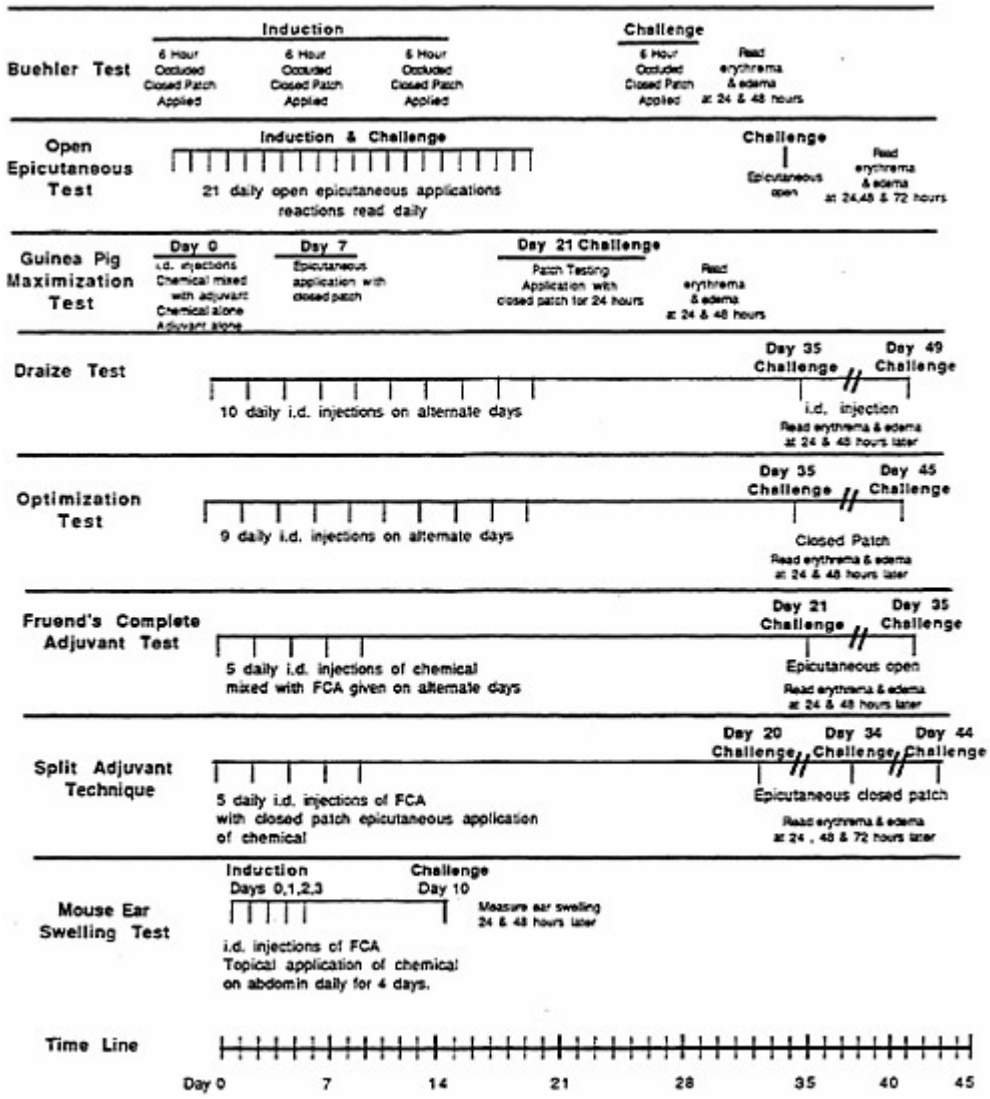

FIGURE 3-2 Schematic representation of selected methods for detecting chemicals producing contact hypersensitivity.

twofold: first, to optimize the penetration of the chemicals through the skin to immunocompetent cells; and second, to amplify any immunologic response that occurs but is not manifested to a level that overcomes "background noise." The Buehler test (Buehler, 1964) maximizes penetration by a topical closed-patched technique that resembles the human patch test. In the optimization test (Maurer, 1985), the chemical is injected intradermally. The guinea pig maximization test described by Magnusson and Kligman (1969) uses both intradermal and epicutaneous exposure. Because there is concern about using intradermal injections for predicting contact hypersensitivity, Jordan (1982) reported the use of dermabrasion as a means of enhancing penetration. Amplification of the response is also accomplished with adjuvants. The Freund's complete adjuvant test and guinea pig maximization test are two examples of assays that employ adjuvants (Klecak, 1985).

Guinea pig hypersensitivity assays, although sensitive and reproducible, are limiting, in that they often require subjective evaluation. Irritating chemicals or colored compounds are difficult to score. For the most part, to differentiate immune from irritating reaction, nonirritating concentrations are used to challenge the animal. This raises concern that not enough chemical has been made available to elicit the immune memory response. Erythema and edema are the end points for the immune response in all the tests described.

Efforts are under way in many laboratories to develop and validate more quantitative "immunologically based" assays for species other than the guinea pig. In vivo murine assays being examined include the ear swelling, cellular influx, and local lymph node proliferation and infiltration tests. Gad et al. (1986) showed the potential usefulness of the mouse as an experimental animal. Recently, Gad reported on the comparison of four methods (the Buehler test, the guinea pig maximization test, the epicutaneous maximization test, and the MEST) with human data to show that all four tests can generate data that are useful for a relative-hazard classification process (Gad, 1988). Kammüller et al. (1989) reported the usefulness of a popliteal lymph node (proliferation) assay in mice.

Stern et al. (1989), by radiolabeling the immune cells of mice before challenge, measured immune-cell localization in the challenge site. Kimber and Weisenberger (1989) have used a node proliferation assay in the mouse to differentiate between immune-system-mediated effects and irritation. Some of the in vitro tests under study include monitoring of lymphocyte blastogenesis, cytokines, and acute-phase proteins. In addition, several assays use cytologic or histologic markers, such as ICAM-1 (intercellular adhesion molecule-1) in mice and cutaneous basophil hypersensitivity in guinea pigs. The in vitro assays, coupled with cell identification with histologic markers, are clarifying the mechanisms of chemical hypersensitivity.

SUMMARY

Hypersensitivity disease resulting from inhalation of, dermal exposure to, or ingestion of chemicals contaminating the workplace and the environment has become an important health problem in industrialized societies. The ability of a chemical, particularly a low-molecular-weight chemical, to induce sensitization is generally related to its ability to couple covalently to body proteins. Sensitization, however, does not necessarily lead to disease.

Inhalation of a variety of chemicals can sensitize individuals and result in asthma, rhinitis, pneumonitis, or chronic granulomatous pulmonary disorder. The increasing prevalence of and mortality from asthma is a serious health problem that needs urgent attention. Animal models suggest that sulfur dioxide and ozone exposures can intensify

allergic asthma. Current standards, for the general environment as well as the workplace, particularly for sulfur dioxide, might be adequate to protect healthy individuals from acute bronchospasm, but may be inadequate to protect individuals who have asthma or who may be disposed to develop asthma.

Chemicals ingested in food can cause food hypersensitivity or food intolerance, a nonimmunologic response. Food hypersensitivity can be associated with a wide range of clinical symptoms that often are difficult to distinguish from food intolerance.

Similarly, exposure of skin to some chemicals can result in allergic contact dermatitis. Chemicals can also act as primary irritants or as agents that induce a nonspecific release of inflammatory mediators. Distinguishing among these conditions can present a diagnostic challenge.

Animal models used to predict the likelihood of chemicals to produce hypersensitivity may be limited in predicting the incidence of response in humans because of hereditary and psychologic factors that influence these reactions. Some models currently under development show promise as research tools to determine mechanisms of sensitization and to establish the toxic potential of xenobiotic substances.

Established and validated biologic markers of hypersensitivity include skin tests, in vitro assays for specific antibodies (IgE, IgG) or cellular immunity (lymphocyte stimulation), provocation challenges, and the cytologic evaluation of bronchoalveolar lavage fluid. These most frequently serve as markers of exposure or susceptibility. Our ability to develop biologic markers for hypersensitivity disease is impaired by our limited understanding of disease mechanisms, predisposing factors, and epidemiology of these disorders, as well as by species differences in response to chemical exposure.

RECOMMENDATIONS FOR FUTURE RESEARCH

IgE Sensitivity

Immunologically Mediated Adverse Reactions

It is difficult to differentiate between IgE-specific responses to chemical haptens and adverse reactions that cause clinical symptoms by nonspecific mechanisms (e.g., the release of mediators). Data are needed about the mechanisms of nonimmunologically mediated sensitization, including identification of the cells and mediators involved. The role of pulmonary inflammation produced by inhaled chemicals in the induction of asthma should be investigated.

Local Versus Systemic Immunity

The role of locally produced IgE, as opposed to systemic IgE, in the induction of clinical symptoms should be addressed, since clinical responses do not always correlate with the levels of systemic IgE. The severity of disease may be controlled by the level of IgE in local mucosal tissues.

Sensitizers

Many chemical sensitizers have been identified. The mechanisms of hapten binding, the translocation to lymphoid tissues, and the establishment of localized immunity in mucosal tissues and skin need to be examined. Studies are needed to determine whether there are differences in the mechanisms of sensitivity to high-versus low-molecular-weight allergens. These studies should include comparisons of initial induction of immunity in lymphoid tissues and the accumulation of immune cells in mucosal tissues and skin that influence localized immune responses.

Individual Differences in Sensitivity

We know that allergic diseases exist, but there is little information available to determine why some individuals become sensitized or have adverse reactions to chemicals and others do not. The differences between individuals need to be identified, and markers useful in identifying susceptible persons need to be developed.

Development of Self-Antigens

It is possible that altered self-antigens result from reactions between low-molecular-weight haptens and tissues. The response to altered self-antigens might be responsible for the production of disease in some individuals. The possible existence of self-antigens after exposure to chemicals should be examined. Such products (adducts) would also serve as markers of chemical exposure.

Effects of Exposure Conditions

Animal data indicate that the incidence and severity of pulmonary and dermal hypersensitivity reactions are related to the amount of exposure. More studies are needed on the influence of dose and dose schedule on sensitization and its clinical manifestation.

Epidemiology

Although information on hypersensitivity and adverse reactions to chemicals is frequently updated, better data are needed to define the magnitude of this problem. This is especially true for adverse reactions to ingested chemicals. There are few epidemiologic studies to indicate the incidence of hypersensitivity or intolerance to food additives.

IGE AND CELLULAR IMMUNITY

Cellular and Antibody Immunity

Cellular and antibody responses have been identified in hypersensitivity disease, such as in hypersensitivity pneumonitis. However, data are still needed to clarify the roles played by antibody and cellular immunity in the production and maintenance of disease.

Individual Variability

Most exposed individuals develop an immune response to inhaled antigens. The reasons that only a few individuals develop lung disease are not known, but it seems likely that disease is produced selectively because of disorders in immune regulation in the lung. More data are needed to differentiate the exposures and responses that lead to immune-mediated disease from those that produce immunologic changes but not disease. For example, many exposed individuals have large numbers of lymphocytes in their lungs, yet are asymptomatic. What is the function of these immune cells? Is it possible that they provide immune protection by assisting in the recognition and elimination of offending antigens?

Local Versus Systemic Immunity

Systemic immune responses can be measured in patients with hypersensitivity disorders. However, the role of systemic responses, compared with the induction of local immunity, is not understood. Local immune responses in tissues could be considerably more important than systemic immunity in the production of clinical symptoms.

Organic Versus Chemical Hypersensitivity

Many biologic materials have been identified

that can induce hypersensitivity pneumonitis. Relatively few chemicals, however, have been associated with this disease. Studies are needed to determine whether immune-system responses to chemical haptens are as likely to induce hypersensitivity pneumonitis as are immune-system responses to inhaled organic antigens.

Effects of Exposure

It appears that peak exposure is more important than time-weighted exposure in the induction of hypersensitivity to chemicals. Data are needed to determine the nature of exposures most likely to lead to induction of sensitivity to chemical haptens. Clarification is needed of both induction of sensitivity and development of disease after brief high-level exposures and the effect of repeated exposure over defined periods.

Particle Composition

Many antigens that lead to hypersensitivity are complex (high-molecular-weight) materials. Data are needed to illuminate the effects of particle composition on the induction of sensitivity and clinical disease.

Chemical Haptens

Additional data are needed on the mechanisms involved in the induction of immunity to chemical haptens, such as TDI and beryllium. In most instances, the biomolecules bound by the hapten have not been identified.

4

Autoimmune Diseases

This chapter deals with autoimmune diseases caused by exposure to environmental chemicals. The first portions discuss current understanding of the problem, that is, the definition, scope, and relative contributions of innate genetic susceptibility to environmental agents in producing autoimmune diseases. The next section deals with the possible mechanisms by which xenobiotics can induce autoimmunity. It includes a consideration of the lessons that can be learned from the study of animal models. The end of the chapter discusses the availability of potential biologic markers of autoimmunity and of autoimmune diseases.

DEFINITION OF THE PROBLEM

Autoimmune diseases are those in which an individual's own immune system attacks one or more tissues or organs, resulting in functional impairment, inflammation, and sometimes permanent tissue damage. Autoimmune diseases result from the loss of immune tolerance to self-antigens, and an immune response to one or more relevant tissue antigens can be demonstrated. Sometimes, however, when no specific antigen can be detected, an autoimmune process can be inferred if there is inflammation of a tissue or group of tissues that show no evidence of infection and if the condition responds to immunosuppressive therapy. Exposure to xenobiotic substances is associated with certain autoimmune diseases; Table 4-1 provides a partial list.

The autoimmune response can produce disease directly by means of circulating antibody, indirectly through the formation of immune complexes, or as a consequence of cell-mediated immunity. In most cases, more than one pathogenetic mechanism manifests itself. In myasthenia gravis or α-methyldopa-induced autoimmune hemolytic anemia, antibodies against the acetylcholine receptor at the neuromuscular junction or the red blood cell membrane can be confirmed. One example of prominent cellular immunity is autoimmune thyroiditis, in which the lymphocytes infiltrate the thyroid gland. Immune complexes also can be involved, as in lupus nephritis.

INCIDENCE OF AUTOIMMUNE DISEASES

Autoimmune diseases vary widely in different populations, displaying both geographic

and temporal variations. The prevalence of some putative autoimmune diseases is listed in Table 4-2. Crohn's disease was not described until 1932 (Crohn et al., 1932). Rheumatoid arthritis is also relatively new and is less common than other forms of arthritis manifested in Old World skeletal remains (Woods and Rothschild, 1988). The

TABLE 4-1 Xenobiotics Incriminated in Human Autoimmunity[a]

Xenobiotic	Association
Heavy metals	
Gold	Immune-complex glomerulonephritis
Cadmium	Immune-complex glomerulonephritis
Mercury	Immune-complex glomerulonephritis
Pharmaceuticals	
Lithium	Autoimmune thyroid disease
Penicillin	Autoimmune hemolytic anemia
Penicillamine	Myasthenia gravis
	Pemphigus
	Autoimmune thyroid disease
	Autoimmune hemolytic anemia
α-Methyldopa	Autoimmune hemolytic anemia
	Autoimmune hepatitis
Pyrithioxine	Pemphigus
α-Mercaptopropionylglycine	Pemphigus
Captopril	Pemphigus
Amiodarone	Autoimmune thyroid disease
Oxyphenisatin	Autoimmune hepatitis
Halothane	Autoimmune hepatitis
Organic solvents, industrial chemicals	
Hydrazine	Systemic lupus erythematosus
Polybrominated biphenyl	Autoimmune thyroid disease
Polychlorinated biphenyl	Autoimmune thyroid disease
Vinyl chloride	Systemic sclerosis
Silica dust	Systemic sclerosis
Chemicals in foods, food additives	
Tartrazine	Systemic lupus erythematosus
Alfalfa sprouts	Systemic lupus erythematosus
Adulterated rapeseed oil	Systemic sclerosis

[a] Some of the associations reported here are controversial and are included merely for the sake of completeness. Inclusion in this table does not mean that the members of the subcommittee believe that each association is firmly established.

incidence of Crohn's disease is increasing in several geographic areas. In central Israel, the incidence increased from 0.33/100,000 in 1970 to 3.1/100,000 in 1979 (Fireman et al., 1989). Over similar periods, increases have been documented in Alberta, Canada (Pinchbeck et al., 1988), and in northern Europe (Binder, 1988), suggesting a xenobiotic or infectious environmental contribution. Trends and geographic variations are useful, if difficult, in clarifying the epidemiology and etiology of autoimmune disease.

TABLE 4-2 Autoimmune Disease Related to Specific Xenobiotic Exposure with Some Annual Incidencea

Disease	Substance	Annual Incidence
Systemic lupus erythematosus	Pharmaceuticals, hydrazine, tartrazine, alfalfa sprouts	6 to 35 per 100,000
Autoimmune hemolytic anemia	Pharmaceuticals	
Myasthenia gravis	Penicillamine	2 to 5 per million
Pemphigus	Penicillamine, pyrithioxine, α-mercap-topropionylglycine, captopril	
Glomerulonephritis	Pharmaceuticals, heavy metals (mercury, cadmium, gold)	
Autoimmune thyroid disease	Polybrominated biphenyl, polychlorinated biphenyl, lithium, penicillamine, amiodarone	
Autoimmune hepatitis	α-Methyldopa, oxyphenisatinn, halo-thane	
Scleroderma	Vinyl chloride, silica dust	4.5 to 12 per million

[a] Some of the associations reported here are controversial and are included merely for the sake of completeness. Inclusion in this table does not mean that members of the subcommittee believe that each association is firmly established.
Source: Bigazzi (1988) and Wyngaarden and Smith (1988).

SUSCEPTIBILITY VERSUS EXPOSURE

There is a familial clustering of many autoimmune diseases, suggesting a genetic predisposition. The human leukocyte antigen (HLA) system, which is a classification of cell-surface glycoproteins on lymphocytes and macrophages, is an important marker of susceptibility for 40 or more diseases. The influence of this system can play a dominant role in the development of autoimmune disease. The association between the HLA-B27 (class I antigen) haplotype and ankylosing spondylitis (suspected to be of autoimmune origin) is a striking example. Ninety percent of patients and 9% of normal subjects having this haplotype have a relative risk of 87 (87 times the normal rate of disease). The risk varies from country to country, and in the United States there is a significant

racial difference; HLA-B27-positive blacks have a lower risk than whites (McDevitt, 1985).

For rheumatoid arthritis, as for most other autoimmune diseases, the role of genetics is much less pronounced; 50% of patients and 20% of healthy persons have the HLA-DR4 haplotype for a relative risk of 4 (McDevitt, 1985). The mechanism by which this haplotype influences susceptibility to autoimmune disease is still unknown, but several possibilities have been proposed: The class II MHC (major histocompatibility complex) product binds the autoantigen firmly and presents it to the reactive T cell; the class II MHC product expressed by the thymic epithelium shapes the T-cell repertoire; or an unknown disease-susceptibility gene is in linkage disequilibrium with the class II MHC gene.

A number of other genetic traits are associated with an increased prevalence of autoimmune disease. Studies of human populations have shown that certain immunoglobulin allotypes (genetically determined markers on immunoglobulin molecules) are associated with a greater risk of developing particular autoimmune diseases, such as Graves' disease and insulin-dependent diabetes mellitus. Polymorphisms of the β chain of the T-cell receptor, as determined indirectly by restriction fragment length analysis of the genes, also seem to be associated with susceptibility to some autoimmune diseases (Demaine and Welsh, 1988).

Experimentally induced autoimmune diseases require that particular genes encode the variable regions of the T-cell receptor (Heber-Katz, 1990). If such findings pertain to humans, an important new biologic marker will have been discovered.

XENOBIOTIC-INDUCED AUTOIMMUNITY

Several autoimmune diseases associated with exposure of susceptible individuals to organic chemicals are listed in Table 4-2 (Bigazzi, 1988; Kammüller et al., 1988). It is clear that illnesses that meet the diagnostic criteria for systemic lupus erythematosus (SLE) can occur after exposure to a number of pharmaceuticals, including hydralazine, procainamide, phenytoin, and isoniazid (Weinstein, 1980). With hydralazine-induced lupus, susceptibility has been linked to the patient's rate of acetylation of the drug (Godeau et al., 1973; Perry, 1973; Reidenberg and Martin, 1974; Strandberg et al., 1976; Batchelor et al., 1980). Because many cases of lupus are not related to drug use, the distinction between drug-induced and idiopathic lupus has been made. However, some clinicians have challenged the notion that lupus not related to pharmaceutical exposure is idiopathic. Occupational exposure to the organic solvent hydrazine causes SLE in slow acetylators with the HLA-DR3 phenotype (Reidenberg et al., 1983). Tartrazine, a yellow dye used as an artificial coloring agent in food and drugs, has been associated with SLE-like illness (Pereyo, 1986). Ingestion of alfalfa sprout causes SLE-like illnesses in monkeys (Malinow et al., 1982).

Some patients with a genetic predisposition do not develop autoimmunity, whereas patients without a family history of the associated HLA haplotype can. Attention has focused increasingly on environmental influences. Ingestion of dietary iodine can lead to the expression of autoimmune thyroiditis. Pollutants such as heavy metals have been implicated in some cases of immune-complex glomerulonephritis. Infections and hormonal balance also can play a role in the induction of an autoimmune state.

In some cases, a combination of diet, chemical exposure, genetic susceptibility, infection, and hormonal balance can act synergistically to induce an autoimmune state; in others, an overwhelming environmental

exposure or some other single factor could be the cause. Individuals with a genetic predisposition could be at increased risk from a given environmental exposure, as was seen in the Spanish toxic oil syndrome.

The Spanish toxic oil syndrome, an epidemic of disease in Madrid and a surrounding region that began in May 1981, was probably related to ingestion of anilinedenatured rapeseed oil, although the actual toxicant has never been identified definitively. A disease developed in approximately 20,000 people beginning about one week after they had ingested the adulterated cooking oil. Their signs and symptoms were fever, rash, pruritus, interstitial pneumonitis, dyspnea, eosinophilia, thrombocytopenia, arthralgias, myalgias, malaise, intrahepatic cholestasis, gastrointestinal symptoms, intrahepatic cholestasis, and sometimes lymphadenopathy (Noriega et al., 1982). Most of the victims made an uneventful recovery, but approximately 15% developed features suggestive of autoimmune disease, with a scleroderma-like illness, Sjögren's syndrome; pulmonary hypertension; Raynaud's phenomenon; and dysphagia (Fernández-Segoviano et al., 1983; Alonso-Ruiz et al., 1986). There was an increased incidence of the HLA-DR3 and HLA-DR4 phenotypes among those who developed chronic disease (Vicario et al., 1982; Kammüller et al., 1988). Another epidemic occurred in the Netherlands in 1960 when 20,000 of 600,000 individuals who ate a new brand of margarine developed erythema multiforme (Mali and Malten, 1966). These examples illustrate the implications of environmental xenobiotics in a variety of autoimmune disorders.

A somewhat similar picture of progressive systemic sclerosis has been associated with exposure to trichloroethylene (Lockey et al., 1987). An outbreak of disease with strikingly similar signs has been described in individuals ingesting preparations of L-tryptophan (Belongia et al., 1990). There are case reports from Japan (Kumagai et al., 1979, 1984), Europe (Byron et al., 1984), and the United States (Varga et al., 1989, and references therein) of women who developed features of autoimmune disease, such as scleroderma, after receiving mammary injections or implants of silicone for cosmetic reasons. There is controversy about this association, because the calculated incidence in the United States among women with augmentation mammoplasty is no greater than that found in the population at large. Studies using animal models have suggested that silicone can trigger persistent fibrosis and inflammation, but have provided no evidence of autoimmunity (Ballantyne et al., 1965).

MECHANISMS

In most cases of autoimmunity associated with xenobiotic exposure, the precise mechanism by which the xenobiotic substance induces an autoimmune process is unknown. In some areas, however, the pathogenesis of these disorders is coming into focus. Drug-induced immune cytopenias are among the best studied examples of these conditions, partly because of the accessibility to the target tissue and the ability to monitor changes over time. For example, four mechanisms have been identified for drug-induced immune hemolytic disorders (Petz and Garratty, 1980). The drug can attach to the cell membrane, where it interacts with a drug-specific antibody. Alternatively, the drug could modify the cell membrane so that the patient's immune system regards the cell as foreign. Immune complexes formed between the drug and its respective antibody could adhere to the membrane to produce injury. A fourth mechanism involves red-cell sensitization due to production of red-cell autoantibody and is, in that sense, the only true autoimmune form of these reactions. Procainamide (Kleinman et al., 1984), α-methyldopa (Worlledge et al., 1966), and nomifensine

(Salama et al., 1989) are all examples of drugs involving red-cell sensitization. Procainamide also is associated with other autoimmune phenomena (Blomgren et al., 1972; Henningsen et al., 1975).

The immunopathy is thought to arise by blocking of the normal immunosuppression against self-antigens. Consistent with this is the observation that low doses of cyclophosphamide, also known to suppress suppressorcell activity differentially, can reliably induce red-cell autoantibodies in mice (Hutchings et al., 1985). Exogenous chemicals can induce the production of novel adducts that might serve as triggers of an immune response. For example, halothane-exposed guinea pigs and rabbits develop trifluoroacetylated (TFA) proteins, which evoke anti-TFA antibodies. Some of these animals show liver injury that resembles the hepatitis seen in some patients after halothane anesthesia (Hubbard et al., 1989).

Several recent observations suggest that immune-mediated damage occurs through a combination of mechanisms. Sera from patients with hemolytic anemia caused by drug-specific antibodies have occasionally been shown to react with red blood cells alone, suggesting the presence of true autoantibodies (Florendo et al., 1980; Habibi, 1985), which are produced by a host to its own tissues. Autoantibodies are found along with drug-dependent antibodies in patients with drug-induced immune neutropenia (Salama et al., 1989) and thrombocytopenia (Lerner et al., 1985).

The administration of high doses of a cephalosporin, one of a class of drugs thought to act principally as haptens, has recently been shown to induce red-cell, neutrophil, and platelet antibodies in dogs that can be demonstrated ex vivo in the absence of the drug (Bloom et al., 1988). These observations serve to further blur the line between hypersensitivity and xenobiotic-induced autoimmune disorders. The above mechanisms have been well studied, and similar mechanisms are known to play a role in xenobiotic-induced immune damage involving other tissues. In addition, cell-mediated immunity is thought to be important in some diseases with features of autoimmunity. For example, lymphocytes and macrophages infiltrate the myelin sheaths of patients with multiple sclerosis (Adams, 1983).

ANIMAL MODELS

The many animal models of autoimmune diseases that have been studied fall into three groups.

1. *Spontaneous autoimmunity*. A good model is the well-studied New Zealand Black mouse, in which both sexes spontaneously develop autoimmune hemolytic anemia, B-and T-cell defects, hepatosplenomegaly, and glomerulonephritis because of a genetic predisposition (Milich and Gersjwon, 1980).
2. *Experimental autoimmunization*. In these models, animals receive injections of autoantigens and an appropriate adjuvant. An example is experimental allergic encephalomyelitis (EAE), in which demyelinating lesions accompanied by neurologic deficits suggestive of multiple sclerosis are produced by injection of myelin basic protein accompanied by an appropriate antigen. Adoptive transfer of EAE can be performed by infusions of lymphocytes (Paterson, 1960).
3. *Chemically induced autoimmune disease*. The animal is exposed to a chemical that induces an autoimmune disease. Pharmaceutical agents thought to induce autoimmunity in humans have been studied in animals, and species variability and strain variability have been observed. For example, hydralazine and procainamide given to BALB/c strain mice and A strain mice induce antinuclear antibodies in both strains; the A strain mice, but not the BALB/c strain mice, develop glomerulonephritis (Ten Veen and Feltkamp-Vroom, 1973). Canavanine, the arginine analogue, induces double-stranded

DNA antibodies in mice (Prete, 1985). Canavanine occurs in alfalfa sprouts and induces an autoimmune disease similar to lupus in primates (Malinow et al., 1982). The lesson learned from animal models of autoimmunity is that genetic influences, environmental exposure, or in some cases a combination of the two can lead to autoimmune disease. Our knowledge of autoimmune phenomena in humans suggests a similar situation.

BIOLOGIC MARKERS

Physical signs and symptoms can support the validation of biologic markers of autoimmune diseases. For example, characteristic joint deformities are indicative of rheumatoid arthritis. Diagnoses are ultimately made on clinical grounds, with constellations of signs and symptoms forming the patterns from which diseases are diagnosed. Likewise, historical data can be used to confirm both a marker of susceptibility to autoimmunity and a marker of effect. For example, the validity of the biologic marker is supported in cases in which a family history of an autoimmune disease suggests that family members are at increased risk or when historical data for a given region or group of people show a consistently high incidence of a given disease.

MAJOR HISTOCOMPATIBILITY COMPLEX

The HLA system can provide a marker of susceptibility to certain immune-system-mediated diseases, ranging from ankylosing spondylitis (associated with HLA-B27) to pemphigus vulgaris (associated with HLA-Rw4). These associations are not absolute; large numbers of healthy persons have susceptibility markers, and many affected persons do not. The relative risk of these diseases could vary with geography, sex, and race. Nevertheless, these markers are important research tools that have value in elucidating the epidemiology of the diseases, and they can, in selected cases, be an aid to diagnosis. Their clinical utility in autoimmune diseases is limited, and HLA typing should not be done routinely on all patients with autoimmune phenomena.

A closer association of HLA genes with autoimmune disease has been reported when alleles are identified by means of oligonucleotide sequences or individual amino acid products, instead of depending on the usual serologic reagents (Kwok et al., 1988; Nepom et al., 1989).

IMMUNOGLOBULIN ALLOTYPES

The genes of the major histocompatibility complex (MHC) and the genes that control the constant region of the immunoglobulin heavy chain regulate the immune response in experimental animals and humans. In humans, IgG molecules are polymorphic at the Gm locus, and a number of associations between Gm allotypes and autoimmune diseases have been described (Nakao and Kozma, 1988). Farid et al. (1977) reported an association between Gm phenotype f and autoimmune thyroiditis. Field et al. (1984) found a correlation between Glm(2) and increased susceptibility to insulin-dependent diabetes in individuals who had the HLA-DR4, but not the HLA-DR3, haplotype. Thus, there are interactions between MHC and Ig determinants. In the presence of a particular MHC haplotype, a particular Ig allotype will increase the risk of autoimmune disease.

OTHER GENETIC MARKERS

The presence of thyroid-specific autoantibodies is frequently an indicator of autoimmune thyroid disease. Burek et al. (1984) studied the association of a number of genetic

markers with thyroid autoantibodies. They found that four genetic traits—HLA, Gm, ABO blood group, and Rh blood group—showed weak but significant associations with autoantibodies, but that in the aggregate these markers were quite predictive. The predisposition to autoimmunity is polygenic, and the greatest susceptibility is the result of inheritance of a number of genetic traits, which often act through different pathways (Rose and Burek, 1985).

RATE OF ACETYLATION

The liver enzyme acetyl transferase metabolizes many drugs, such as isoniazid and hydralazine. The genes that control the level of this enzyme are polymorphic and separate the population into two groups: slow and fast acetylators. Among patients who ingested low total amounts of hydralazine, 60% of the slow acetylators developed the antinuclear antibodies characteristic of lupus, whereas none of the fast acetylators did (Perry et al., 1970). Persons who are slow acetylators of some drugs have an increased incidence of drug-induced lupus. Hence, slow hepatic acetylation of a drug also is a marker of susceptibility to drug-induced lupus.

Antinuclear Antibodies

Antinuclear antibodies are detected by incubating a tissue section with the subject's serum and then with a fluorescein-labeled antihuman antibody. If the subject is producing antibody to a nuclear constituent, that constituent will fluoresce in ultraviolet microscopy. Antinuclear antibodies are markers for a number of autoimmune diseases, the most notable of which is systemic lupus erythematosus (Ferrell and Tan, 1985). Antibodies to specific nuclear constituents are high specific for certain collagen vascular diseases. These tests are extremely valuable in making specific diagnoses and play a major role in clinical medicine. They can also be important in epidemiologic surveys of xenobiotic-exposed populations.

Specific Tissue Autoantibodies

Serum antibodies to specific tissue antigens detected by immunofluorescence, radioimmunoassay, enzyme-linkedimmunosorbent assay (ELISA), immunoperoxidase, and other highly sensitive techniques have been useful in the diagnosis of certain autoimmune diseases. For example, the antibodies for antigens in the kidney basement membrane found in Goodpasture's syndrome are both diagnostic and pathogenic. Many other tissue-specific autoantibodies are valuable in diagnosis but do not necessarily play a role in pathogenesis. Autoantibodies of thyroglobulin and thyroid peroxidase, for example, are characteristic of chronic thyroiditis; autoantibodies to islet cells of the pancreas are found in many patients with insulin-dependent diabetes; and autoantibodies that react with actin of smooth muscle are prominent in chronic active hepatitis (Bigazzi et al., 1986). Similar autoantibodies, however, are common in healthy individuals, and their incidence increases with age. Recent studies suggest that patients with disease, as well as individuals at risk of developing disease, produce autoantibodies to distinct antigenic determinants on the thyroglobulin molecule (Bresler et al., 1990). Tests for specific tissue antibodies are important research tools that provide information about the pathogenesis of these diseases; they also have predictive value in the diagnosis of many autoimmune disorders.

Histopathologic Examination

The microscopic examination of specimens from tissue biopsies, appropriately fixed and stained, can be valuable in diagnosing autoimmune diseases and can elucidate the pathogenesis and mechanism of

immune-tissue damage. Direct immunofluorescence can detect antibody and complement deposition in tissues. For example, in bullous pemphigoid, an autoimmune disorder that produces bullous skin lesions, heavy depositions of IgG can be detected along the basement membrane between the epidermis and dermis of patients (Diaz et al., 1985).

Immune Complexes

Aggregates of antibody and antigen can be detected in serum and tissues in several disorders, including infectious diseases, autoimmune diseases, malignancies, and serum sickness secondary to administration of drugs. This lack of specificity makes them of little use as markers of autoimmunity, but they are markers of disease activity. Systemic lupus erythematosus is the prototype of an autoimmune disease for which the pathologic changes are due to the deposition of immune complexes in various tissues, especially the kidney. These complexes involve DNA-anti-DNA. Measurement of immune complexes and demonstration of antibodies to native DNA and determination of levels of hemolytic complement in serum are diagnostically useful (Toth et al., 1986).

Complement

Inflammatory disorders can deplete the immune complement, and an abnormally low level of complement (C3 consumption), although not a specific marker of autoimmunity, can be a biologic marker of disease activity in autoimmune disorders, most notably systemic lupus erythematosus (Toth et al., 1986).

SUMMARY AND RECOMMENDATIONS

Autoimmune diseases in humans are common and cause a great deal of morbidity and mortality. Xenobiotic exposures can induce autoimmune disorders, which sometimes require a genetic predisposition, in both humans and animals. A great deal is known about drug-induced autoimmunity, but our knowledge of autoimmune diseases arising from environmental exposure is in its infancy, and we do not know the extent to which apparently spontaneous autoimmune disorders are influenced by environmental factors.

Clinical research should determine the extent to which non-drug-induced autoimmune disorders are environmentally induced. If it is found that significant percentages of human autoimmune disorders are environmentally related and that these diseases can go into remission by removing the incriminated chemical or by reducing exposure to it, techniques must be developed to allow clinicians to identify environmental factors in autoimmunity.

Inexpensive and practical tests to screen populations for susceptibility to autoimmune disease should be developed. The best current predictor of susceptibility to autoimmune disease is the HLA class II haplotype. Determinations based on nucleotide base (or amino acid) sequence are more discriminating than are conventional serologic assays, and they are more predictive of autoimmune disease (Nepom, 1989).

Populations exposed to toxic substances because of spills of hazardous materials, proximity to toxic-waste sites, and occupation could then be easily screened for autoimmune phenomena, including subclinical or asymptomatic disease. An example of such a test is the indirect immunofluorescence assay for a battery of autoantigens.

Better data on the epidemiology of autoimmune diseases should be collected continuously. It is important to document changes in the incidence of these diseases and the geographic clustering of cases in acquiring an understanding of environmental factors. Autoimmune diseases do not require reports to local health departments, so

existing epidemiologic data are a patchwork of areas of controversy. This mission would be ideal for the Agency for Toxic Substances and Disease Registry.

The association between autoimmune disease and some pharmaceuticals is well established. Efforts should be made to investigate the prevalence of autoimmune responses and markers among pharmaceutical-manufacture workers. Perhaps health-care workers routinely exposed to these drugs could also be monitored for the prevalence of drug-induced autoimmune disease.

The environmental control unit discussed in Chapter 3 could be an effective tool for investigating the role of xenobiotic substances in autoimmunity. It allows isolation of the patient from the ubiquitous chemical environment. If improvement can be objectively quantitated, chemicals from the patient's daily life can be reintroduced to see which, if any, produce disease. This simple concept is well grounded in common sense, and examination of its utility in the study of autoimmune diseases is merited.

5

The Capacity of Toxic Agents to Compromise the Immune System (Biologic Markers of Immunosuppression)

There is increasing awareness and concern within the scientific and public communities that chemical pollutants can suppress immune processes and thus cause increased development of neoplastic and infections diseases. Adverse effects on humans treated with immunosuppressive drugs, numerous studies employing experimental animals, and, to a lesser extent, isolated cases of altered immune function in humans inadvertently or occupationally exposed to xenobiotic substances support these concerns. There is no definitive evidence, as yet, that persons who live near contaminated sites or chemical-manufacturing plants have been immunologically compromised to the extent that they are at increased risk of disease. Nonetheless, there is reason to believe that chemical-induced damage to the immune system might be associated with pathologic conditions, some of which could become detectable only after a long latency. Likewise, exposure to immunotoxic xenobiotics can present additional risk to individuals with immune systems that are already fragile, for example, because of primary immunodeficiency, infancy, or old age.

Most of the experimental data on the effects of xenobiotics on immune function have been generated from animal models. The value of incorporating immunologic data for toxicologic assessment of drugs, chemicals, and biologics for evaluation of human hazard is increasingly accepted. However, as in other areas of toxicology, it is difficult to extrapolate change in a given area of immune function in experimental animals to the incidence of clinical or pathologic effects in humans.

One should not use such a term as "chemical AIDS" in reference to chemical-induced immune dysfunction. Acquired immune deficiency syndrome (AIDS) is a well-defined disease of known viral etiology that bears no resemblance to potential chemical-induced immune-system changes. AIDS and the effects of commonly used immunomodulating drugs can be useful, however, as examples of the damage that can result from a compromised immune system in animals and humans. In addition, infection with the HIV-1 virus frequently occurs in persons concomitantly affected by other immunosuppressive agents, such as addictive drugs, malnutrition, herpesvirus-6, and Epstein-Barr virus. These agents could serve as cofactors that predispose an individual to HIV-1

infection, as well as confounding the resulting immune response.

CONSEQUENCES OF IMMUNOSUPPRESSION

The study of human immunodeficiency disease syndromes reveals a clear association between the suppression or absence of an immunologic function and an increased incidence of infectious or neoplastic disease. Numerous examples of such deficiency diseases have been reported and are well characterized in humans (Table 5-1). A deficiency in one or more immunologic functions can lead to severe, recurrent infections throughout life. These infections can be bacterial, viral, fungal, or protozoan, and the predominant type of infection depends on the associated immunologic lesion. Some infections can be treated with antibiotics or gammaglobulin, and in some cases the immunologic defect can be restored by bone marrow transplantation. However, other immunodeficiency diseases are much more severe. For example, children born with reticular dysgenesis have no white blood cells and usually die from infectious disease in the first year of life; children born with ataxia telangiectasia rarely survive past puberty. These diseases of genetic deficiency are more severe than those caused by environmental toxicants, because they are the result of the absence of part of the immune system. They demonstrate well-characterized consequences of immunosuppression. These same diseases would be expected to be associated with specific immunosuppression, whether the cause were genetic or environmental.

There are more than 60 inbred hybrid and mutant strains of rodents with well-defined immunodeficiencies (NRC, 1989c). Many of these animals have diseases that are comparable to the human immunodeficiency diseases listed in Table 5-1. An example is the beige mouse (the result of a recessive mutation on chromosome 13), which is a model for the human Chediak-Higashi disease syndrome. This defect results in reduced cell-mediated and natural killer immune function. Animal models have well-characterized, specific immunologic defects and known increased susceptibilities to infectious, neoplastic, and autoimmune diseases. Studies of human immunodeficiency diseases and the counterpart animal models emphasize the potentially serious consequences of immunosuppression, whether it occurs as a result of heredity, aging, or nutrition or is acquired as a result of exposure to xenobiotics.

For some time, immunosuppressive agents have been used in treating autoimmune diseases and as adjunctive therapy in organ transplantation procedures to prevent rejection by the recipient. Studies in this area have provided information on the potential clinical effects of chronic low-level immunosuppression. In addition, experimental studies with these compounds have provided comparative data between experimental animals and humans on immunosuppression that should have direct application to risk assessment.

Immunosuppressive treatments, such as x-irradiation, neonatal thymectomy, or the use of immunosuppressive drugs, result in an increased incidence of parasitic, viral, fungal, or bacterial infections. There is a well-established association between the therapeutic use of chemical immunosuppressants, such as those used in organ transplant therapy or in cancer chemotherapy, and an increased incidence of infections and neoplastic disease in humans (Ehrke and Mihich, 1985). For example, in a study of renal graft recipients undergoing immunosuppressive treatment, a 10-fold increased incidence of monoclonal gammopathies was observed (Radl et al., 1985). In another study, 50% of transplant patients developed cancer within 10 years after the operation (Penn, 1985). The tumors detected in these patients were heterogeneous and included skin and lip

tumors (21-fold increase), non-Hodgkins lymphomas (28- to 49-fold increase), Kaposi's sarcoma (400- to 500-fold increase), and carcinomas of the cervix (14-fold increase). Infections also are common in transplant patients on immunosuppressive therapy. In one study, 30% of cardiac-transplant patients treated with cyclosporin developed pulmonary infections within the first year after surgery (Austin et al., 1989). The most common pathogen was cytomegalovirus (11%), followed by *Pneumocystis*

TABLE 5-1 Consequences of Immunosuppression

Syndrome	Cell Type Affected	Result
DiGeorge syndrome	T cell	Increased bacterial, viral, and yeast infections
Nezelof's syndrome	T cell	Increased bacterial, viral, and protozoan infections
Common variable immunodeficiency (CVD)	B cell (T cell)	Increased bacterial infections
Bruton's disease X-linked infantile hypogammaglobulinemia	B cell	Increased bacterial infections
Selective IgA deficiency	B cell	Increased bacterial infections
Wiskott-Aldrich syndrome	B and T cells; monocytes	Increased bacterial and viral infections
Ataxia telangiectasia (A-T)	B and T cells	Increased bacterial and viral infections
Severe combined immunodeficiency disease (SCID)	B and T cells	Increased bacterial and viral infections
Reticular dysgenesis	Leukocytes	Increased bacterial and viral infections
Adenosine deaminase (ADA) deficiency	Th cells (direct); B cells (indirect)	Increased bacterial and viral infections
Chediak-Higashi syndrome	Phagocytes, NKs, and Tc cells	Increased bacterial infections
Chronic granulomatous disease	Phagocytes (primarily neutrophils)	Increased bacterial infections
Complement deficiency C1-C8	—	Increased bacterial infections

Source: Adapted from Coleman et al. (1989).

carinii (10%) and *Aspergillus* (4%). It also has been postulated that the occurrence of some B-cell lymphomas and hepatocellular carcinomas in immunosuppressed individuals (e.g., patients with X-linked lymphoproliferative disorders) are due to their inability to control various viral infections, such as herpes simplex, human papilloma, and infections caused by Epstein-Barr virus (Purtillo and Linder, 1983). AIDS provides another example of the consequences of altered immune function in which the loss of immune responsiveness is associated with disease, most notably from *Pneumocystis carinii* and other opportunistic pathogens and the development of Kaposi's sarcoma, a rare form of cancer.

Infections that arise from immunosuppression will depend on the degree of suppression and potency of the infectious agent. The nature of the exposure can thus be an overriding factor in determining the nature of the infectious process. It is not surprising, for example, that the complex immune system can affect in several ways the equally complex development of cancer. Because the immune system normally provides a defense against viruses, the suppression of the immune system also can result in an increase in viral-oncogene-dependent tumors. Likewise, because tumors can generate specific nonself markers on their surfaces, similar to transplantation antigens, it is natural to expect some tumors that would normally be rejected by the host to develop under conditions of immunosuppression.

For purposes of illustration, the various effects of cyclosporin A (CsA) on the immune systems of humans and experimental animals will be compared with respect to effective doses. CsA is a fungal cyclic undecapeptide first described in 1976 (Borel et al., 1976). It is an immunosuppressive drug widely used to reduce transplant rejection, a cell-mediated immune response (Klaus and Hawrylowicz, 1984). The effects of CsA on graft survival and on the generation of an immune response have been studied in animal models of many species to predict the effects of an immunotoxicant on the human immune system. CsA has become the drug of choice in the clinical management of graft rejection, despite an increase in the risk of various infections. In humans, the daily clinical dose of CsA is 3-10 mg/kg of body weight for various indications; however, recent studies have shown that, independent of the route, the maintenance of plasma concentration of 250 nanograms/ml of CsA is needed to achieve resistance to organ rejection (Andrieu et al., 1988; Kerman et al., 1988; Martinet et al., 1988; Schmidt et al., 1988; Talal, 1988; Yocum et al., 1988; Baker et al., 1989; Sullivan and Shulman, 1989; Szer, 1989; Werner et al., 1989).

Although occasionally CsA does not alter the immune systems of patients (Chatenoud et al., 1989; Müller et al., 1989), the vast majority exhibit altered immune function. CsA reduces T-cell numbers and function during activation (Baker et al., 1989), and it inhibits the proliferative response of T cells and the secretion of interleukin-1, interleukin-2, and interferon (Kerman et al., 1988; McKenna et al., 1988; Pelletier et al., 1988). In HIV-seropositive patients, CsA reduces the number of peripheral blood CD8 (cytotoxic) T cells associated with the class I MHC (major histocompatibility complex) molecules and increases the number of CD4 (helper) T cells associated with the recognition of class II MHC molecules.

Most of the work examining CsA's in vivo effects on the immune system has been performed in rats and mice. However, in work with baboons and dogs, administration of CsA (20-40 mg/kg) inhibited the proliferative response of peripheral-blood lymphocytes to mitogen and antigen and prolonged pancreatic graft survival (Du Toit and Heydenrych, 1988; Kneteman et al., 1989; Zeitz et al., 1989). In rats, as in humans, administration of 5-30 mg/kg per day prolonged the survival of allogeneic grafts and reduced the severity of autoimmune disease (Bain et al., 1988; Tilney et al., 1988; Hall et al., 1989;

James et al., 1989; Oluwole et al., 1989; Ricordi et al., 1989; Rodrigues et al., 1989). Numerous studies show that in vivo administration of CsA can modulate the immune response in rodents. In rats and mice, administration of CsA reduced the medulla of the thymus, changed splenic morphology, and modulated T-cell proliferation, lymphokine secretion, and the accumulation of transplant-specific cytotoxic T cells (Hiramine et al., 1988, 1989; Muthukkumar and Muthukkaruppan, 1988; Orosz et al., 1988; Tanaka et al., 1988; Yoshimura et al., 1988; Armas et al., 1989; Fukuzawa and Shearer, 1989). Administration of CsA affected the susceptibility to infection with murine cytomegalovirus (MCMV) by reducing the immune response (Selgrade et al., 1989). Dean and Thurmond (1987) have compiled the results of immunotoxicity studies of CsA from a number of experimental animal species used in toxicology (Table 5-2). Comparative studies indicate that rats are slightly more susceptible to the immunosuppressive effects of CsA than are mice, but the consistency was good between the test species.

Although exceptions exist, rodent studies have been predictive for both the type of immune effects and the dose required to achieve the effects for humans given various immunosuppressive therapeutic drugs in clinical trials. The most notable exceptions are glucocorticoid steroids, which are thymolytic in rodents, but not in humans. Although more comparative immunotoxicity studies are required, rodents are now the most appropriate animal model for examining the immunotoxicity of non-species-specific compounds, according to established similarities of toxicologic profiles and ease of performing immunologic evaluations. It remains critical, however, to establish the pharmacokinetic and metabolic properties of the test compounds in humans and experimental species.

Animal data on CsA can be applied to predict immune-system effects in humans. Understanding of the mechanisms of action of immunotherapeutic agents, such as CsA,

TABLE 5-2 Species Comparison of Immune Responses Suppressed by Cyclosporin A

Species	Response	CsA Dose, mg/kg	Antigen, Model
Mouse	AB production	50-300	SRBC, DNP (Ficoll, Dextran), marrow graft SRBC, BCG, oxazalone
	CMI (DTH)	100-300	
	GVH reaction	50-250	
Rat	AB production	20-50	SRBC, DNP-KLH, MHC marrow graft, lymph node assay
	GHV reaction	10-60	
Guinea pig	CMI (DTH)	10-100	BCG, OVA, DNCB, DNFB
Dog	CMI (DTH)	15-30	Marrow graft
Rhesus monkey	AB production	50-250	SRBC

Abbreviations AB, antibody, BCG, bacillus Calmette-Guérin; CMI, cell-mediated immunity; DNCB, dinitrochlorobenzene; DNFB, dinitrofluorobenzene; DNP, dinitrophenol; DNP-KLH, DNP-keyhole limpet hemocyanin; DTH, delayed-type hypersensitivity; GVH, graft versus host; MHC, major histocompatibility complex antigens; OVA, ovalbumin; SRBC, sheep red blood cell.
Source: Dean and Thurmond (1987).

and the relationship between the effective biologic dose and the various immunologic values, therapeutic effects, and adverse effects will provide a better basis for extrapolation of animal data to human risk. Many animal studies have shown that high-dose exposure to environmental contaminants and other nontherapeutic agents can modulate the immune system. Adverse effects after low-level chronic exposure remain to be confirmed.

ENVIRONMENTAL CONTAMINANTS

Human Studies

Although they are not as well established as are the effects of therapeutic drugs, environmental toxicants are implicated in a number of reports that describe increased rates of neoplastic disease or infection in humans associated with immune-system changes. For example, a cluster of Hodgkin's diseases was reported in individuals from a small town in Michigan (Schwartz et al., 1978). Reduced CD4:CD8 ratios and natural-killer-cell function have been reported in asbestos workers (Lew et al., 1986). Another unconfirmed report describes a 4-year study of workers engaged in the manufacture of benzidine, a human bladder carcinogen, and suggests that individuals with depressed cell-mediated immunity (as shown by skin tests) demonstrated precancerous conditions and subsequent neoplasms (Gorodilova and Mandrik, 1978). On the other hand, no cases of neoplastic diseases were registered in workers with normal immunologic responses.

There are a number of better-substantiated clinical studies. For example, there have been observations of abnormal antibody production, prolongation of allograft rejection, and decreased resistance to disease in humans occupationally exposed to silica (Uber and McReynolds, 1982). A series of reports from Taiwan have described immunologic changes (Yu-Cheng diseases) in individuals exposed to rice oil inadvertently contaminated with polychlorinated biphenyls and dibenzofurans (Lee and Chang, 1985). These individuals primarily demonstrated altered T-cell function and increased rates of sinopulmonary infection.

Because considerable in vivo and in vitro data have been accumulated on the immunotoxic effects of lead, it can serve as an example of the way metals can affect the immune system. The adverse effects of lead vary with age. In the young, central nervous system function is the major target, whereas in adult workers, renal toxicity and anemia predominate. Because lead has a long half-life, the body burden takes considerable time to reach its maximum with a constant intake. The body burden, over time, may be a better estimate of the biologically effective dose than either the exposure concentration or the blood lead concentration. There are several reports that lead alters immunity in humans. Lead workers with elevated blood lead had decreased levels of salivary IgA and an increased incidence of colds and influenza (Ewers et al., 1982), impaired mitogen responses to phytohemagglutinin (Jaremin, 1983), and increased numbers of suppressor T cells (Cohen et al., 1989). Children with high blood lead concentrations have been reported to have diminished levels of IgM, IgG, and IgA (Wagnerová et al., 1986); others infected with *Shigella enteritis* had prolonged diarrhea (Sachs, 1978). There also are reports that elevated concentrations of lead do not alter immunity in humans (Reigart and Graber, 1976; Kimber et al., 1986). Nevertheless, it appears that high blood lead concentrations can damage the immune system.

There are several examples in which immunologic changes have been ascribed to exposure to various environmental pollutants, but have not been associated with any clinical changes. For example, women who have been chronically exposed at low levels to groundwater contaminated with the pesticide

aldicarb exhibited an altered number of T cells, including decreased CD4:CD8 ratios (Fiore et al., 1986). Immunologic effects also have been reported in individuals exposed to methyl isocyanate, an intermediate in the production of carbamate pesticides, after an industrial accident in 1984 in Bhopal, India (Deo et al., 1987). The effects of immune response included an increase in the number of CD4 and total T cells, but decreases in lymphocyte mitogenesis. Several persistent immune alterations, also primarily cell-mediated, have been reported in Michigan residents who ingested dairy products contaminated with polybrominated biphenyls (Bekesi et al., 1978), although Silva et al. (1979) were unable to detect any immune abnormalities in a similarly exposed cohort. Individuals occupationally exposed to 2,3,7,8-tetrachlorodibenzo*p*-dioxin (TCDD), which is one of the subregistries established by the ATSDR (Jennings et al., 1988), also demonstrated immune changes. Jennings showed that antinuclear antibodies and immune complexes were detected significantly more frequently in the blood of dioxin-exposed workers. In addition, the number of leukocytes in peripheral blood cells was elevated in dioxin-exposed workers. Some of these changes were not confirmed in a later study (Evans et al., 1988), perhaps because of a technical error; other observations were confirmed. Benzene-induced pancytopenia, a classic symptom of chronic benzene exposure, is an immunodeficiency disease by virtue of the reduced number of immunocompetent cells that result from altered marrow function (Snyder, 1984). In fact, lymphopenia is common after exposure to organic solvents (Browning, 1965; Capurro, 1980). Alterations in the number of some cell types (decreases in CD3 and CD4) were reported to occur in solvent-exposed workers (Denkhaus et al., 1986); the effects might have some specificity.

In addition to environmental chemicals, a large number of therapeutic substances, as well as abused recreational drugs, can alter immune function in humans. Among these are diphenylhydantoin, ethanol, cocaine, and isobutyl nitrites (Newell et al., 1984; Specter et al., 1986).

Although most of the xenobiotics that alter the human immune system also can affect experimental animals, clinical studies often have been criticized for incomplete or inconsistent diagnosis of immunodeficiency, lack of clinical changes, small group size, inability to establish exposure levels, or lack of reproducibility. As might be expected, there are several studies in which immune functions were not shown to be affected after the subjects were exposed to presumably high levels of chemicals, even though the agents, such as heavy metals and TCDD, are known to be immunotoxic in animals (Reggiani, 1980; Kimber et al., 1986). Furthermore, there is no evidence that xenobiotics can influence immune systems in the general population (except through occupational or inadvertent exposure).

The question of population-wide immune system effects would be difficult to answer, because the immunologic effects one might expect in the general population would likely be subtle. The tests routinely used for clinical assessment of immunosuppressed function in humans are not very sensitive. The difficulty in identifying a recently exposed, well-defined cohort is substantial. There is considerable immunologic variability in the general population. A consensus exists that further clinical studies with better-defined cohorts and more sensitive tests will be required to assess the true potential of xenobiotics to affect human health.

Experimental Studies

A growing body of research in immunotoxicology has shown that many xenobiotic substances cause immunosuppression in laboratory animals (Table 5-3). Immunologic effects often are accompanied by increased susceptibility to challenge with infectious

agents or tumor cells. Animal studies, primarily those which use rodents, have provided a large information base about potentially immunotoxic chemicals, suggestive evidence of the mechanisms for their effects, and an appreciation that the immune system is susceptible to chemical injury. The susceptibility of the immune system is due as much to the general properties of a chemical (e.g., its reactivity to macromolecules) as it is to the complex nature of the immune system. Because the cellular events responsible for immune processes also are involved in embryogenesis, many immunosuppressive xenobiotics would be expected to be developmental toxicants.

TABLE 5-3 Classes and Examples of Chemicals Causing Immunologic Changes

Class	Examples
Polyhalogenated aromatic hydrocarbons	TCDD, PBBs, PCDF, PCBs, hexachlorobenzene
Metals	Lead, calcium, arsenic, methyl mercury
Aromatic hydrocarbvons (solvents)	Benzene, toluene
Polycyclic aromatic hydrocarbons	DMBA, B[*a*]P, MCA
Pesticides	Trimethyl phosphorothioate, carbofuran, chlordane, malathion
Organotins	TBTO
Aromatic amines	Benzidene, acetyl aminofluorene
Oxidant gases	Nitrogen dioxide, ozone, sulfur, dioxide
Particles	Silica, asbestos
Natural products	Selected vitamins, antibiotics, vinca alkaloids, estrogen, plant alkaloids, mycotoxins
Drugs of abuse	Ethanol, cannabinoids, cocaine, opioids
Therapeutic drugs	Diphenylhydantoin, lithium
Others	Nitrosamine, BHA

Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PBBs, polybrominated biphenyls; PCDF, polychlorinated dibenzofuran; PCBs, polychlorinated biphenyls; DMBA, dimethylbenzanthracene; B[*a*]P, benzo[*a*]pyrene; MCA, methylcholanthrene; TBTO, bis(tris-*n*-butylin)oxide; BHA, butylated hydroxyanisole.

To date, animal data have not been used to any significant extent in the assessment of human risk resulting from exposure to immunosuppressive environmental pollutants. Extrapolating short-term, high-dose animal studies to chronic low-dose human exposure

is always a problem. Moreover, this problem is compounded in that the end points (infection and neoplasia) of immune-system deficiency are secondary to other confounding factors. To use immunotoxicity data from animal studies in the quantitative assessment of risk (the prediction of the incidence of disease at a given human dose), we would have to be able to predict the disease incidence that results from a given degree of immunodeficiency. However, animal immunotoxicology is now at a point where it should be used in identification of pollutants that have the potential to induce immunodeficiency and in the estimation of the degree of hazard that environmental agents have, according to their immunosuppressive potency. Because of the potential for tragic outcomes associated with exposure to immunosuppressive environmental pollutants, prudent public health concerns indicate the need for a remedy. The following discussion concerns some of the environmental toxicants shown to alter immune function at doses at which toxicity in other organ systems is not readily apparent.

Aromatic Hydrocarbons

Probably the most extensively studied class of environmental pollutants are halogenated aromatic hydrocarbons (HAHs), including dibenzo-*p*-dioxins, dibenzofurans, polychlorinated biphenyls (PCBs), and polybrominated biphenyls (PBBs) (Vos and Luster, 1989). These compounds, many of which are widespread in the environment, are primarily used in commercial production of industrial chemicals, pesticides, flame retardants, and heat conductors. Dioxins and PCBs produce myelosuppression, immunosuppression, thymic atrophy, and inhibition of immune complement system components in almost all species tested, including primates. The most potent dioxin, TCDD, is an extremely potent immunosuppressant in mice. A dose of 1-2 μg/kg of body weight is all that is required to reduce immune function by 50%. As probably occurs with a number of immunosuppressive HAHs, the specific effects of TCDD on the immune system can vary, depending on the age of the animal at the time of chemical exposure. For example, the primary effect of perinatal TCDD exposure is persistent suppression of cellular immunity, a condition that mimics neonatal thymectomy. In contrast to perinatal exposure, TCDD exposure in adult mice, while still inducing deterioration of thymic tissue (predominantly cortical lymphoid depletion), causes a transient antiproliferative response in rapidly dividing cell populations, including hematopoietic cells and B cells. The marked and persistent suppression of T-cell function seen in neonates is not manifested in adults, although suppression of cytotoxic T-cell response and altered members of regulatory T cells have been reported.

Regardless of age at the time of exposure or the target tissue examined, immunosuppression by TCDD, as well as by PCBs, is believed to be mediated through stereospecific and irreversible binding to an intracellular receptor protein (the Ah genotype) found in the cellular targets for TCDD, including lymphoid tissue, bone marrow cells, and the thymic epithelium (Thomas and Faith, 1985). The Ah genotype was determined primarily in immune studies comparing inbred strains of Ah-responsive and nonresponsive mice in which the ability of TCDD to cause immunotoxicity correlated with the presence of the Ah locus (Vecchi et al., 1983; Tucker et al., 1986). In addition, a good correlation exists between the binding affinities of various HAHs and their ability to induce immunotoxicity (Silkworth et al., 1984; Tucker et al., 1986).

The role, if any, of microsomal enzyme induction in the cellular mechanisms responsible for immunotoxicity after TCDD binds to its receptor is unknown. The observation that the thymic epithelium contains a high concentration of receptor has led to the

suggestion that TCDD causes maturational defects in developing thymocytes via inadequate epithelial support (Greenlee et al., 1985). There is evidence, however, that alterations other than that of thymic epithelium function are responsible for immunosuppression. These alternative mechanisms include direct thymolysis events (McConkey et al., 1988), alterations in regulatory T-cell function (Kerkvliet and Brauner, 1987), alterations in B-cell differentiation (Luster et al., 1988), and stem cell inhibition (Fine et al., 1989).

Polycyclic aromatic hydrocarbons (PAHs), another well-studied class of compounds, are formed as products of incomplete combustion of fossil fuels, tobacco, and coke and in automobile exhaust. Selected PAHs, including benzo[*a*]pyrene (B[*a*]P), 7,12-dimethylbenzanthracene (DMBA), and 3-methylcholanthrene (3-MC), have been shown to produce immunosuppression and myelotoxicity; they also are carcinogenic (Dean et al., 1986). There is controversy about whether the immunosuppressive properties of PAHs are a cofactor for carcinogenicity, because they allow tumor antigen (neoantigen) to bypass normal host immune surveillance. Humoral immunity is suppressed after exposure to several PAHs, including B[*a*]P, DMBA, and 3-MC, although humoral immunosuppression after DMBA exposure could reside at the level of T-cell regulation (Dean et al., 1990). The finding of reduced progenitor B cells in exposed animals suggests that B cells also are directly targeted early in their maturation (Ward et al., 1984). B[*a*]P has been shown to impair the production of interleukin-1, implicating chemical-induced defects in accessory cell function as a contributing factor in decreased production of antibody-forming cells (Lyte and Bick, 1986). Cell-mediated immunity also is inhibited by PAHs. Cytotoxic T-cell activity in mice is suppressed after in vitro and in vivo exposure to DMBA or 3-MC (Wojdani and Alfred, 1984; Dean et al., 1985a, 1986).

Hexachlorobenzene (HCB), a fungicide and an intermediate in a variety of chemical syntheses, has been shown to have immunosuppressive properties in rodents. HCB suppresses both cellular and humoral immunity in adult mice (Loose et al., 1978) and is particularly toxic to T-cell function after in utero exposure (Barnett et al., 1987). HCB stimulates immune responses in rats after adult exposure (Vos and Luster, 1989) and perinatal exposure (Vos et al., 1983).

Benzene

The immunotoxicity of benzene, for which a subregistry has been established at ATSDR, has been the subject of considerable research in humans and animals, with particular emphasis on its hematologic and leukemogenic potential (Snyder, 1984). In humans and experimental animals, the predominant hemopathy associated with benzene exposure is pancytopenia, with associated bone marrow hypoplasia (Laskin and Goldstein, 1977). Although hematopoietic progenitor cells are particularly susceptible to benzene, the mature circulating lymphocyte also responds to benzene in an antiproliferative response (Snyder et al., 1980). Chronic benzene exposure has been associated with depressed serum levels of IgA, IgG, and immune-system complement in humans. Despite the myelotoxic effect produced by benzene in experimental animals, the exact relationship between benzene-induced immunosuppression and an increase in leukemia has not been determined. Using pharmacokinetics to determine amounts of total benzene metabolized in 24 hours, Beliles and Totman (1989) have established the biologically effective dose. Using pharmacokinetics as a basis for across-species extrapolation of experimental data (benzene produces leukemogenesis at dose levels of 100-300 ppm in animals), they were able to show that the human risk of leukemia estimated from the incidence of leukemia in mice was quantitatively comparable to the risk predicted from occupational epidemiologic studies.

Metals

A large experimental data base exists on the immunosuppressive properties of metals, their inorganic salts, and organometallic compounds (Koller, 1980). Inorganic substances, including heavy metals, such as lead, cadmium, mercury, and nickel, have been studied, and the effects of exposure have been reported to range from suppression to enhancement of the immune system. Some metals are reported to induce autoimmune disease. Mercury and to a lesser extent gold and lead have been shown to induce polyclonal B-cell activation, and considerable effort has been devoted to understanding this autoimmune phenomenon (Bigazzi, 1988). Heavy metals also are sulfhydryl-alkylating agents and as such bind with high affinity to cellular sulfhydryl groups. This suggests that, at sufficient dose levels, these compounds can interfere with normal cell-to-cell communication by altering various membrane properties, thus causing immunosuppression. This observation is strengthened by the observation that the suppressive effects of lead, at least in vitro, can be reversed by the addition of exogenous thiol reagents. Organotin compounds, used as heat stabilizers, biocides, and industrial catalysts in the production of foams and rubber, also are immunotoxic in rats. These compounds target the thymus, causing severe thymic atrophy and suppression of cell-mediated immune responses (Seinen and Penninks, 1979).

Lead, in general, tends to suppress immunity in animals. It is well accepted that lead increases host susceptibility to numerous infectious agents and increases both tumor growth and development in animals (Koller, 1990). Lead also impairs cell-mediated immunity, particularly the delayed-type hypersensitivity response (Müller et al., 1977; Faith et al., 1979). Although lead affects IL-2 (interleukin-2), it does not appear to inhibit interferon (Gainer, 1974; Blakley et al., 1982; Exon et al., 1985). Furthermore, natural-killer-cell activity appears to be unaffected by lead (Talcott et al., 1985). Some macrophage activities, such as phagocytosis, oxygen metabolism, vascular clearance, and antigen presentation, are suppressed by lead. Mitogen responses are quite variable in animals exposed to lead (Lawrence, 1981; Burchiel et al., 1987; Koller, 1990). In vitro exposure to lead results in increased plaque-forming-cell responses; it stimulates the proliferation of immune cells; and it inhibits microbial killing, antigen presentation, and propagation of bone-marrow-derived macrophages. It has no effect on IL-2-receptor expression, on phagocytosis, or on IL-1 production, but it results in an increased density of Ia antigen (the murine class II MHC antigen) on B cells (Lawrence, 1981; Kowolenko et al., 1988; Koller, 1990). The differences in results obtained with lead in animal studies usually can be credited to variations in species, strains, or experimental metallothione levels, induction of which is highly variable among species and strains (Ohsawa et al., 1986). It is noteworthy that oral intake of lead by rats at a dose of 10 ppm, which does not otherwise damage their health, has been demonstrated to suppress immunity (Koller et al., 1983a,b). Thus, the immune system of rodents appears to be a sensitive target organ of lead toxicity. This may explain why rats tend to develop renal cancer after chronic administration of lead, whereas nephrotoxicity in humans does not progress to neoplasia.

Complex Mixtures

Most of the animal studies discussed above involved the administration of doses of toxicants at levels that are higher than those normally found in the environment. Although many of the chemicals are immunotoxic at doses that do not produce other toxicities, there is still the question of whether the general public is at risk of immunosuppression, particularly after chronic low-level exposure to chemical mixtures. One study on the immune system of mice has

examined the effects of a complex mixture of 19 organic and six inorganic environmental toxicants commonly found in contaminated groundwater at hazardous-waste sites (Germolec et al., 1989). The authors note that although the chemical configuration does not actually occur in the environment, it is representative of a highly contaminated groundwater sample. The mixture (Table 5-4) was administered to the mice in their drinking water over a period of 14-90 days and was found, at least at the high levels, to suppress the number of granulocyte-macrophage progenitor cells in the bone marrow, the generation of specific antibody-forming cells in the spleen, and the ability of the mice to fight an infection of *Plasmodium yoelii*, although it did not affect other indicators of targetorgan toxicity. The authors note that although several of the components of this complex mixture had been shown to have similar immunologic and myelotoxic effects, earlier studies with the individual components suggest that none of the individual contaminants was present at sufficient concentrations to be solely responsible for the observed effects. Therefore, the effects of the complex mixture of groundwater contaminants on the immune system are likely to result from a composite of influences of the individual components. Controlled studies that examine the effects on the immune systems of laboratory animals of complexes of environmental contaminants found at environmentally relevant levels should help provide an understanding of the real immunologic risk of living in a contaminated environment.

Miscellaneous

There are many compounds for which there are no human data, but for which there are confirmatory animal data. Among these are dimethylnitrosamine (Thomas et al., 1985), phenolic food additives (Archer and Johnson, 1978), certain pesticides (*O,O,S*-trimethyl phosphorothioate—a contaminant in malathion), and several other organophosphate pesticides (Rodgers et al., 1985). For a complete listing of these putative immunotoxic xenobiotics and specific references, the reader is directed to more detailed books and reviews (Koller, 1990; Gibson et al., 1983; Dean et al., 1985a, 1986; Luster et al., 1987; Vos and Luster, 1989; Rodgers et al., in press).

INHALATION AND IMMUNOSUPPRESSION

Inhaled pollutants can enhance susceptibility to or severity of viral, bacterial, and neoplastic diseases. This could be related to, or a direct consequence of, the impaired immune function. Pulmonary immunocompetence has been assessed after exposure to phosgene and ozone. Exposure of rats to 1.0-ppm phosgene for 4 hours results in a significantly enhanced and prolonged pulmonary influenza virus infection (Ehrlich and Burleson, in press). Influenza-virus-specific cytotoxic T-lymphocyte activity in the lungs is suppressed after exposure to phosgene gas (Ehrlich et al., 1989). Phosgene inhalation also results in suppressed pulmonary natural-killer-cell (NK) activity (Burleson and Keyes, 1989), which remains suppressed through day 4 and returns to normal on day 7 after exposure. Phosgene inhalation suppresses NK activity in the spleen. It is therefore important to assess systemic immunotoxicity after inhalation exposures.

Suppressed pulmonary NK activity was observed after uninterrupted ozone exposures (23.5 hours/day) at 1.0 ppm for 1, 5, or 7 days. The suppressed immune function returned to normal 10 days after exposure stopped (Burleson et al., 1989). Van Loveren et al. (1990) report that inhalation exposure to ozone for 7 days at 0.2 or 0.04 ppm stimulates NK activity, but exposure at 0.8 ppm suppresses pulmonary NK activity.

Subchronic inhalation exposure to isobutyl nitrite results in decreased thymus weight and decreased white-blood-cell counts.

TABLE 5-4 EPA Survey Concentrations of Groundwater Contaminants and Composition of a Complex Chemical Mixture Representing a Contaminated Groundwater Sample

	Average EPA Survey Concentrations (ppm)	Maximum EPA Survey Concentrations (ppm)	Maximum Concentrations Used in the Study (ppm)[a]
Acetone	6.90	250.0	106.00
Aroclor 1260	0.21	2.9	0.02
Arsenic	30.60	3680.0	18.00
Benzene	5.00	1200.0	25.00
Cadmium	0.85	225.0	102.00
Carbon tetrachloride	0.54	20.0	0.80
Chlorobenzene	0.10	13.0	0.20
Chloroform	1.46	220.0	14.00
Chromium	0.69	188.0	72.00
Diethylhexyl phthalate	0.13	5.8	0.03
1,1-Dichloroethane	0.31	56.1	2.80
1,2-Dichloroethane	6.33	440.0	80.00
1,1-Dichloroethylene	0.24	38.0	1.00
1,2-*trans*-Dichloroethylene	0.73	75.2	5.00
Ethylbenzene	0.65	25.0	0.60
Lead	37.00	31000.0	140.00
Mercury	0.34	50.0	1.00
Methylene chloride	11.20	7800.0	75.00
Nickel	0.50	95.2	13.60
Phenol	34.00	7713.0	58.00
Tetrachloroethylene	9.68	21570.0	6.80
Toluene	5.18	1100.0	14.00
1,1,1-Trichloroethane	1.25	618.0	4.00
Trichloroethylene	3.82	790.0	13.00
Xylenes	4.07	150.0	3.20

[a] The highest dose level of the mixture used in the study was a 1:5 dilution (20%) of the technically achievable stock mixture, which is not shown.
Source: Germolec et al. (1989). Reprinted with permission; copyright 1989, the Society of Toxicology.

Injection or inhalation of isobutyl nitrite suppresses splenic and peripheral blood NK activity (Lotzová et al., 1984). Subchronic inhalation exposure to isobutyl nitrite also results in decreased thymus weight, decreased liver weight, decreased white-blood-cell counts, mild focal hyperplasia, and vacuolization of the epithelium lining bronchi and bronchioles (Lynch et al., 1985).

Arisine gas, used in the manufacture of semiconductor microchips, was used for inhalation exposure in mice to test for systemic immunotoxicity (Rosenthal et al., 1989). The exposure caused several hematopoietic effects: a decrease in erythropoiesis, as indicated by a reduction in erythroid-colony-forming units and femur cells; splenomegaly; and decreases in red blood cells, hematocrit, and hemoglobin with increases in white-blood-cell counts and mean corpuscular volume of red blood cells.

Alveolar macrophages provide an important

first line of defense and thus interact with inhaled xenobiotic substances and microorganisms. Alveolar macrophages exposed to xenobiotics can impair immunologic function or participate directly in disease induction. Alveolar macrophages from rabbits exposed to ozone at 0.6-0.95 ppm for 3 hours exhibit a suppressed ability to phagocytize streptococci (Coffin et al., 1968). In mice, ozone exposure at as little as 0.08 ppm for 3 hours results in an enhanced mortality of those exposed to group C streptococci (Coffin and Gardner, 1972; Miller et al., 1978). Suppressed pulmonary bactericidal activity was reported after a 4-hour exposure to ozone in mice (Goldstein et al., 1971). The ingestion of mineral dusts, such as quartz and asbestos, results in pulmonary fibrosis. Phagocytosis of asbestos by alveolar macrophages results in an increase in IgG receptor sites, enhanced ability to spread across a glass substrate, and more extensive cytoplasmic processes (Miller and Kagan, 1976). The phagocytic activity of alveolar macrophages is suppressed after inhalation of nitrogen dioxide (Gardner et al., 1969; Acton and Myrvik, 1972; Suzuki et al., 1986), which also results in impaired alveolar macrophage bactericidal activity (Valand et al., 1970) and increased susceptibility to *Klebsiella pneumoniae* (Ehrlich and Henry, 1968), *Streptococcus pyogenes* (Gardner et al., 1977), influenza virus (Henry et al., 1970; Ito et al., 1971), and cytomegalovirus (Rose et al., 1988).

Various compounds have been studied for their role in altering humoral immunocompetence in the lung by measuring the plaqueforming-cell (PFC) response to sheep red blood cells (SRBCs) as a measure of pulmonary humoral immunity. Chronic inhalation of cigarette smoke inhibits the PFC response of lung-associated lymph node cells to SRBCs (Sopori et al., 1989). Fly ash from a fluidized-bed combustor, fly ash from a pulverized-coal combustor, and quartz particles administered to Fischer-344 rats all significantly increased the number of lymphoid cells in the lung-associated lymph nodes. In contrast, quartz and fly ash from a pulverized-coal combustor suppressed the PFC response to SRBCs, but fly ash from the fluidized-bed combustor had no effect (Bice et al., 1987a). Exposure to nitrogen dioxide (Schnizlein et al., 1980), B[*a*]P (Schnizlein et al., 1982), and diesel exhaust (Bice et al., 1985) enhanced the PFC response to SRBCs in lung-associated lymph node cells.

Bronchoalveolar lavage fluid (BALF) contains numerous biochemical indicators of lung damage, including cells, antioxidants, proteins, enzymes, cytokines, growth factors, arachidonic acid, and metabolites. BALF analysis has been used to assess the pulmonary toxicity of many inhaled pollutants (Henderson et al., 1979a,b, 1985a,b, 1986, 1988; Henderson, 1984, 1988, 1989). BALF thus provides biologic markers that allow direct extrapolation of results from animal studies to investigations on human subjects. Most cells in BALF consist of alveolar macrophages. However, neutrophils provide an indicator of inflammatory response to infection and to inhaled xenobiotics.

Exposure of human subjects to low concentrations of ozone results in an increase in the appearance of neutrophils in BALF (Koren et al., 1988). Arachidonic acid metabolites have been measured in rat BALF after exposure by inhalation to phosgene. Phosgene exposure results in decreased levels of prostaglandin E-2 and leukotrienes in rat BALF. Production of arachidonic acid metabolites was measured by rat and human alveolar macrophages after in vitro exposure to phosgene (Madden et al., 1991). Monomethylhydrazine and dinitrogen tetroxide are toxic irritants in rocket fuel that result in pulmonary edema (DeJournette, 1977) and should be evaluated for pulmonary immunotoxicity.

The above categories of pulmonary immunologic responses can provide information to evaluate the immunotoxicity of inhaled chemicals and to compare sensitivity across

species for use in risk assessment. This cascade of pulmonary immune-system functions can now be used to obtain meaningful results for the extrapolation of data from animals to human risk assessment.

SKIN AND IMMUNOSUPPRESSION

Several studies have established that dermal exposure to some chemicals, drugs, or environmental conditions can lead to selective immunosuppression in laboratory animals (Cooper et al., 1985; Furue and Katz, 1988; Halliday et al., 1988). The list includes ultraviolet light, cold, CsA, DMBA, and various tumor promoters. Immunosuppression is primarily characterized in the animals as an inability to elicit contact hypersensitivity responses to topically applied skin-reactive chemicals, although more generalized immunosuppression has been observed. The mechanisms for these events are unknown but appear to be associated with inhibition of accessory cell function of Langerhans cells at low dose levels and induction of suppressor T cells at higher dose levels. The relevance of these changes regarding actual disease, such as skin tumors or infections, is unknown.

MYELOTOXICITY AND IMMUNOSUPPRESSION

Blood-cell lineages are derived from pluripotent cells, which in adults are primarily found in the bone marrow. Within the marrow microenvironment, these self-renewing cells mature into committed progenitor cells, which can be found in peripheral blood and tissues. The continued development of these cells is under the control of various growth factors, many of which originate in bone marrow stromal cells, which also provide a supporting matrix for development of hematopoietic cells. Hematopoietic cells involved in the immune system include monocytes, granulocytes, and lymphoid precursor cells. A variety of evidence, including the use of long-term bone marrow cultures, has demonstrated the importance of the microenvironment in regulating myeloid and lymphoid development. Bone marrow is a sensitive target for therapeutic drugs and environmental toxicants, most likely because stem cells turn over rapidly. In fact, bone marrow hematotoxicity is the dose-limiting toxicity in many patients in antineoplastic or immunosuppressive therapy.

Although not as sensitive as in vitro proliferative assays, alteration in bone marrow cellularity and differential cell counts are used as indicators of hematotoxicity. The quantitation of pluripotent hematopoietic stem cells (PHSCs) is determined in rodents by in vivo assays of spleen colony-forming units (CFU-S). More committed progenitor cells can be identified and enumerated operationally by their ability to proliferate in the presence of specific colony-stimulating factors (CSF). In vitro assays also have been developed to monitor the formation of stromal-cell-dependent colonies. These assays can establish selective effects on the stromal microenvironment, growth factor production, and direct stem cell proliferation.

A linear relationship between hematotoxicity and immunosuppression has not been established, although it is generally agreed that inhibition of normal lymphoid or myeloid stem cell development will be manifested as altered immune function, provided that the alteration is of sufficient magnitude or duration. In this respect, the toxicity of several environmental contaminants has been attributed, at least in part, to their direct effect on the hematopoietic system. For example, blood dyscrasia, including leukopenia and bone marrow hypoplasia or aplasia, and acute myelogenous leukemia have been attributed to benzene exposure (Laskin and Goldstein, 1977), and some benzene metabolites inhibit bone marrow hematopoiesis in vitro. Benzene also is carcinogenic in

animals and can cause leukemia at doses of 100-300 ppm. Heavy metals, such as arsenic, cadmium, copper, gold, iron, lead, and zinc, also cause hematologic changes in humans. Lead, for example, causes an increase in the number of reticulocytes and a concomitant increase in basophilic stippling (Albahary, 1972). Chronic lead exposure can lead to anemia.

In laboratory animals, many halogenated aromatic hydrocarbons, including chlorinated dibenzo-*p*-dioxins and biphenyls, produce myelotoxicity at relatively low dose levels (Vos and Luster, 1989). PAHs, such as B[*a*]P and DMBA, also produce myelotoxicity in laboratory animals but require higher dose levels (Legraverend et al., 1983). Other chemicals shown to suppress hematopoietic function in laboratory animals include asbestos, selected mycotoxins, glycol ethers, and organophosphate pesticides. Clinical case studies have suggested an association between occupational exposure to organophosphate pesticides and hematotoxicity in humans (Jenkyn et al., 1979).

DIFFICULTIES IN ESTABLISHING HUMAN RISK

Establishing a relationship between potential immune-function changes and actual diseases in humans has been difficult and controversial. For example, Lagakos et al. (1986) reported a high incidence of leukemia and recurrent infections in children of East Woburn, Massachusetts, exposed to drinking water contaminated with industrial solvents. Trichloroethylene (TCE) was the primary contaminant (267 ppm), but lesser amounts of tetrachloroethylene (21 ppm), 1,2-*trans*-dichloroethylene, and 1,1-trichloroethylene were found. Because the analysis was performed several years after the contamination occurred, the initial levels are unknown, but they are assumed to have been higher; a decrease of several orders of magnitude in 2 years in another well contaminated with TCE has been shown (Landrigan et al., 1987). Fagliano et al. (1987) have reported an increase of leukemias in a population in New Jersey exposed primarily to elevated concentrations of TCE, tetrachloroethylene, and other trihalomethanes in drinking water. Increased childhood leukemias have been observed in a population in Arizona exposed to TCE at detected levels of 8.9 and 29.0 ppb (Flood et al., 1989). However, the investigators state that available information was insufficient to conclude that there was a relationship between the environmental contaminants, the population exposure, and the observed childhood leukemia mortality. Studies by Byers et al. (1988) of family members in the East Woburn group demonstrated an increased number of individuals with altered ratios of T-cell subpopulations, autoantibodies, infection, and recurrent rashes. TCE was incriminated as the most likely contaminant, because Sanders et al. (1982) reported that TCE in the drinking water of mice suppresses both humoral and cell-mediated immunity. Dichloroacetic acid, a metabolite of TCE, was reported by Katz et al. (1983) to increase lung lesions caused by parasites in dogs. Unfortunately, as with most epidemiologic investigations, neither the concentrations at the time of exposure nor the effective biologic dose have been estimated or measured. Recently, the Agency for Toxic Substances and Disease Registry has established a registry for persons exposed to TCE (Burg, 1990). This registry could be useful in establishing a basis for the verification of the suspected relationship between animal bioassay results and the human health effects of TCE.

FACTORS THAT AFFECT SUSCEPTIBILITY

Age and External Factors

Some groups of individuals could be at increased risk of developing immunosuppression

as a result of exposure to environmental contaminants. Exposure in utero, when the immune system is developing, could have long-term effects on the ability of an individual to generate an immune response (Osburn and Schultz, 1973). Infants and young children also can have increased susceptibility to immune modulation by environmental toxicants, as childhood is the time when primary immunity is often developed. As a person ages, the immune system begins to decline (which leads to increased susceptibility to infection) and there is an increase in the incidence of tumors (Lange, 1978; Makinodan and Kay, 1980). Because immune function declines as an individual ages, the effect of potentially immunosuppressive chemicals can be more pronounced in adults than it is in children.

Exposure of laboratory animals to TCDD results in thymic atrophy and immunosuppression. When TCDD is administered to adult animals, suppression of the cell-mediated and humoral immune responses occurs and lasts for 1-2 months. However, the effects of TCDD on the thymus and on immune-system responses are more severe and long-lasting if TCDD is administered both before and after birth rather than only after birth (Vos and Moore, 1974; Faith and Moore, 1977; Luster et al., 1979). Thymic atrophy and cell-mediated immunosuppression also are extensive after perinatal exposure, at which time the immune system is being developed in utero (Thomas and Hinsdill, 1979; Fine et al., 1989). Upon perinatal exposure to TCDD, there is a significant reduction in early lymphopoiesis. These studies show that the developing fetal or neonate immune system could be at greater risk of suppression if it is exposed to environmental toxicants.

Other factors that can enhance the effects of immunosuppressive compounds on the immune system are smoking, diet, malnutrition, stress, and disease. For example, smoking has been shown to modulate pulmonary leukocyte function, and smokers could be more susceptible to modulation of pulmonary immune response by ozone or nitrous oxide. Further studies should be conducted to explore more fully the interactions of these risk factors with immunosuppressive environmental xenobiotics. A consensus exists that studies using better defined cohorts and more sensitive tests will be required to assess the potential of xenobiotics to impair human health.

Metabolic Differences

The absorption, metabolism, distribution, and excretion of a given chemical can differ within animal species and between animals and humans. Males and females also can metabolize xenobiotics differently. Because there are sometimes variations in biotransformation of chemicals, careful attention should be given to comparing responses between species.

Metabolic rates vary as a function of body size, and smaller animals metabolize faster. Thus, mice metabolize a material more rapidly than do rats. The differences in metabolic rate can serve as a basis for scaling doses across species. However, across-species scaling is more reasonably based on compound-specific experimental results from several species rather than on arbitrary scaling factors (Davidson et al., 1986).

The route of administration also can affect pharmacokinetics and result in different disease end points. For example, toluene diisocyanate (TDI) is a potent sensitizer when it is inhaled, but it produces tumors when it is ingested. This is presumably because a carcinogenic metabolite forms in the acidic environment of the stomach. There could be marked differences between species in the metabolites formed, and this possibility needs to be explored. When marked differences in biotransformation or unexpected deviations in the metabolic rate of a compound are encountered or are not investigated, comparative-toxicity studies

must be performed in animals to provide a basis for predicting human risk.

Pharmacokinetic studies should be coupled with toxicity studies to establish which dose paradigm or marker paradigm is useful as an estimate of the biologically effective dose. The usefulness of this exercise has already been illustrated in the discussion of the desirability of maintaining a plasma CsA level of 250 ng/ml, the appropriate paradigm of biologically effective dose in this instance.

Species Differences

As alluded to above, one difficulty in extrapolating to humans from animals is differences between species. The differences between species may result from metabolic differences or from actual differences within the immune system (mice, for example, do not produce basophils). Therefore, care should be taken to acknowledge and minimize these differences.

IMPORTANCE OF MECHANISTIC STUDIES

The induction of disease via immunosuppression is essentially a two-stage process: suppression followed by initiation of the infectious or neoplastic response. Therefore, studies of the mechanisms by which the immune status is altered and, therefore, means by which the individual becomes more susceptible are necessary to establish the validity of markers for predicting disease and the degree of their predictability. When possible, mechanistic studies should be conducted with the appropriate consideration of the biologically effective dose and temporal susceptibility of the target site.

Most studies on the mechanisms of immunotoxicant action are performed to allow a further understanding of the effects a compound can have on the human immune system and to show potential differences between animal models and humans. That is, if the site of action of a compound can be determined, and the importance of the analogous site to the function of the human immune system can be established (as discussed with CsA), then the ability of the animal model to predict the risk to human health of exposure to a given chemical can be more fully evaluated. An additional benefit can arise by further clarifying the processes by which the immune system responds to and eliminates foreign materials. In this way, chemical toxicants can be used as tools to dissect specific parts of an immune response. For example, one study showed that *O,S,S*-trimethyl phosphorodithioate, a contaminant in the pesticide malathion, inhibits cytotoxic T-lymphocyte (CTL) activity, an indicator of effector T-cell function, at an early postrecognition step (Rodgers et al., 1988). The study determined that because this chemical is a potent esterase inhibitor, there is an esterase whose activity is necessary at an early post-recognition step for lysis of tumor target cells by CTLs that is distinct from the *N*-benzyloxycarbonyl-L-lysine thiobenzylesterase of CTL granules. This is one example of how a chemical can be used to dissect an immune response, and it demonstrates that immunotoxic chemicals can be powerful tools in basic immunologic research.

SUMMARY

There is an increasing awareness within the scientific and public communities that toxic chemicals can suppress the immune system. These concerns are supported by numerous studies that use experimental animals and, to a lesser extent, by isolated human experience. There is not a great body of evidence that persons in the general population with putative exposure have been immunologically compromised. However, it is well established that treatment with immunosuppressive therapeutic agents and

infection with viruses, such as HIV, result in an increase in the incidence of infection and neoplasia. Exposure markers of immunosuppressive agents are blood or tissue concentrations of the parent compound or its metabolites. The variations of immune-system markers related to immunosuppression can result from various factors, such as age, lifestyle, and adjunctive exposure, as well as from exposure to immunosuppressive agents.

Disease is induced via immunosuppression in a two-stage process: In addition to the immune-system deficiency, there is an initiation of an infectious or neoplastic response. Animal bioassays based on treatment with a putative immunosuppressant followed by exposure to an antigen are therefore most useful to identify the test agent as a potential hazard. Studies of the mechanisms by which the immune status is altered and the degree to which the subject is rendered more susceptible are necessary to establish the validity of extrapolating findings to humans. When possible, mechanistic studies should be conducted with the appropriate consideration of biologically effective doses.

Animal immunologic bioassays are useful to identify possible hazards associated with human exposure to xenobiotics; to explain possible differences in susceptibility between individuals and species; and to develop a rational basis for the management of risk in medicine, in the workplace, and among members of the general public. These bioassays could be useful, because they can suggest possible mechanisms in the classification of carcinogens.

Although the hazards suggested by animal immunotoxicologic bioassays need to be further validated by comparison to humans, there is an unacceptable risk of potential adverse effects among the general public from unnecessary, continued exposure to immunosuppressive agents. Continued exposure to the pollutants at doses causing immunosuppression is unwarranted. However, retrospective studies of exposed persons could be useful, if exposure levels can be reconstructed. Studies of consistently exposed workers, for example, could be useful because of the likelihood of better monitoring of exposure concentrations that are higher than those encountered by the general public, but lower than those producting immunodeficiency in experimental animals. Modification of clinical monitoring to obtain correlations between findings from animal studies and the patient could prove to be useful, although direct application to a healthy population could be difficult.

RECOMMENDATIONS

There is no definitive evidence that individuals living in the vicinity of contaminated sites or chemical manufacturing plants have been immunologically compromised sufficiently to be at increased risk of disease. To elucidate more fully the level of human risk of increased disease after exposure to an immunosuppressive xenobiotic, persons who are occupationally exposed or exposed after an accident should be tested for possible immunotoxic effects. The higher the exposure, the greater likelihood of finding a measurable effect. Several suggestions are made to make this proposition feasible.

A profile of assays for the assessment of the immune function of human populations should be developed (a set of suggested assays can be found in Chapter 7). Within this development, the establishment of normal ranges and the validation of these assays should be emphasized so that the effects of response to immunotoxicants can be monitored. A team of scientists and technicians well versed in the assays within this profile should be appointed to respond to the need for immunotoxicity testing in case of accidental or occupational exposure. This could be handled through the federal government for the establishment of the core group. At the time of an accident, a scientist with expertise in the immunotoxicity of

the chemical in question should be included. Within this context, a questionnaire should be used to assess the increased incidence or duration of infection after exposure to an immunosuppressive chemical. Population background levels should be established for various complaints associated with infection, and the contribution of stress should be determined. Prospective, longitudinal studies of exposed human populations should be done, using the battery of tests described in Chapter 7. These studies will require funding arrangements and well-defined control populations. Ethical, controlled clinical studies that use human volunteers deliberately exposed to potential immunotoxicants should be considered.

Most immunotoxicologic evaluations are performed in germ-free, healthy, well-fed young adult animals. Although this is the standard of practice, some groups could be more susceptible to immunosuppression as a result of factors that affect the response, such as age, malnutrition, and exposure to other chemicals that weaken the immune system. Animal models should be devised to test the effects of age, malnutrition, pregnancy, and other factors on the susceptibility of an individual to immune-system suppression. This would be best accomplished through comparison of the dose-response curves for a given chemical in healthy, young adult animals.

One variable that has not been well defined in immunotoxicology is the level of immune-system suppression necessary to produce increased susceptibility to disease. Animal models are now used to determine the ability of a chemical to increase risk of disease. The end point used in these models is death, which could be caused by factors other than immunosuppression. Models should be developed that measure the effect of a chemical on the immune response and that can be used to evaluate the susceptibility of an animal to a pathogen. These data should then be compared to the effects of the chemical at the same doses on other immune-system responses. Through this type of analysis, perhaps a definition of the amount of immunosuppression necessary to increase the risk of disease could be established.

The tests routinely used for clinical assessment of immunosuppression in humans are not very sensitive. This could result from normal variations in immune function, or it could stem from the types of cells (peripheral blood cells) available for analysis. Assays that have reduced interassay variability and reduced biologic variability and are therefore more likely to detect changes at lower exposure levels should be developed. One way to reduce variability is to establish standard operating procedures.

Extrapolation from animals to humans is difficult because of metabolic differences and other factors. These differences should be taken into account and minimized wherever possible in the interpretation of data. Also, wherever possible, mechanistic studies should be incorporated into the risk-assessment profile of an immunotoxicant. Once the point of action of a xenobiotic is established, it can be determined whether the same site is present in the human immune system. Mechanistic data also can allow a determination of whether a given xenobiotic can induce increased susceptibility to human disease by illuminating the importance of the mechanism to human defense against disease.

6

Animal Models for Use in Detecting Immunotoxic Potential And Determining Mechanisms of Action

This chapter deals with approaches to and methods for using experimental animals to identify xenobiotic substances that produce injury to the immune system and to determine the mechanisms by which injury occurs. The contributions and limitations of animal models are discussed in relationship to extrapolation to humans. Specific approaches and methods are outlined and discussed. As discussed in Chapter 3, immunogenicity of a xenobiotic substance in experimental animal models should provide a flag to indicate the potential of the xenobiotic to produce a hypersensitivity response in humans.

Although every effort should be made to assess the impact of environmental exposure on the human immune system in human populations, experimental animals are the best surrogates for detecting harmful xenobiotic substances and for determining their mechanism of action. With some exceptions, the immune and metabolic systems of humans and experimental animals are similar enough that animal models can provide the conduit to detect immunotoxic chemicals. Most agents that suppress the immune system in humans produce similar results in rodents, and the mechanisms for immunosuppressive action are similar in experimental animals and humans. Data obtained from animal immunotoxicity studies are an important component of the evaluation of health risks associated with environmental chemical exposure.

ANIMAL IMMUNOTOXICITY BIOASSAYS

Much attention has been given to the need to verify immunotoxicity bioassays that appraise immune function after exposure to xenobiotics. Such bioassays should meet several criteria:

1. They should be reproducible within a laboratory and between laboratories.
2. They should be specific to the part of the immune system to be assessed.
3. They should be sensitive enough to measure normal and abnormal immune function.
4. They should be able to measure alterations in immune function caused by exposure to known immunotoxicants.

Control compounds known to alter immune

function, such as cyclophosphamide, should be included in experiment design until the investigators have confidence in the reproducibility and usefulness of the assay in their laboratory. The ability of the assay to predict the potential incidence of human disease must be considered if the assay is to assist in regulatory decisions. The ability of the assay to forecast the effects in humans also should be considered and should be discussed by the investigators when presenting their results.

The development of assays to determine the immunosuppressive potential of chemicals has relied mainly on the use of the mouse, whereas the potential to induce hypersensitivity is most often studied in the guinea pig. Although the mouse is not used routinely in initial toxicologic evaluations, except in the determination of carcinogenic potential, the mouse immune system is well characterized, and most of the necessary reagents for immunotoxicity testing are specific for the mouse.

Because the rat is the animal used most often in initial toxicologic evaluations of chemicals, most of the pharmacokinetic and toxicologic data available are for this species. An effort has been made to use the rat to optimize and verify methods that assess immune function and immunotoxic effects and to develop the reagents necessary to assess immunotoxicity in the rat. Several assays have been optimized and found to be sensitive and reproducible in the rat. One laboratory has optimized a system to examine several areas of immunotoxicity simultaneously in the rat; however, each assay also can be assessed individually (Koller and Exon, 1985; Exon et al., 1984, 1986). All the caveats given here for mouse assays apply to assessments of rat immune-system function. Recent studies have examined cytomegalovirus infection in the rat, but no host-resistance models are currently considered feasible for routine immunotoxicologic evaluation.

Table 6-1 lists several series evaluations that can be used in succession for hazard evaluation. That is, if results of several of the assays in the first group are positive, the investigator should proceed with selected assays in the following groups. Mechanistic procedures are included in Table 6-1. Elucidation of the mechanism by which a chemical affects the immune system provides important information for hazard evaluation.

Some of these procedures are performed in vivo, but the majority are conducted in vitro. Since the immune system is extremely complex and is regulated by other body systems, application of immunotoxicologic data for risk-assessment purposes must be based on data obtained from in vivo exposures. However, both in vivo and in vitro immune procedures that have been verified are appropriate to evaluate chemical-induced immune dysfunction.

Several reviews discuss the various tests used to assess immune function in mice and rats after exposure to potentially immunotoxic compounds (Vos, 1977, 1980; Speirs and Speirs, 1979; Dean et al., 1982; Luster et al., 1982, 1988; Bick et al., 1985; Koller and Exon, 1985; Exon et al., 1986). Some assays are preferred because of their ease of incorporation into current toxicologic examinations. However, many of these procedures are sensitive to modulation by xenobiotic levels that do not produce adverse effects in other organ systems. Other immunotoxicity assays are preferred because they respond to modulation by toxicants. These assays are more difficult to incorporate into routine toxicologic evaluations. The advantages and disadvantages of each assay are discussed below. Table 6-2 summarizes the sensitivity and predictivity of these assays.

Pathologic Evaluation

Pathologic evaluation as an initial screen for immunotoxic chemicals can be useful because it is incorporated into the standard toxicologic assessment of new chemicals

(Vos, 1977). If the pathology serves as a basis for assessing potential immunotoxic effects, then it is not necessary to use more animals than those required in the standard toxicologic bioassay. The areas studied by immunopathology include hematologic values, as well as the weight, histopathology, and cellularity of lymphoid organs. Hemograms

TABLE 6-1 Approaches to Animal Immuntoxicity Testing

Rapid Screen
Pathologic evaluation of lymphoid organs
T-cell-dependent antibody response
Enzyme-linked immunosorbent assay
Plaque-forming-cell assay
Further identification of immunotoxicity
Cell-mediated immunity
Lymphoproliferation
Mixed-lymphocyte reaction, phytohemagglutinin, concanavalin A, lipopolysaccharide, anti T-cell receptor complex, anti-immunoglobulin + interleukin-4
Delayed hypersensitivity response
Cytotoxic T-cell response
Nonspecific
Natural-killer-cell cytotoxicity
Macrophrage bactericidal activity and interleukin-1 tumor necrosis factor activity
Interleukin-2 activity
Immune-cell surface markers
Colony-forming units (spleen); or (granulocyte/monocyte)
Host-resistance models
Listeria monocytogenes
Streptococcus pneumoniae or *pyogenes*
Influenza virus
B16F10 melanoma tumor
PYB6 tumor
Mechanistic studies
Interleukin-2 receptor expression
Ia receptor expression
Transferrin receptor expression
Mac 1 and Mac 2 receptor expression
F4/80 receptor expression
mRNA for cytokines
Complement components
Antibody-dependent cell-mediated cytotoxicity
Respiratory burst (macrophages, polymorphonuclear leukocytes)
Antigen presentation

should include a complete blood-cell count; enumeration of the number of white blood cells; and a differential count of the lymphocytes, polymorphonuclearneutrophils, basophils, eosinophils, and monocytes. The lymphoid organs that should be examined are the thymus, spleen, lymph nodes, and bone marrow. Bone marrow is sensitive to

TABLE 6-2 Validated Rodent Immunoassays

	Sensitive[a]	Predictive[b]
Pathology (M,R)[c]		?
Humoral immunity		
Plaque-forming cell (M, R)	×	×
Enzyme-linked immunosorbent assay or radioimmune assay (R)	×	×
B-cell markers, immunoglobulin (M, R)	×	
Cell-mediated immunity		
Delayed-type hypersensitivity (M, R)	×	×
Mixed-lymphocyte reaction (M)	×	×
Cytotoxic T cell (M)	×	×
T-cell markers (M, R)	×	
CD3, CD4, CD8, Thyl (M)	×	
Nonspecific		
Natural-killer-cell cytotoxicity (M, R)	×	×
Macrophage phagocytosis (M, R)		×
Macrophage bactericidal activity (M, R)	×	×
Macrophage antitumor activity	×	×
Interleukin-2 (M, R)	×	×
Interleukin-1 (M, R)	×	×
Prostaglandin E-2 (M, R)	×?	
Bone marrow		
CFU-S (M)	×	
CFU-C	×	
Host resistance[d]		
Streptococcus (M)	×	×
Listeria (M, R)	×	×
Influenza virus (M, R)	×	×
Herpes simplex virus (M)		×
Plasmodium (M)	×	
PYB6 tumor (M)	×	×
B16F10 melanoma (M)	×	×

[a] Sensitive: procedures with inherently small variation or sensitive to a small degree of immune modulation.
[b] Predictive: procedures corollary to immune function.
[c] M, mouse; R, rat.
[d] Sensitive for detection of pathogenesis but not immune function.

compounds that block division of rapidly proliferating cells. Until now, very few data have been accumulated on the ability of the changes in these measures of immunopathology to predict disease states, and they generally are thought to be ineffective biologic markers of immunotoxicity. Functional alterations of markers related to immune dysfunction are not likely to correlate with changes in histopathologic markers.

Humoral Immunity

The humoral immune response results in the production of antibodies by differentiated B cells that recognize the immunizing antigen (Ehrich and Harris, 1945; Raidt et al., 1968), and initial studies can be used to determine the number of splenic or peripheral-blood B cells. However, as with histopathologic evaluation, an alteration in the number of splenic or peripheral B cells is not a sensitive indicator of chemical insult to the immune system. Therefore, a determination of immune function should be conducted. The generation of a primary humoral immune response requires the interaction of macrophages, regulatory T cells, and B cells (Mosier, 1967; Gorczynski et al., 1971; Miller et al., 1971; Jakway et al., 1975). Because of this complexity, the ability of immunocytes to generate a primary immune response is a sensitive indicator of immune dysfunction caused by immunotoxicants.

The generation of humoral immune responses can be performed both in vivo and in vitro. After in vivo immunization by intravenous injection of the T-dependent antigen (sheep red blood cells), the response can be measured either by quantitation of the serum antibody titer to the antigen (measured by immunoassay or hemagglutination) or by counting the cells that produce antigen-specific antibodies (through determining the number of plaque-forming cells) (Jerne and Nordin, 1963; Cunningham, 1965). The humoral immune response also can be assessed by the ability of immunocytes to proliferate in response to a mitogen, such as lipopolysaccharide (Andersson et al., 1972). However, some compounds that cause changes in serum antibody titer or in the number of B cells that produce specific antibody do not alter mitogenic responses (Sikorski et al., 1989; Cao et al., 1990).

A decrease in the resistance of mice to influenza virus is associated with suppression in the number of plaque-forming cells and in the mitogenic responses of B cells (Luster et al., 1988). The production of antibodies that opsonize (alter bacteria to enable more efficient phagocytization) and fix complement is involved in the elimination of streptococcal infection. In addition, a decrease in humoral immune responses results in increased parasitemia after infection with *Plasmodium yoelli* (Luster et al., 1988).

Cellular Immunity

A cellular immune response results in the generation of effector cells that phagocytize or lyse invading antigens. For example, in an immune response to an alloantigen (an antigen responsible for transplant rejection), the effector cell is a cytolytic T cell (Lindahl and Bach, 1976). Therefore, initial studies could involve a differential count of T cells by cytofluorometry. The same count should be performed in both the spleen and the thymus. However, as discussed above for B cells, simple counting of T cells is not as accurate an indicator of immune-system responses as is the measurement of effector function.

Two assay systems have been used to measure the effect of environmental toxicants on the in vivo generation of cell-mediated immune responses. One is the generation of a delayed hypersensitivity response to antigens, such as keyhole limpet hemocyanin or sheep red blood cells. This response has been measured by the area of induration

formed, the ability of radiolabeled monocytes to migrate and become macrophages, or the amount of radiolabeled albumin that infiltrates the area, all as a result of antigen challenge (Lefford, 1974; Holsapple et al., 1984). The second measure that can be made after either in vivo or in vitro exposure to antigen is the generation of cytotoxic T lymphocytes to alloantigen (Cantor and Boyse, 1975a,b). The level of response is assessed by the ability of immunized cells to lyse target cells that have the same major histocompatibility locus as that of the immunizing antigen. Both these assay systems involve complex interactions of many cell types.

Another assay used to determine the effects of environmental toxicants on the cellular arm of the immune system assesses the ability of immunocytes to proliferate in response to alloantigen. This assay is called a mixed-leukocyte response (MLR). Although MLR does not measure the ability of the effector cell to eliminate antigen, it is sensitive to perturbation by chemicals known to affect cellular immunity. MLR is generally more sensitive to changes than are the proliferative responses to mitogens (Bach and Voynow, 1966; Harmon et al., 1982).

The mitogens used to stimulate the proliferation of T cells are the plant lectins phytohemagglutinin and concanavalin A (Andersson et al., 1972). Some studies have shown that the concentration of mitogens most likely to stimulate T-cell proliferation is also optimal for the generation of suppressor cells (Dutton, 1972; Rich and Pierce, 1973; Redelman et al., 1976).

A change in the ability of the host to resist influenza virus, herpes simplex virus, *Listeria monocytogenes*, and *Plasmodium yoelli* infections and to eliminate the tumor PYB6 has been shown to correlate with alterations in MLR (Harmon et al., 1982; Luster et al., 1988). Changes in T-cell responses to mitogen and cell-mediated immune responses, such as a delayed hypersensitivity response, also are correlated with alterations in the ability of the mouse to eliminate *Listeria monocytogenes*, herpes simplex virus, PYB6, and *Plasmodium yoelli*.

Nonspecific Immunity

The immune system responds to and eliminates antigens before the generation of a specific immune response through nonspecific mechanisms. Natural killer cells can kill sensitive tumor targets (such as YAC-1 for mouse and K562 for human) upon their initial exposure (Reynolds and Herberman, 1981). Although the mechanism of cytolysis could be slightly different, natural killer cells eliminate sensitive tumor cells by a method very similar to that of cytotoxic T lymphocytes. A decrease in natural-killer-cell activity is associated with the reduced ability of an animal to respond to cytomegalovirus and to eliminate PYB6 and B16F10 tumors (Luster et al., 1985).

Standard assays for nonspecific leukocyte function include quantitation of peritoneal macrophage number (basal and in response to in vivo stimuli), quantitation of polymorphonuclear cells, and leukocyte phagocytic ability (basal and stimulated). Although these measurements are easy to perform, they are not sensitive to chemical perturbation. Additional estimates of macrophage function suggested are the quantitation of ectoenzymes, bactericidal activity, and tumoricidal activity. Alterations in polymorphonuclear-cell function and leukocyte phagocytic ability have been correlated with changes in resistance to *Streptococcus pyogenes* infection. Resolution of an infection with *Listeria monocytogenes* requires the appropriate function of macrophages. In addition, modulation of macrophage function has been correlated with changes in the elimination of the B16F10 tumor and *Plasmodium yoelli*.

Soluble mediators of immune responses also are measured to assess immune function.

Interferon and complement serve to eliminate nonspecific complement pathogens. Alterations in interferon levels have been correlated with modulation in resistance to influenza virus. In addition, changes in complement activity have been correlated with alterations in the host defense against *Streptococcus pyogenes* infection.

Bone Marrow

The reservoir of stem cells that replenishes erythroid and immune cells is found in the bone marrow. Because this organ contains many highly proliferating cells, it is sensitive to toxic agents that modulate cellular proliferation (such as antineoplastic agents). Therefore, a change in the cellularity of bone marrow could be a useful indicator of a general toxicity, but is not necessarily specific to the immune system. However, upon stress of the immune system, when it could be necessary to call on the bone marrow reserve, alterations in bone marrow cellularity will lead to immunotoxicity. Two assays are used to assess the effects of xenobiotic substances on the stem cell activity of the bone marrow. One is a determination of the number of cells able to form colonies in the spleen (CFU-S). Additionally, the CFU-GM assay used often in immunotoxicology is based on the ability of bone marrow cells to form colonies of granulocytes and monocytes in vitro in response to the appropriate hormones.

Host Resistance

The models of host resistance now used to assess the integrity of the mouse immune system after exposure to xenobiotics were discussed above. These assays are expensive to run, require special housing to isolate infected animals, and require special facilities to grow the pathogens. Most laboratories undertaking immunotoxicity studies for hazard evaluation will have these models available, but they may not be feasible for individual researchers to undertake. Because the assays are expensive and use a great number of animals, they should not be used for screening. However, once an alteration in one area of immune function is noted, the appropriate model of host resistance could be used to determine the effect of a xenobiotic on the whole animal's response to disease. These host-resistance models are the best available models for illustrating the link between immunosuppression and clinical manifestation of disease end points. Wholeanimal responses could be the best way to predict alterations in immune function, although they might not be sensitive to minimal xenobiotic modulation (Bradley, 1985).

Mechanistic Studies

If the cellular or subcellular site of action in animals is known, the opportunity to determine whether the effect will occur in humans is increased. Often, mechanistic studies can be performed in human cells, and a specific response in an exposed population can be investigated. Indirectly acting chemicals are now being identified. Agents that alter immune function as a consequence of effects on other tissues are only beginning to be investigated. Agents can produce tissue damage, releasing acute-phase reactive proteins, which then modify the immune system. The classical example is casein, which will decrease immune-system activity by causing the release of serum amyloid protein. Agents that alter nervous-system function, kidney function, and liver function are also known to alter immune status.

Because the immune response can be generated in vitro, the actions of a chemical can be investigated at all stages of cellular activity. Several current investigations are directed at determining the biochemical basis for immune-system alterations. Luster et al.

(1985) have shown that benzidine affects the leukotriene pathway in immunocytes. Dean et al. (1985b) reported that dimethylbenzanthracene (DMBA) inhibits interleukin-2 (IL-2) production. Efforts are being made to understand more about pharmacologic receptors on immunocytes and the transduction events that are responsible for immune regulation. Studies by Sanders and Munson (1984a,b) and Fuchs et al. (1988) showed that the β-adrenoceptor activation enhances primary antibody response. Studies by Tucker et al. (1986) and Kerkvliet and Brauner (1987) implicated the Ah (aromatic hydrocarbon) receptor in immunosuppression caused by dioxin. Ah mediates the induction of AAH (aryl hydrocarbon hydrolase) when dioxin and the receptor bind.

Dioxins and polychlorinated aromatic compounds most likely act directly by means of a receptor mechanism. In contrast, cyclophosphamide must be metabolically activated, because its metabolites are the reactive agents. Likewise, the polyaromatic compounds and nitrosamines are metabolized to active chemicals. It is likely that halogenated methanes require activation also.

ASSAYS OF PULMONARY IMMUNOCOMPETENCE

The assays described in the previous section are applicable to situations in which the test chemical can be given orally. Special assays are needed to determine harm from exposure to air pollutants. They should be meaningful in predicting the susceptibility to, severity of, or recovery from disease. They should detect insults to both the humoral and the cell-mediated immune systems, evaluate local pulmonary versus systemic immunity, and provide data that are useful for comparing interspecies sensitivity for risk assessment (G.R. Burleson, EPA, personal commun., 1990).

Several pulmonary immune functions are important in cell-mediated immunity against viral disease and are proposed for use in evaluating immunocompetence: interferon production, alveolar macrophage function, natural-killer-cell activity, and cytotoxic-T-lymphocyte (CTL) activity (Burleson, 1987, and personal commun., 1990). These immune functions increase in the lungs of rats infected with rat-adapted influenza virus. Interferon is measured in the bronchoalveolar lavage fluid (BALF), and alveolar macrophage function is assessed in cells from BALF. Bronchoalveolar lavage is a relatively noninvasive procedure that allows direct comparisons of immune function in animals and humans. Natural-killer-cell and CTL activities are measured in whole-lung homogenate. CTL activity also is detected in the lung-associated lymph nodes (LALNs), spleen, and peripheral blood after viral infection. This pulmonary model for cell-mediated immunity has been used to evaluate the immunotoxicity of inhaled compounds (Burleson and Keyes, 1989; Burleson et al., 1989; Ehrlich et al., 1989; Ehrlich and Burleson, in press). Pulmonary humoral-mediated immunity also should be evaluated after exposure to inhaled pollutants. The important considerations for evaluation of pulmonary humoral-mediated immunocompetence are reviewed by Bice and Shopp (1988).

Changes in antigen elimination: Inhaled pollutants can damage epithelial cells and alter lymphatic clearance of antigens from the lung to the LALNs (Schnizlein et al., 1982; Hillam et al., 1983). Changes in the number or function of alveolar macrophages or neutrophils entering the lung also could alter clearance of antigen (Harmsen et al., 1985, 1987).

Changes in the function of LALNs: Lymphocyte subpopulations and numbers in the LALNs can be altered as a result of inhaled particulate matter eliminated by the LALNs. Insoluble particles remain in the LALNs for long periods (Snipes et al., 1983), and these toxic materials can alter antigen-handling cells and other immune-system functions (Bice et al., 1985, 1987a).

Changes in the recruitment of immune cells

into the lungs: Changes in vascular permeability are important in the recruitment of lymphoid cells and antibody-forming cells into the lung (Bice et al., 1982). Thus, inflammation and lung damage as a result of exposure to pollution could alter the recruitment of immune cells and antibody from the blood into the lung.

Changes in antibody production of the lung: Large numbers of antibody-forming cells are present in BALF from immunized lung lobes (Bice et al., 1980a,b, 1982). Plasma cells in the alveoli and interstitial tissues are at risk from inhaled pollutants (Bice et al., 1987b). Damage to these cells could reduce the production of local antibodies. These are important in pulmonary defense against pathogens. Cells that produce antibody in immunized lung lobes have been reported 3 years after the last antigen exposure (Bice and Muggenberg, 1989). The cells responsible for long-term maintenance of antibody levels in the lung could be important in preventing recurrent pulmonary infections. The cells responsible for long-term antibody production are at risk of damage caused by inhaled pollutants.

Changes in the function of immune-memory cells in the lung: Immune-memory cells are recruited or produced locally after lung immunization (Mason et al., 1985; Bice and Muggenberg, 1988). These cells provide local immune memory against inhaled or aspirated pathogens. Damage to immune-memory cells or to the cells responsible for antigen presentation could result in a loss of localized lung immune memory and lead to recurrent pulmonary infections.

BALF contains numerous biochemical indicators of lung damage, including antioxidants, proteins, enzymes, cytokines, growth factors, arachidonic acid metabolites, and cells. BALF analysis has been used to assess the pulmonary toxicity of numerous inhaled pollutants (Henderson et al., 1979a,b, 1985a,b, 1986, 1988; Henderson, 1984, 1988, 1989). It thus provides biologic markers that allow direct extrapolation of results from animal studies to human subjects. Most of the cells in BALF consist of alveolar macrophages. However, neutrophils provide an indicator of inflammatory response to infection and to inhaled xenobiotics. The above categories of pulmonary immunologic response can provide information to assist in evaluating immunotoxicity of inhaled chemicals and in comparing interspecies sensitivity for use in risk assessment. The severity of change of pulmonary immune functions for humoral and cell-mediated immunity to assess immunotoxicity can now be used to obtain results for the extrapolation of data from experimental animals to humans.

ASSAYS REQUIRING ADDITIONAL DEVELOPMENT

The previous sections listed assays that are generally acceptable for immunotoxicity testing in animals. This section is devoted primarily to animal bioassays that are still in development. Some of these procedures have been used in immunotoxicology testing protocols but require additional testing and confirmation before they can be accepted as validated. Some of the more advanced procedures could require little additional testing to determine their reproducibility; others, used in basic immunology, could require modification or additional development for adaptation to immunotoxicology. Some of these procedures could be inherently insensitive; others might not test for, or correlate with, immune function. Immunotoxicity assays and correlated immune-system biologic markers should be investigated in both humans and animals. The example in a following section relating the effects of ultraviolet light and suppression of Th/Ts ratios is an example of the type of cross-discipline activity that needs encouragement.

Subpopulations of immunocytes can be identified and enumerated by specific surface antigens that are peculiar to an individual cell type, but studies in animals suggest that immunomodulatory chemicals and drugs may not target the same subpopulation of immunocytes

that is affected in humans. Thus, enumeration of immunocyte surface markers as correlates of immune function has not been of much value. Nevertheless, the ease of these procedures and the remote possibility that a correlation could occur suggest that further comparative investigation is in order. In addition, markers of cellular activation that are modulated on the basis of the differentiation status of the cell, such as Tal or class II MHC proteins, may be useful in detecting immunomodulation by xenobiotics.

Lymphokine production and activity have been studied on a limited basis for selected species. Additional information is needed to ascertain the effect of xenobiotics on lymphokine production and activity in animal species and to elucidate the ability of such alterations to correlate with changes in host resistance. Assays are available for the 10 known animal interleukins and several of the cytokines. Animals also produce interferon and other cytokines that are similar to those in humans. Those that require additional investigation for applicability in immunotoxicity testing are tumor necrosis factor and the factor that stimulates CFU-GM in bone marrow (Aggarwal et al., 1985; Pestka et al., 1985; Nathan, 1987; Malkovsky et al., 1988). As new lymphokines are discovered, measurement of immunohormones to detect xenobiotic-induced immune dysfunction deserves attention.

Further exploration of the macrophage as an indicator of xenobiotic-induced immunotoxicity could be of considerable value. Evaluation of cell-surface markers, such as the Mac-1 (type 3 complement receptor) (Beller et al., 1982; Garner and Elgert, 1986), Mac-2 (activation marker) (Ho and Springer, 1984; Garner and Elgert, 1986), F4/80 (activation marker) (Austyn and Gordon, 1981), and Ia antigen (Beller et al., 1980; Bhattacharya et al., 1981), could prove a valuable asset in assessing immune dysfunction. Other properties of the macrophage, such as respiratory burst, antigen presentation, monokine secretion, and activity, cytostasis, cytotoxicity, protease activity, and size heterogeneity, require additional investigation to assess their ability to reflect modulation by xenobiotics (Nathan, 1987).

Other markers that require additional investigation in animals are the antibody-dependent cytotoxic cells (Cordier et al., 1976), transferrin receptor (Neckers and Cossman, 1983), IL-2 receptor (Cantrell et al., 1988), Ia receptor, and complement activity (Ehlenberger and Nussenzweig, 1977). Some of these have been used, and a few appear promising as sensitive indicators of immunomodulation by xenobiotics, but all require additional investigation before they can be considered as validated assays for immunotoxicology. Other procedures that deserve additional attention are detection of mRNA levels (Lindsten et al., 1989) and analysis of bone marrow factors, such as colony forming unit-granulocyte (CFU-G), colony forming unit-basophil (CFU-B), and stromal-cell culture.

USE OF IMMUNOTOXICITY BIOASSAYS

The animal assays used to study the effects of xenobiotic exposure on the immune function of rodents have proved to produce consistent results in several laboratories and are correlated with a change in host resistance. Some assays are of less value, because they lack the ability to detect changes in the immune functions at low levels. Table 6-1 summarizes the status of these assays. Of all animal studies conducted thus far, the generation of a primary immune response to antigen seems to be the most responsive to modulation by xenobiotic substances.

Considerations in the Design of Immunotoxicity Testing

The first choice of an experimental animal

for toxicologic investigation is the one whose toxicokinetics of the test chemical are similar to those of the human. If the metabolism of the chemical is not known, the best experimental animal is usually the one best for measuring the response. The immune systems of most commonly used animals present similar targets and operate in a similar manner. Although the mouse was the experimental animal of choice because much of what is known about the immune system was discovered in the mouse, the rat is equally useful for most aspects of immunotoxicity assessment. The dog is an important experimental animal in toxicology, but has not yet been used widely in studies of immunotoxicity. Primates are now beginning to be used to determine how chemicals target their immune systems. Many of the reagents available for human immune assessment can be used in cynomolgus and rhesus monkeys.

The duration of exposure also is an important consideration. For most studies aimed at determining target-organ toxicity, 90 days of exposure is believed to be adequate to produce and demonstrate most toxicities. Exceptions are made for studies of developmental, reproductive, and genetic effects. For target organs not closely aligned with metabolism and excretion (liver and kidney) of a chemical, 90 days of exposure can mask an effect. The long exposure allows induction of tolerance mechanisms to take place. This could be an important consideration for the immune system, which, for the most part, is not in the mainstream of chemical metabolism and excretion.

The cells of the immune system are activated by the introduction of an antigen and, if the level of the chemical in the lymphoid tissue is decreased because of enhanced metabolism or excretion, an effect that could occur with short exposure could be missed with a longer exposure. An exposure less than 90 days is justified in immunotoxicology because of the fairly rapid turnover of most of the immunocompetent cells once they have been stimulated by an antigen. Except for memory cells, most cells of the immune system perform their function in 3-14 days. Thus, an exposure that maximizes the levels of the chemical in the lymphoid tissues for this period should provide the best chance of showing the effect on the immune system. Exposure of 14-30 days is usually adequate to demonstrate immunotoxicity.

This minimal time requirement for hazard evaluation constitutes a significant advantage over other types of assays that often require much longer periods. However, the prediction of disease end-point incidences is complicated by the necessity of challenges with infectious or neoplastic agents during the duration of immune suppression. Reversibility of effects on the immune system is related to the chemical and the age of the experimental animal. Most of the effects of immunosuppressive antimetabolites, such as azathioprine and methotrexate, are readily reversed. Most immune responses are restored within 2 weeks when alkylating agents, such as cyclophosphamide, are used. However, high doses of alkylating agents can injure pluripotential stem cells that might not be regenerated. In animals, the immune responses could be intact when the animals are challenged, but, if the stem cells are again insulted by administration of antimetabolites, a more severe and prolonged depressed response will occur. The reversibility of the effect on the immune system is important information for hazard evaluation.

As do other systems of the organism, the immune system has many compensatory mechanisms, and in the normal individual a large reserve exists. The immune system has several levels of protection, and innate immunity has several components, as does acquired immunity. Even when innate immunity is sufficient to handle a particular pathogenic insult, the acquired immune system is activated to provide future protection. There is overlap between humoral and cell-mediated immune responses to pathogens. Although one system can predominate in protecting against a given pathogen, the other often will back up or synergize with

the primary system. For example, neutrophil bactericidal activity can be sufficient to protect from a streptococcal insult, but IgM and IgG antibodies afford future protection. When neutrophil activity is inadequate, IgG antibody and complement might be sufficient to remove the invader. In addition, antibody-coated bacteria are more efficiently recognized and killed by tissue macrophages. Similar examples can be shown for cooperation between T-and B-cell-mediated protective activity.

Because dose is a function of the amount of the xenobiotic received and the duration of exposure, because most agents have several target organs leading to both quantitative and qualitative changes in the dose-response curve, and because the immune-system response can be influenced by other target-organ toxicities, care must be taken in selecting doses for immunotoxicity assays, To use these assays, one must be aware of the temporal consideration, especially important to ensure system responses. The dose should not overwhelm the functional capacity of other systems or the metabolic capability of the test animals, and the apparent immune changes should not be the result of influences by other systems. Minimal modulation of the nervous and endocrine systems can result in magnification of the effect in the immune system. Contrary to general belief, the immune system can remain intact when other toxic manifestations are great. In a series of experiments with tetraethyl lead and chlordane, animals surviving a median lethal dose (LD) had intact immune systems. Animals that are moribund because of chemical exposure often can respond normally to antigens. Immune alteration can be secondary to other effects. Acute-phase reactive proteins, produced and released in some hepatotoxic events, can lead to immunosuppression. It is important to know whether the immune system is the most sensitive target, so that appropriate exposure levels can be established and at-risk groups identified.

Immunotoxicity as a Basis for Risk Assessment

Immune function can be defined as the normal, special, or proper action of immunocytes and their secretory products. Action may be expressed if a macrophage phagocytizes foreign debris; B lymphocytes produce antibody that "neutralizes" foreign antigen; T lymphocytes, natural killer cells, or lymphokine-activated killer cells kill tumor cells; or cytokines regulate the immune network. Several immune procedures are available to assess xenobiotic-induced dysfunction in animals. Fewer assays are available to evaluate immune dysfunction in animals. Measuring immune function is essential and critical in interpreting and translating data from animals to humans and in understanding the relationship of immunocompetence to disease resistence. In using immunotoxicity data for risk analysis, one must be cautious to ensure that the data are a correlate of immune function, i.e., disease resistance. The immune system can be a sensitive target organ, compared with conventional toxicologic procedures used to evaluate the toxicity of drugs and chemicals. Although it is not possible to predict the outcome of exposure and human disease incidence from animal immunotoxicity bioassays, they are useful in the hazard evaluation phase of risk assessment. Additionally, immunotoxicity data may clarify the mechanisms whereby agents induce adverse effects that are manifested as other end points, such as cancer.

Chemical suppression of humoral immunity: The immunotoxicity of *trans* -1,2-dichloroethylene (DCE) was assessed in a 90-day study by Shopp et al. (1985). In the cell-mediated immunity assays, no dose-related decreases were seen in either sex. Humoral immune status was assessed by measuring quantitation of spleen IgM antibody-forming cells (AFCs), hemagglutination titers to sheep erythrocytes (SRBCs), and spleen response to the B-cell mutagen lipopolysaccharide

(LPS). Cell-mediated immunity was assayed by delayed-type hypersensitivity (DTH) to SRBCs, popiteal lymph-node proliferation in response to SRBCs, and spleen-cell response to challenge with concanavalin A. In males on day 4 after treatment, there was a significant decrease in AFCs at 17, 175, and 387 mg/kg per day, but the decreases were significant at 175 and 387 mg/kg per day only when the data were calculated on the basis of spleen cells. No dose-dependent effects were noted in the DCE-treated female mice. In both sexes, there were no changes in hemagglutination titers after DCE treatment and spleen responsiveness to LPS was unaltered in male mice. However, females exposed at 452 mg/kg per day did have an enhanced response to LPS. In addition, lymphocyte responsiveness in the absence of the mitogen (LPS) was decreased at 224 and 452 mg/kg per day in females only. This suggests a no-observed-adverse-effect level (NOAEL) of 22 mg/kg per day in female mice. The impact of the decrease in spleen AFCs in male mice at all three dose levels is uncertain, but the decrease is significant only at the 175 and 387 mg/kg per day on the basis of AFCs. Therefore, the NOAEL in male mice is taken to be 17 mg/kg per day.

This study illustrates three areas of uncertainty in the use of immunotoxicity assays. The first, in translation of animal data to human risk, is the difficulty in measuring a given indicator of adverse effect in the same compartment in animals and humans. Rodent immunotoxicity studies most often center on effects in the spleen, as in this study, or in lymph node, thymus, or cells from the target site, such as alveolar macrophages. For the most part, these compartments are the sites of immune function. Thus, the results give the best prediction of the potential actions of a xenobiotic substance on the immune system. In contrast, the major source of immunocompetent cells available for determination of human immune status is peripheral blood. The immune compartments and stages of differentiation of cells in the peripheral blood and in the lymphoid tissue are different. Correlations must be made from knowledge of the action of immune cells in each of the tissues. Unless there is a major breakdown in immune-system function or the challenge by the secondary agent occurs at the right time, an immune-system alteration induced by a xenobiotic is not likely to be detected in specific disease end points.

The second uncertainty of extrapolation of animal data to human risk is the variability in measurement of the indicator of toxicity. Not only are fewer human assays available, but the variability in the human population is considerable. In most immunotoxicity studies, inbred animals are used to decrease variation between individuals. Thus, animal studies are more likely to detect an immunosuppressive xenobiotic substance. Because of genetic differences among humans and because of the many factors that can influence the response of the immune system, it is difficult to detect immunosuppression at low exposure levels. Likewise, immunosuppression can lead to infectious diseases caused by opportunistic microorganisms if simultaneous exposures (i.e., to pathogenic microorganisms and xenobiotics) occur.

The third uncertainty relates to the temporal separation of cause and effect with respect to immune-system suppression. There is a continuum of consequences of immunosuppression that is related to the degree of suppression. The most serious consequence is the time required to recover from an infection. Major immunosuppression is readily detected, and the hazard is easily described. Moderate to minor immunosuppression is less readily detected, but the consequences can be considerable. Prolongation of an infection or development of the disease can occur when moderate immunosuppression occurs. Assuming that this variation in immune-system activity represents an indication of the potential for disease, uncertainty factors of 10 for variability

within the test species (in this case mice), 10 for variation between animals and humans, and 10 if one applies this information derived from a short-term study to a life-time exposure could seem reasonable. Using these uncertainty factors suggests that an intake of 0.0175 mg/kg per day over the lifetime of humans would probably not produce adverse effects.

A role for mechanistic studies in the management of risk: Understanding of the mechanisms of immunosuppression, most frequently developed from animal experiments, can facilitate our understanding of risk and play an important role in the selection of alternatives used to decrease risk. Light (ultraviolet-B, 290-320 nm) suppresses cell-mediated immune responses in the skin (De Fabo and Noonan, 1983). In mice, the suppression occurs regardless of whether the sensitizer (antigen) is applied locally or at a site distant from the site of UV exposure (Noonan and De Fabo, 1990). This depression of the immune system decreases immune responses to conact sensitizers (Noonan et al., 1981) and to alloantigens (Williams et al., 1990) and is critical to the growth of UV-induced tumors (Noonan and De Fabo, 1990). Urocanic acid (UCA) is a major UV-absorbing component of the skin (stratum corneum). UV light converts the *trans* isomer of UCA to the *cis*-UCA isomer, an immunosuppressive agent (Noonan et al., 1988). In mice, the immunosuppression is affected by the generation of suppressor T cells (Ross et al., 1987) and defective in antigen-presenting cells (Noonan et al., 1988). Frentz et al. (1988) have shown that in patients (with nonmelanoma skin cancers) with a history of UV exposure, the ratio of circulating helper T lymphocytes (Th) to suppressor T lymphocytes (Ts) was abnormally low ($p < 0.010$) compared with that in patients with a history of x-ray exposure or controls. The low Th:Ts ratio (CD4:CD8) was associated with an increase in the absolute number of Ts cells. The immunodeficiency in the skin would enhance the survival of initiated tumor cells, increasing the likelihood of progression of these cells to skin carcinomas. This information is important in the understanding of the risk associated with changes in stratospheric ozone (De Fabo et al., 1990). On the basis of the information presented above and the report by Reeve et al. (1989) that topically applied UCA enhanced the number of skin tumors and the malignancy rate in hairless mice, several cosmetic companies have removed UCA from some products, such as moisturizing creams.

Disruption of normal modulation in the immune system: Malathion is a widely used organophosphate pesticide that acts through inhibition of acetylcholinesterase. Inhibition of acetylcholinesterase of red blood cells or pseudocholinesterase in the serum is the standard by which organophosphate toxicity is measured (i.e., this is thought to be the most sensitive indicator of organophosphate toxicity). Studies have shown that administration of doses of malathion that inhibit acetylcholinesterase suppresses the humoral immune response to antigen (Casale et al., 1983). However, recent studies showed that administration of noncholinergic doses of malathion enhanced the immune response to antigen (Rodgers et al., 1986). In this strain of mice, using purified malathion (>99.9% pure), administration of malathion at 715 and 900 mg/kg through oral lavage led to 0% and 10% inhibition of serum pseudocholinesterase, respectively. Further studies showed that enhanced macrophage function contributed to this enhanced immune responsiveness after malathion administration (Rodgers and Ellefson, 1990). Most recent studies were conducted to determine the NOAEL for malathion on the mouse immune system. For this, the respiratory burst of peritoneal cells was used as a measure (Rodgers and Ellefson, in press). The respiratory burst of macrophages is instrumental in the bactericidal and tumoricidal activity of leukocytes. On the other hand, an enhanced respiratory burst of leukocytes has been associated with tissue and DNA damage. An enhanced respiratory burst of

leukocytes is thought to be involved in the pathogenesis of rheumatoid arthritis, ischemia-reperfusion injury, and arthrogenesis. Therefore, an alteration in the level of respiratory burst activity, either increased or decreased, can be detrimental to the host. For this measure of immune function, an unusual dose-response curve was noted. There are dose-dependent changes after malathion at above 150-300 mg/kg (administered orally) and below 1 mg/kg. Between these doses of malathion, there is an elevation in the respiratory burst activity of peritoneal cells, compared with vehicle or naive controls, but not relative to one another (i.e., plateau noted). In this study, lowest-observed-adverse-effect level (LOAEL) and NOAEL for oral administration of a single dose of purified malathion for enhanced respiratory bursts of peritoneal cells were 0.25 (120% of control) and 0.1 mg/kg, respectively. Thus, an uncertainty factor of 10 for variability within species and 10 for rodent to human extrapolation would lead to a relatively safe dose of 0.001 mg/kg. This seems appropriate for occasional exposures to malathion.

SUMMARY

A large body of data exists on animal models, some of which have been verified and many more of which are in development. Over the last decade, several important animal models have been developed to detect the immune potential of chemical agents and to elucidate immunologic mechanisms of injury. The following are two of the most productive areas of development:

1. Animal models are essential for detecting immunosuppressive potential. They provide the means whereby dose-response and mechanistic studies can be performed to provide a basis for hazard evaluation as related to human risk.
2. When possible, the experimental design for animal studies should be applicable to humans with respect to the following:

a. Ability to measure the status of the immune system.
b. Use of a route of administration that emulates human exposure.
c. Matching of the toxicokinetics (i.e., absorption, distribution, and metabolism of the xenobiotic in the experimental animal and humans).

Experimental animals provide an important and necessary means for detecting immunotoxic compounds and for determining their mechanisms of action in the induction of disease. Many of the methods described in this chapter can be used to detect immunotoxic chemicals. They have been validated in laboratories, and several of them can be used for in-depth mechanistic investigations. The principles of toxicology that are applied to assessing immunotoxic chemicals cover the selection of the following: experimental animals; route and duration of exposure; sensitivity, reproductibility, and interpretability of methods; and ability of these bioassays to be applied to hazard evaluation.

RECOMMENDATIONS

The Subcommittee on Immunotoxicology recommends that studies on the toxicity of xenobiotic substances to human immune function include the generation of primary cellular and humoral immune responses to an antigen, such as keyhole limpet hemocyanin. Inclusion of this assay in studies of alterations in immune responsiveness to xenobiotics should vastly enhance their usefulness.

It will be necessary to develop models that detect potential immunosuppressive chemicals in species other than rodents. Dogs and nonhuman primates are widely used in toxicity studies, and attention should

be given to their use in immunotoxicity investigations.

Models need to be developed for closer extrapolation to humans by using severe combined immunodeficiency and transgenic and congenic mouse and human cell lines.

The relationship between indicators of immune status in peripheral blood and lymph nodes (spleen) should be established. Because the business end of the immune system is in the immune system's organs and the available marker medium in humans is blood, the relationship of these components should be well defined with agents that are known to alter the immune system.

The mechanisms by which classes of chemicals produce immune alterations should be determined. Mechanisms classified according to chemical family will assist the regulatory establishment in making decisions related to risk assessment. Knowledge of the mechanism of immune-system injury will enhance extrapolation to humans and give a rational basis to remediation.

There should be investigation of the role of immunocompetent cells in metabolizing chemicals to reactive agents that are either immunosuppressive or that are recognized as nonself and elicit a hypersensitivity reaction.

In vitro immunologic assays that can be used for rapid detection of potential immunosuppressive agents need to be developed to determine cellular and subcellular sites of action.

There needs to be investigation of the relationships between specific chemically induced immunosuppression and quantitative and qualitative changes in host resistance to microorganisms, parasites, and neoplastic diseases.

In host-resistance assays, research should be directed to measure more sensitive end points than mortality. Some suggested approaches include measurement of the generation of an immune factor that is known to eliminate a pathogen after exposure. For example, measurement of viral-induced natural-killer-cell function might be more sensitive and relevant to human disease than is death of a rodent.

7

Human Immune-System Biologic Markers of Immunotoxicity

This chapter deals with markers that could be useful for assessing immunotoxicity in the human immune system. It also discusses currently available clinical tests, and a clinical testing regimen is proposed.

Immunity results from many complex interacting mechanisms that involve antigenspecific and antigen-nonspecific elements. The specific immune system employs two broad classes of cells that react with antigens: B cells and T cells. B cells are precursors of the antibody-secreting plasma cells of the humoral immune system, which in turn produce the five major classes of immunoglobulin molecules. T cells consist of an array of subtypes of cells: those that mediate important immunoregulatory functions, such as help or suppression; those involved in effector functions, such as the direct destruction of antigen-bearing cells; and those that make soluble products called lymphokines. Together, these T cells play a central role in delayed hypersensitivity or cellular immune response. In addition to the antigen-specific elements, there are numerous populations of cells (e.g., monocytes and natural killer cells) and factors (e.g., complement or interferon) that act in conjunction with specific mechanisms of immunity.

TESTS FOR ASSESSING IMMUNITY

An unusual susceptibility to infection and sometimes to autoimmune phenomena or allergy is a characteristic of a meaningful defect in immunity, whether primary or secondary (Waldmann, 1988). The first clue to the nature of the immunologic defect is often provided by the kind of infection. In general, patients with impaired humoral immunity have an increased incidence of recurrent infections with high-grade encapsulated bacterial pathogens, such as *Pneumococcus* and *Hemophilus influenzae*, that lead to chronic sinopulmonary infection, meningitis, and bacteremia. When cellular immunity is intact in such patients, they have less severe problems with fungal and viral infections. Abnormalities of T cells and thus of cell-mediated immunity predispose individuals to infection by a wide variety of agents, including viruses that lead to disseminated infections, particularly herpes simplex, varicella zoster, and cytomegalovirus; fungi that lead especially to mucocutaneous candidiasis; and parasitic organisms, including the protozoan *Pneumocystis carinii*. Infection, of course, can cause as well as result from immunodeficiency. A variety of infectious agents, such as the human immunodeficiency

virus (HIV), can have specific and nonspecific effects on the immune system.

Many tests have been developed to assess immunity (Bentwich et al., 1982, 1988; Rosen et al., 1986). A systemic approach to the evaluation of immunocompetence, which is based on simple screening procedures followed by appropriate specialized tests of immune function, usually permits the definition of the immune defect. Such an approach should include evaluation of the humoral immune system (B-cell system), of the cellular immune system (T-cell system), and of nonspecific resistance (polymor-phonuclear leukocytes, natural killer cells, immune complement). It should be emphasized that although some exogenous agents can alter several elements of the human immune system, others have a primary effect only on a single element. For example, low doses of cyclosporin A selectively affect T cells by acting on lymphokine (interleukin-2) production. Conversely, anticonvulsive drugs, such as phenytoin, act primarily on the humoral immune system, leading to selective deficiency of IgA.

Many of the screening tests were developed to define the position of the defect in the events of cellular maturation and regulatory cellular interaction that lead to profound hereditary immunodeficient states. These tests are not sensitive enough to detect modest immunodeficiency caused by toxic agents. It should be emphasized that normal individuals show a wide range of responses in the tests discussed below. Thus, when studying one person, it should not be concluded that a modest variation from the normal range for an immunologic test is caused by a putative toxic agent. There are numerous testing methods and variations for many immunologic factors and these are constantly evolving. Many of the standard tests are discussed by Reese and Betts (1991) and Aloisi (1988).

TESTS OF THE HUMORAL IMMUNE SYSTEM

The evaluation of the human humoral immune system involves the measurement of serum concentrations of immunoglobulins, the assessment of antibody formation after immunization, the measurement of "natural antibodies," and the enumeration of circulating B cells.

Immunoglobulin Concentration

There are five major classes of immunoglobulin: IgM, IgG, IgA, IgD, and IgE. There are two subclasses of IgA: 1 and 2; and four subclasses of IgG: 1-4. Several methods are available for measuring serum immunoglobulin concentration, including single-radial diffusion, double diffusion in agar gel, immunoelectrodiffusion, radiommunoassay, enzyme-linked immunosorbent assay (ELISA), and automated laser nephelometry. Electrophoresis is not satisfactory for the quantitation of immunoglobulins, but it is useful in the detection of monoclonal immunoglobulins (M-components). The single-radial-diffusion assay is widely used. Gel diffusion methods are very sensitive to differences in diffusion constants and thus to differences in molecular size. It is not possible to measure the concentration of immunoglobulin in body fluids unless the molecules measured in the fluid are the same size as those in the standards. Thus, reliable measurements cannot be made of such proteins as low-molecular-weight IgM in abnormal plasma or IgA in external secretions—where it appears as a dimer rather than as the monomer present in the standard sera—unless special standard preparations that contain immunoglobulins of the same size are used. Furthermore, the use of goat or sheep antisera can give spuriously high estimates for serum IgA in patients with selective IgA deficiency because many of

these patients have circulating antibodies to the ruminant proteins.

The serum concentration of each of the major immunoglobulin classes, with the exception of IgD, which appears predominantly on the cell surface, should be determined. Furthermore, IgG subclass determinations are of increasing importance. The determinations, however, must be well standardized because antisera vary in quality. Because serum immunoglobulin concentrations vary with age and environment, appropriate norms must be used. Patients can manifest a deficiency in all immunoglobulin classes (common variable hypogammaglobulinemia or X-linked lymphoproliferative syndrome after infection by Epstein-Barr virus) or, alternatively, they can have a deficiency of only a single class (IgA in selective-IgA deficiency as a primary defect or after phenytoin therapy). They can even have reduced amounts of only a single subclass (IgG2 deficiency in some persons who have recurrent *Hemophilus influenzae* infections).

The concentration of immunoglobulins cannot be used as the sole criterion for the diagnosis of immunodeficiency. Diminished immunoglobulin concentrations can result from loss into the gastrointestinal tract as well as from decreased synthesis. An indication of loss can be obtained by measuring serum albumin, which usually is lost concomitantly. Normal total immunoglobulin concentrations or even total immunoglobulin subclass concentrations do not exclude humoral immune deficiency. The response to antigenic stimulation with both protein and polysaccharide antigens must be defined if immunodeficiency is strongly suspected. Failure to respond to one or more classes of antigens has been observed in patients with normal or high levels of immunoglobulin classes, regardless of whether they have isolated immunoglobulin class or subclass deficiency. Specifically, patients with the Wiskott-Aldrich syndrome can exhibit normal or even elevated immunoglobulin concentration, yet have multiple infections because of their failure to make specific immune responses, especially when they are exposed predominantly to polysaccharide antigens.

Antibody Formation

Antibody-mediated immunity (humoral immunity) can be assessed by specific antibody responses to specific antigens to which the population is commonly exposed. Humoral immunity after immunization can be assessed the same way. Protein and polysaccharide antigens should be used. Live vaccines (bacillus Calmette-Guérin) including those for poliomyelitis, rubella, and mumps should not be given when a profound primary or secondary immunodeficiency is suspected. Tests for the following are recommended:

Natural Antibodies: Isohemagglutinins are natural antibodies to blood group A or B antigens found in all normal individuals except those with type AB red cells. By 3 years of age, 98% of normal persons with type A, B, or O blood have isohemagglutinin titers of at least 1:16. Patients with the Wiskott-Aldrich syndrome can have normal immunoglobulin levels yet lack isohemag-glutinins as an indicator of their antibody-deficient state. Other natural antibodies that can be assayed include heterolysins (e.g., against sheep or rabbit red blood cells), antistreptolysin, and bactericidal antibodies against *Escherichia coli*.

Antibody Response to Immunization: Commercial diphtheria-tetanus (DT) vaccine can be given in recommended doses. Blood is taken 2 weeks after each injection and tetanus and diphtheria antibodies are determined. Three doses of killed poliomyelitis vaccine (1.0 mL intramuscularly, at intervals of 2 weeks) can be used. Blood is taken 2 weeks after the last injection and antibody is

usually determined by virus neutralization. In patients who have been immunized with DT or diphtheria-pertussis-tetanus (DPT) vaccine, one booster injection is given, followed by determination of antibodies.

Additional Active Immunization: Polyribose phosphate (PRP), the *Hemophilus influenzae* capsular polysaccharide, is a potent but harmless antigen. A single dose (0.05 mg subcutaneously) is sufficient to immunize a healthy person. Immunization of young children with PRP conjugated to a protein carrier is now becoming a standard practice. Consequently, to measure antibody responses purely to carbohydrate antigens, pneumococcal or meningococcal polysaccharides free of carrier proteins should be used. Blood is drawn after 2 weeks and antibody is determined. These and other pure polysaccharides are not useful (and could be contraindicated) in children under 1 year of age. Furthermore, interpretation of results in children under 5 years is difficult. Keyhole limpet hemocyanin (KLH) is another useful protein antigen.

B Cells

B cells are counted by immunofluorescence detection of membrane-bound immunoglobulin or B-cell = specific antigens (CD19 and CD20). Monocytes can be distinguished from B cells by peroxidase or esterase staining, by ingestion of IgG-coated latex particles, or by the use of monoclonal antibodies specific for monocytes.

Precursor B cells can be identified among bone marrow cells with purified fluorochrome-labeled antibodies to detect cytoplasmic μ heavy chains in cells that do not have demonstrable surface immunoglobulin or cytoplasmic light chains.

Further information about the nature of the defects in immunoglobulin production can be obtained by the use of in vitro immunoglobulin biosynthesis studies. Several such procedures have been developed to study defects in the maturation of B cells into immunoglobulin-secreting plasma cells. Although some antigen-specific systems have been proposed, in most cases, the peripheral blood mononuclear cells from the patient to be studied are cultured with a polyclonal activator of B cells, such as pokeweed mitogen or *Staphylococcus aureus* Cowen strain activator. Immunoglobulins synthesized and secreted by the plasmacytoid cells generated can be determined by specific radioimmunoassay or ELISA of the supernatant fluid or by defining the number of immunoglobulin-secreting cells by a reverse hemolytic plaque assay. Modifications of the reverse hemolytic plaque assay have been used in attempts to define helper and suppressor T-cell activity. A description of the details of any specific approach is beyond the scope of this report, and there is no consensus that any one procedure reflects in vivo regulatory T-cell function in all cases.

CELLULAR IMMUNE SYSTEM

Several tests are commonly used to assess cell-mediated immunity, including those that enumerate T cells and T-cell subsets, identify delayed skin reactions, and measure in vitro stimulation of lymphocytes to proliferate and form blast cells. Other in vitro tests measure T-cell effector or regulatory function. As is the case for humoral immunity, a series of simple tests is available to screen for defects in cell-mediated immunity. A white blood cell count and differential should be obtained, and the absolute lymphocyte count should be calculated by multiplying the total white cell count by the percentage of lymphocytes. Children have higher absolute lymphocyte counts than adults do, so age must be considered in evaluating lymphocyte numbers. Lymphocyte counts consistently below 1,500/mm^3 indicate lymphocytopenia and can signify a defect in the T-cell system. The proportion of circulating T cells in the mononuclear cell preparation can be determined

by the sheep red blood cell rosette method or, more usually, by immunofluorescence with the use of CD2 or CD3 monoclonal antibodies. Normally, T cells constitute 55-80% of peripheral blood lymphocytes. Normal values reported for absolute numbers of circulating T cells are 1,620-4,320/mm^3 for the first week to 18 months of life and 590-3,090/mm^3 after 18 months of age (Fleisher et al., 1975).

Some sets of T cells have been defined through the use of monoclonal antibodies. The association of a particular T-cell subset defined with a monoclonal antibody with a given function has caused some confusion in the analysis of immunologic data in immunodeficiency states. For example, CD4-positive cells have commonly been associated with helper functions and CD8 cells have been associated with cytotoxic functions. This dichotomy is an oversimplification. The CD4 population has been shown to contain not only helper cells but also memory cells and cytotoxic cells for targets bearing class II MHC (major histocompatibility complex) molecules and suppressor-inducer cells (Engleman et al., 1981; Meuer et al., 1983). The CD8 population contains cells that can recognize antigen presented by macrophages, and they can augment and amplify the interaction of CD4 cells with B cells. Thus, describing CD4 cells as helper cells and CD8 cells as suppressor-cytotoxic cells is not justified. More important, it has become clear that the CD4 cells and CD8 cells recognize foreign antigens in the context of distinct major histocompatibility antigens. CD4 cells recognize antigen in the association with class II MHC human leukocyte antigen, (HLA-D) molecules, and CD8 cells recognize antigens in association with class I MHC (HLA-A, HLA-B, and HLA-C) (Engleman et al., 1981; Meuer et al., 1983). Abnormalities in the number of CD4 or CD8 cells can be associated with abnormalities in the ability to recognize antigens and regulatory functions of T cells that can lead to immunoincompetence or to autoimmunity.

Skin Testing

The ability of patients to manifest preexisting T-cell immunity has been evaluated in vivo using a series of skin test antigens that normally produce a response. The prototype is the tuberculin skin test. Because delayed cutaneous hypersensitivity, a localized immunologic skin response, depends on functional thymus-derived lymphocytes, it is used in screening for T-cell-mediated immunodeficiency. The antigens generally used are mumps, trichophyton, purified protein derivative (PPD), *Candida* or *Monilia*, tetanus, and diphtheria. At least five of the antigens listed below must be used to ascertain defective cell-mediated immunity. All skin tests are by intradermal injection of 0.1 mL of appropriate dilutions of the antigen and should be read in 48-72 hours for maximal diameter of induration. A negative test is not informative in young children because they might not have acquired immunity.

1. Tuberculin: 0.1 mL, containing 2-10 international units (IU) of Tween-stabilized soluble PPD. If negative, the test should be repeated using 50 IU.
2. *Candida* or *Monilia*: Initially test at 1:100 dilution. If no reaction, test at 1:10 dilution.
3. Trichophyton: Use at 1:30 dilution.
4. Mumps: Use undiluted; read at 6-8 hours for early Arthus reaction (antibody mediated) and then at 48 hours for delayed cellular hypersensitivity.
5. Tetanus and diphtheria fluid toxoids: Use at 1:100 dilution.
6. KLH: Use 100 µg in 0.1 mL intradermally 2 weeks after immunization with 2.5 mg KLH subcutaneously.

In Vitro Stimulation of Lymphocytes

A common test of lymphocyte function is

to determine the capacity of such cells to enlarge and convert into blastlike cells that synthesize DNA and incorporate thymidine after in vitro stimulation. Lymphocytes can be activated by mitogens, such as phytohemagglutinin (PHA), concanavalin A (Con A), or pokeweed mitogen (PWM). PPD, candidin, streptokinase, tetanus, and diphtheria also can activate lymphocytes if the patient has already encountered these antigens. Allogeneic cells used in the one-way mixed-lymphocyte culture (MLC) and antibodies to T-cell surface molecules, such as CD3, CD2, and CD43, involved in signal transduction also stimulate T-cell proliferation. T-cell lymphocytic blastic transformation can be assessed directly by measuring blastogenesis and proliferation of cells; expression of activation antigens such as CD69 or CD25, the IL-2 receptor; and release of mediators. The blastogenic response is assessed by ^{3}H-or ^{14}C-labeled thymidine incorporation for 16-24 hours, followed by DNA extraction techniques or cell precipitation on filter paper and subsequent liquid scintillation counting.

The interpretation of mitogenic responses to various stimuli must be made with caution regarding the type of responding cell. For example, PHA stimulates T cells but it also can stimulate B cells when it is bound to particulate matter. PWM stimulates a response in T and B cells, although T cells must be present for B cells to be stimulated. The MLC reaction is the result of T-cell reactivity to MHC-encoded peptides displayed on the surface of B cells and monocytes. It should be noted that the T cells in the population of normal irradiated or mitomycin-C-treated lymphocytes used as the stimulators can secrete factors that induce blastogenesis by the patient's lymphocytes. Because this can be misleading, it is preferable to use B-cell lines or T-cell-depleted normal cells as the stimulators.

Activated T cells express IL-2 receptors, transferrin receptors, and class II MHC molecules not present or present in low numbers on resting T cells. T-cell populations to be assessed for their capacity to express these receptors are stimulated with a soluble lectin, such as PHA, and examined 3 days after stimulation by direct or indirect immunofluorescence using monoclonal antibodies to the IL-2 (CD25) or to transferrin receptors or to class II MHC molecules. For indirect immunofluorescence, an irrelevant mouse monoclonal immunoglobulin and a fluorochrome-labeled antimouse immunoglobulin are used as a control for potential Fc binding of mouse monoclonal cells. Fc is the fragment of an antibody that binds to antibody receptors on cells and to $C1_q$, the subunit of the first component of complement.

Activated T cells and monocytes synthesize and secrete IL-2, -4, -5, -6, -7, -8, interferon, and other cytokines. The supernatants of peripheral-blood mononuclear cells stimulated by soluble PHA can be assessed for IL-2 by determining their capacities to stimulate ^{3}H-thymidine uptake by mouse IL-2-dependent, cultured T-cell lines. There are specific in vitro systems to assay the other cytokines.

OTHER TESTS

There are several assay systems for biologic markers of nonspecific immunity. As noted before, all patients should have an absolute peripheral white blood cell count as well as a differential test to define the proportions of the white blood cell types. Lymphocytopenia can be associated with primary immunodeficiency diseases but can also occur secondarily to viral infections, malnutrition, stress, and autoimmune diseases or to hematopoietic malignancy. Neutropenia has many causes and often is associated with bacterial abscesses. Bone marrow aspiration or biopsy is important for exclusion of other diseases, for identification of plasma cells and pre-B cells, and for diagnosis of obscure infections. NK (natural killer) cells are large

granular lymphocytes. They are cytotoxic cells that are effective without prior sensitization. The proportion of NK cells can be identified with appropriate monoclonal antibodies, including CD16, which identifies a protein of 50-60 kilodaltons (kD) molecular weight on large granular lymphocytes and granulocytes and CD57. Functional assays of NK activity involve the ability of the appropriate mononuclear cells to kill specific NK targets, such as the K562 cell in which the cell-mediated cytolysis in vitro is quantitated by ^{51}Cr release from the target cells.

Apart from neutropenia, there are defects of phagocytic function that affect polymorphonuclear or mononuclear phagocytes. Neutrophil function depends on movement in response to chemotactic stimulus, adherence, endocytosis, and killing or destruction of the ingested particles. Phagocyte mobility depends on the integrity of the cytoskeleton and contractile system. Directional mobility can be mediated by receptors. Endocytosis depends on the expression of membrane receptors for IgG, C3b, and iC3b and on the fluidity of the membrane. Defects in intracellular killing of ingested microorganisms usually result from failure of the "respiratory burst" that is critical to production of superoxide radicals and hydrogen peroxide. The organisms cultured from lesions of patients with this type of defect are generally catalase positive and include staphylococcus, *Escherichia coli, Serratia marcescens*, fungi, and nocardia. Patients with defects in mobility and in adherence and endocytosis usually have infections of the skin, periodontitis, and intestinal or perianal fistulae. On the other hand, patients with normal endocytosis and defective killing tend to have chronic granulomas. The measurement of the nitroblue tetrazolium dye reduction by actively phagocytosing leukocytes has been accepted as a standard measure for the adequacy of the respiratory burst. Assays for bacterial killing yield highly variable results, depending on the bacterial species used in the assay. Chemotaxis and contractibility of phagocytes can be assessed.

The classic complement system consists of nine components (C 1-9) and a series of regulatory proteins (C1 inhibitor, C4 binding protein, and properdin factors H and I). Many biologic activities important in the inflammatory response and in host resistance to infection take place at various points in the classical or alternative pathways of complement activation. Three clinical states should raise the suspicion of a deficiency of a complement component: systemic lupus erythematosus, recurrent infections of the type seen in hypogammaglobulinemia in a patient with normal immunoglobulin levels, and severe neisserial infection. In the laboratory, the measurement of serum hemolytic complement (CH50) is an important test. In inherited complement deficiencies, with the exception of hypercatabolism of C3, serum hemolytic complement is usually absent and rarely above 10% of the normal value. More detailed analysis of complement components requires functional and antigenic measurements of the individual components, usually best performed in laboratories that focus on the complement system.

OPPORTUNITIES FOR DEVELOPMENT OF BIOLOGIC MARKERS THAT ASSESS THE EFFECT OF IMMUNOTOXICANTS

Primary Humoral Immune Responses

Although the tests for humoral, cellular, and nonspecific immunity have been of great value in studying profound hereditary immunodeficiency states, they are not sensitive enough to meaningfully detect modest immunodeficiency in populations of individuals exposed to immunotoxic agents. Furthermore, the available tests usually are directed toward evaluating a complete immune response, such as cell-mediated immunity, without defining the nature of the immunologic

damage (antigen recognition, lymphokine production, lymphokine receptor expression, effector response). Finally, most of the available procedures, including tests for serum immunoglobulin levels, isohemagglutinin titers, response-to-recall skin test antigens, and proliferative responses to recall antigens, tend to assess the capacity of the individual to make a secondary recall response rather than to make a primary response to a new antigen. The ability to develop an immune response to a new agent is a more sensitive measure of immune impairment than is the inducement of secondary immune response. Furthermore, a test that requires a primary immune response permits a thorough analysis of the events of antigen processing and initial recognition.

This section discusses the scientific basis for finding biologic markers that can be used in assessing the effects of toxic agents on the immune system. The primary response to KLH is one such marker. The individual to be studied is immunized subcutaneously with 2.5 mg of KLH. Two weeks later, delayed hypersensitivity responses are assessed in an intradermal skin test with 100 µg KLH. The antibody response to KLH is determined in serum obtained 2 weeks after the KLH immunization. It would be of value to develop other agents for primary immunization to extend the repertoire beyond KLH. However, the subcommittee does not recommend the use of dinitrochlorobenzene for skin testing because it is mutagenic and causes necrosis.

Activation Antigens on Lymphocyte Surfaces and in Serum

The success of the human immune response requires that T and B cells change from a resting to an activated state when resting cells first encounter a foreign pathogen. Appropriately processed and presented antigens react with antigen receptors on the lymphocyte surfaces to trigger T-or B-cell activation. As part of the activation step, the lymphocyte expresses an array of cell surface antigens that are, for the most part, induced receptors for growth factors. These are not found on the surface of resting cells. The activation antigens that have been extensively studied on the surface of T cells include the 55-kD IL-2 receptor peptide defined by CD25 antibodies, the transferrin receptor, the insulin receptor, and class II MHC molecules (Waldmann, 1986). Additional activation antigens have been identified, including those designated CD30 and CD69 by the Nomenclature Committee of the Fourth Conference on Human Leukocyte Differentiation Antigens (Knapp et al., 1989). CD69 identifies a homodimer that appears within the first few hours after lymphocyte activation. B and T cells display surface activation. For example, the antigen defined by CD23, the low-affinity Fc-∈ receptor is absent on resting cells but is expressed on activated B cells. The ability of a person to express specific growth factor receptors on appropriately activated B and T cells can be determined by immunofluorescence using appropriate specific monoclonal antibodies in conjunction with other studies of the in vitro stimulation of lymphocytes. For example, PHA-stimulated peripheral blood mononuclear cells can be assessed with a CD25 antibody 48-72 hours after stimulation with PHA for the cells' expression of the 55-kD peptide of the IL-2 receptor.

A series of cell surface receptors is released into the body's fluids, including the serum. The concentrations of the released receptors in the serum can be assessed using an appropriate ELISA technique with two monoclonal antibodies that recognize distinct epitopes on the peptide. Released forms of the activation antigens (antigens that are expressed on activated but not normal resting cells), the 55-kD IL-2 receptor (CD25), CD8, CD23, CD30, and the transferrin receptor have been identified in the serum (Rubin et al., 1985). The levels of some of these receptors in the serum have been

correlated with antigenic exposure and with specific disease states. For example, normal individuals have measurable amounts of IL-2 receptors in their serum, and some patients with lymphoreticular malignancies or selected autoimmune diseases and individuals receiving allografts have elevated serum levels of this receptor. The cell surface expression and subsequent release into the body fluids, including serum, of soluble IL-2 receptors appear to be a consequence of cellular activation of various cell types that could play a role in the regulation of the immune response. Thus, the analysis of serum levels of IL-2 receptors and other activation antigens could provide a new approach to the in vivo analysis of lymphocyte activation. Theoretically, elevated levels of these activation antigens result from exposure to viruses and chemicals that are toxic to the immune system.

Synthesis and Secretion of Lymphokines After Lymphocyte Activation

After activation, the T and B cells in peripheral-blood mononuclear cells express the genes that encode a series of lymphokine molecules (IL-1 to IL-10) and colony-stimulating factors. As a consequence, the cells synthesize and secrete measurable quantities of these lymphokines that are involved in the control of T and B cells and in eosinophil and basophil growth and differentiation. Biologic assays (such as IL-2-dependent, cultured-T-cell line proliferation in response to IL-2), radioimmunoassays, and ELISA procedures have been developed to quantitate the concentration of lymphokines and colony-stimulating factors. Furthermore, with the molecular cloning of genes that encode each of these lymphokines, one can quantitate messenger RNA transcription for each of the lymphokines after appropriate lymphocyte activation. In general, for the lymphokines produced by T cells, peripheral-blood mononuclear or T-cell populations are activated with Con A, PWM, or insolubilized CD3 antibodies, and then the appropriate assays are used to quantitate the specific lymphokines produced and secreted into the media. Different patterns of lymphokine secretion have been observed in different subsets of T cells, with γ interferon and IL-2 produced by one subset and IL-4 and IL-5 by another subset of long-term cloned CD4 T-cell lines from mice. Thus, an assay of the pattern of increased lymphokine production could be of value in pinpointing the action of an immunotoxicant on a particular subset of immune-system cells. Furthermore, one can see depressed production of a lymphokine at doses of an immunomodulatory agent that do not affect other aspects of the human immune response. For example, at appropriate doses cyclosporin A inhibits the production of IL-2 without abrogating the induction of IL-2 receptor expression.

Proliferative Responses to Super-Antigens

A series of antigens, the "super-antigens," including *Staphylococcus* enterotoxin A and *Staphylococcus* enterotoxin B, have been defined by White et al. (1989). These antigens are recognized by all T cells that use a particular variable-region β-T-cell receptor. For example, *Staphylococcus* enterotoxin is recognized by subsets of murine T cells that use the Vß3 and Vß8 T-cell receptor genes, whereas *Staphylococcus* enterotoxin A is recognized by populations of Vß11-bearing T cells. The enterotoxins are called superantigens because they can interact with large numbers of T-cell receptors based only on Vß usage and because of a relaxed MHC restriction pattern of recognition. Used in proliferation assays, these agents could be of value in assessing the effect of xenobiotics on the human cellular immune system.

Self-HLA-Restricted Cell-Mediated Cytotoxicity

Immunization with antigens elicits cytotoxic T-lymphocytes (CTLs), which are antigen-specific and restricted to lyse target cells that share MHC gene products with the CTLs. The CTLs are believed to be important in recovery from viral infections. T cells develop receptor specificity for the host MHC gene products expressed on the thymic epithelium prior to antigenic exposure. T-cell receptors that express specificities for foreign antigens and MHC class I gene products are selected when an antigen is encountered on the host's antigen-presenting cells. The maturation of such CTLs requires helper T (amplifier) cells and could be regulated by suppressor T cells. Self-restricted CTLs specific to influenza A-Hong Kong virus can be generated in vitro from human peripheral blood mononuclear cells of patients who have been exposed to influenza virus. The generation of CTLs depends on T cells and monocytes in culture. The peripheral-blood mononuclear cells can be assessed for the patient's capacity to generate self-HLA-restricted, cell-mediated cytotoxicity to viral antigens. When this approach is used in patients with common variable hypogammaglobulinemia, the capacity to produce virus-specific self-restricted CTL is variable but usually relatively normal. In contrast, patients with the immunodeficiency states, ataxia-telangiectasia and the Wiskott-Aldrich syndrome, are unable to produce a significant increase of virus-specific CTLs in vitro. CTL enumeration might be of value as a biologic marker for assessing the effect of immunotoxic agents on the human immune system.

TABLE 7-1 Tier 1 (All Persons Exposed to Immunotoxicants)

I.	Humoral immunity
	Immunoglobulin class concentrations in serum (IgM, IgG, IgA, IgE) and immunofixation electrophoresis.
	Natural immunity: Antibody levels to ubiquitous antigens (e.g., anti-A and anti-B group substances in individuals of non-AB blood type).
	Secondary antibody responses to proteins (e.g., diphtheria, tetanus, poliomyelitis) and polysac-charides (e.g., pneumococcal, meningococcal).
	Note: In immunization studies, live microorganisms should not be given to persons suspected of being severely immunocompromised.
II.	Lymphocytes
	Enumeration of B and T cells in blood.
	Surface analysis of CD3, CD4, CD8, CD20.
	Secondary delayed-type hypersensitivity reaction (e.g., candida, diphtheria, tetanus).
	Alternative: Multiple antigen skin test kit.
III.	Autoantibody titers (to red blood cells, nuclei [ANA], DNA, mitochondria, IgE [rheumatoid factor]).

PROPOSED TESTING REGIMEN

Because the immune system has a large functional reserve, toxic damage is likely to be measurable only after significant impairment. Nevertheless, it is possible to propose a series of well established or relatively well established procedures that can be used to follow individuals or groups known or suspected to have been exposed to an immunotoxicant or potential immunotoxicant. Other less well established but promising tests can be expected to become available and should be considered according to circumstances.

All tests should be performed once or twice a year by a physician on persons who are suspected of exposure to immunotoxicants so that latency and changes over time can be assessed. Finally, aliquots of fresh serum and pelleted white blood cells should be stored at -70°C for each individual studied at each time.

For group studies, a tiered approach, a series of tests done in sequence, is recommended. Tier 1 (Table 7-1) is proposed for evaluation of individual persons exposed or potentially exposed to an immunotoxicant. The combined Tier 1 and Tier 2 (Table 7-2)

TABLE 7-2 Tier 2 (All Persons with Abnormal Tier 1 Test Results and a Fraction of the Total Exposed Population To Be Determined by Statistician)

I.	Humoral Immunity
	Primary antibody response to protein and polysaccharide antigens.
	Note: There is a need to develop a panel of antigens that can be used in sequential studies on a given individual since a particular antigen can be used only once to assess a primary response.
II.	Cellular immunity
	Proliferative response to mitogens (PHA, Con A) and possible antigens such as tetanus.
	Primary DTH reaction (KLH).
	Note: Here, too, there is a need for a panel of standard antigens for sequential testing.
	These could be the same as those used to assess primary antibody responses.
III.	NK cells, monocytes, and other T-and B-cell markers
	CD5, CD11, CD16, CD19, CD23, CD64; class II MHC on T cells by two-color flow cytometry for coexpression of class II and a T-cell marker such as CD3.
IV.	Serum levels of cytokines (e.g., IL-1, IL-2, IL-6) and of shed or secreted cellular activation markers and receptors (e.g., CD25).
V.	Class I and II MHC antigen typing.

regimens should be followed in those exposed and showing abnormalities in Tier 1. Tier 3 (Table 7-3) should be considered for those persons who exhibit abnormalities in Tier 2 tests or for a random fraction of population tested using Tier 2.

SUMMARY

Toxic agents can injure the immune system in any of its broad capabilities. Affected persons run the risk of developing diseases, such as serious infections and neoplasia.

A broad series of tests has been developed to assess humoral (antibody mediated) and cellular (T-cell mediated) immunity as well as nonspecific resistance (mediated by NK cells, complement, etc.). A testing regimen that involves a series of currently recommended assays is proposed that can be used to follow individuals or groups known or suspected to have been exposed to a potential immunotoxicant. A tiered approach is proposed, beginning in Tier 1 with a series of simple tests to be done on all individuals to screen for immunodeficiency.

In this regimen, Tier 2 tests would be performed on individuals with abnormal Tier 1 tests and on a fraction of the total exposed population. Tier 3 would be used for those who test positive in Tier 2 and for a random fraction of the Tier 2 population.

Although the tests for humoral, cellular, and nonspecific immunity have great value in studies of profound hereditary immunodeficiency states, they have not been evaluated for their ability to detect more modest immunodeficiencies that might be observed in individuals exposed to immunosuppressive immunotoxic agents. Therefore, the tests for immune-system biologic markers that have been proposed here for risk assessment should be validated prospectively in populations exposed to putative immunotoxicants and in control groups for their ability to predict the development of disease associated with immunodeficiency.

To accomplish this, efforts should be made on an individual patient basis to establish the degree of exposure to specific immunotoxicants. This could be done with retrospective exposure analysis, but the use of markers of exposure—blood concentrations and tissue concentrations—might be a more

TABLE 7-3 Tier 3 (To Be Considered for Those with Abnormalities in Tier 2 Tests or for a Random Fraction of the Entire Population in Tier 2)

If a proportion of CD 16 cells of Tier 2, III is abnormal: nonspecific killing of a tumor cell line to test for NK function.
If primary DTH reaction in Tier 2, II is abnormal: cell proliferation in response to phorbol ester and calcium ionophore, anti-CD3 antibody, and a staphylococcal enterotoxin B (experimental).
Generation of secondary cell-mediated immune reactions (proliferation and MHC-restricted cytotoxicity) in vivo, e.g., with influenza virus (experimental).
Immunoglobulin subclass levels in serum (IgA1, IgA2, IgG1-4).
Antiviral titers (e.g., influenza, parainfluenza, cytomegalovirus, HIV) in serum (no deliberate immunization).

appropriate approach. Nevertheless, it is imperative that the dose-response relation ship be examined.

RECOMMENDATIONS

Because available tests can lack the sensitivity required to detect modest immunodeficiency, a major focus should be on devising more sensitive tests for markers of immune impairment.

Biologic marker tests that focus on the ability to develop a primary response to a new antigen should be developed and validated. Such tests could be more sensitive than are the available tests that examine secondary recall responses. Furthermore, a test requiring a primary immune response permits an analysis of the events of antigen processing and recognition.

Tests should be developed that examine the expression of activation antigens, including lymphokine receptors (such as CD25, the IL-2 receptor) expressed on the surface of lymphocytes. An analysis of serum concentrations of the released form of these antigens also should be performed. Theoretically, the expression of such cell surface molecules that are not present on normal resting cells but are expressed following lymphocyte activation could be induced as a consequence of exposure to immunotoxicants.

8

Application of Biologic Markers Of Immunotoxicity in Epidemiology

This chapter addresses the use of biologic markers of immunotoxicity in epidemiologic research on environmental health problems and how this use can contribute to traditional environmental epidemiology. The characteristics of biologic markers used in population research are distinguished from the characteristics that affect their use in laboratory studies. Appropriate epidemiologic study designs are reviewed, and four case studies are presented to illustrate strengths and weaknesses in methodology.

EPIDEMIOLOGY

Epidemiology is the study of disease patterns in human populations. One use of epidemiology is to determine whether environmental factors, such as toxicants, drugs, infectious microorganisms, parasites, physical stress, or psychologic stress, can cause or be associated with disease or dysfunction. This objective can be met by comparing groups of people to determine differences in the frequency of a particular disease or risk factor. What can biologic markers of immunotoxicity contribute to this study of disease patterns?

The most common problem in conducting epidemiologic studies is the problem of identifying and quantifying exposure to the putative cause(s) or suspect agent(s). Epidemiologic studies have been classified into three groups, or types:

- Cross-sectional studies or snapshots in time.
- Retrospective studies, usually involving attempts to establish the fact or amount of exposure so as to relate it to current disease or disability.
- Prospective or cohort studies, in which the fact or amount of exposure is established at some specific time and cohorts of exposed and unexposed persons are followed over time to determine whether any disease appears more often among exposed than among unexposed or less exposed persons.

Each of the types of epidemiologic studies has some virtues and some drawbacks. A cross-sectional study can be done at an instant in time—comparing how frequently the suspected cause appears among the diseased persons with the relative frequency of the presence of the "cause" among the nondiseased. The major problem with cross-sectional studies is that it may be difficult to establish the temporal sequence necessary to

a causal relationship. Did the cause actually precede the effect, or did the effect really appear earlier and thus possibly give rise to the presumed cause?

Retrospective studies, or case-control studies, deal better with the temporality of a presumed cause-effect relationship. Persons are identified today as having or not having developed the disease of interest, and then the attempt is made to discover whether the presumed cause occurred earlier (or more often) among the diseased than among the nondiseased. Some of the early work relating cigarette smoking to lung cancer proceeded along these lines. Persons with lung cancer were identified "cases." Persons similar in what were considered characteristics possibly associated with the disease—but currently not known to have the disease—were identified "controls." Smoking histories in cases were compared with smoking histories in controls—and cigarette smoking was found to be much more common among the cases.

What was sought was the development of disease among smokers in contrast to development of disease among nonsmokers. What was found was sort of an inverse: smoking among diseased in contrast to smoking among nondiseased. For rare events, some simple and easily examined assumptions permitted the inversion of the data into the form necessary to make a cause-effect inference. This demonstration prompted a great growth in use of case-control studies for rare diseases, in which logistical problems were easily and inexpensively overcome. What the inversion demonstration did not overcome was the potential for recall bias—unequal remembering of exposure to putative excesses by persons with a disease (looking for a reason for the disease), compared with the recollections of persons without the disease, who had no special reasons to try to remember something. A variety of techniques and devices have been developed to reduce the recall bias (Ozonoff, 1987), but none has been completely successful.

Cohort studies have been proposed as an answer to the recall-bias problem. Following persons known to have been exposed and persons known (or believed to have been) unexposed over time should reduce or eliminate the recall bias. However, even these studies have their problems. They may take a very long time, particularly for studies of diseases with a long interval between exposure and disease development. Rare diseases require that very large cohorts be followed. For causes that have consequences other than the disease of interest (deaths from other diseases), exposed persons will be differentially removed from the populations followed, making the estimation of a cause-effect relationship more difficult to demonstrate or quantify.

Each of these types of studies can have difficulties in establishing or estimating exposure. In studying industrial populations in particular, establishing exposure or quantifying peripheral exposure may be particularly difficult. It is here that biologic markers may become useful to the study of exposure response in human populations.

CONTRIBUTION OF BIOLOGIC MARKERS TO EPIDEMIOLOGY

What biologic markers in general and immune markers in particular can contribute to epidemiology is increased accuracy (e.g., no recall bias) and perhaps some better quantification. With appropriate biologic markers, analysts may be able to establish not only the fact of exposure but also the quantity of a foreign material that reaches a target tissue. Instead of studying cases of overt disease only, analysts could study the early manifestations of exposure. This would allow research to proceed more quickly, thus making possible early intervention or prevention. Biologic markers also are useful in identifying whether there are host or susceptibility factors that predispose some groups of people to disease or confound assessments of exposure-disease relationships

(Hulka et al., 1990). The accurate classification by exposure or disease is of primary importance in epidemiology. Misclassification usually leads to underestimating a cause-effect relationship and leads to a false-negative result, although sometimes it can lead to a false-positive result (i.e., that there is a relationship when none really exists). As an example, instead of assuming persons were exposed to a carcinogen because of their proximity to a landfill, an analyst can determine whether a person was exposed to that carcinogen as shown by DNA adducts in peripheral lymphocytes.

Many immune-system biologic markers can be used in epidemiologic studies as indicators of exposure, early effect, or susceptibility. If immune-system markers are to be used this way, they need to be shown to be valid indicators or surrogates. Some characteristics of validity in the broadest terms have been described in other chapters. For epidemiologic studies, immunotoxic markers must have laboratory validity and population validity. A marker may be accurate in the laboratory in representing a biologic event, but be of uncertain meaning because of wide population variability or low predictive value (Schulte, 1987, 1989).

VARIABILITY IN REFERENCE POPULATIONS

Variability in immune-system markers within a population can be attributable to genetic, environmental, or biorhythmic influences rather than to the exposure that is the object of a study. The analytic techniques used to measure immune-system markers are rarely rigidly reproducible and also contribute to variability. Therefore, evaluation of the differences observed in epidemiologic studies will need to take variability into account before ascribing observed health effects solely to exposure to toxicants. Current data suggest that most markers of immunotoxicity will show considerable overlap between exposed and unexposed populations. Valid data on the sources of variability are obtainable only from carefully designed and executed studies (Edwards et al., 1989; Shopp et al., 1989). Determining the sources of variability and improving the precision of the marker measurements are among the highest priorities of researchers who use biologic markers in public-health studies.

SENSITIVITY, SPECIFICITY, AND PREDICTIVE VALUE

The use of the terms sensitivity and specificity as they relate to populations under epidemiologic study must be distinguished from their use with respect to laboratory methods (Griffith et al., 1989). Laboratory sensitivity indicates the lowest level of an analyte that can be measured reliably by the analytic technique. Laboratory specificity indicates the ability of the analytic technique to exclude identification of substances other than the desired analyte.

Sensitivity and specificity as used in population studies are measures of the accuracy of a test. Sensitivity is the ability to identify a true positive correctly. If the event (exposure) did occur, sensitivity is the measure of the proportion of the cases that the test (or marker) indicates did occur? Specificity is the ability of a test or marker to identify a true negative correctly. If the event did *not* occur, in what proportion of the cases does the test say that the event did not occur? Predictive value is a measure of the potential usefulness of the test in identifying an exposed individual in the population (positive predictive value) or identifying an unexposed individual in the population (negative predictive value). Several examples (derived from Ozonoff, 1987) are given in Table 8-1.

For rare conditions (low prevalence in the population), even very good measures (markers) give poor positive prediction, as shown in Example 1. When there is high prevalence in a population, even substantially

TABLE 8-1 Three Examples of the Relationship Between Exposed Subjects and the Presence (+) or Absence (-) of Markers Illustrating the Interaction of Prevalance, Sensitivity, Specificity, and Predictive Value

EXAMPLE 1: Highly accurate test, used when there is low population prevalence (10 in 1,000 = 1%):			
Sensitivity = 100% = 10/10			
Specificity = 95% = 940/990			
	Exposed		
	Yes	No	
+	10	50	60
-	0	940	940
	10	990	1000
EXAMPLE 2: Highly accurate test, used when there is high population prevalence (100 in 1,000 = 10%):			
Sensitivity = 100% = 10/10			
Specificity = 95% = 940/990			
	Exposed		
	Yes	No	
+	100	45	145
-	0	855	855
	100	900	1000
EXAMPLE 3: Less accurate test, used when there is a higher prevalence (100 in 1,000 = 10%):			
Sensitivity = 100% = 10/10			
Specificity = 95% = 940/990			
	Exposed		
	Yes	No	
+	100	180	280
-	0	720	720
	100	900	1000

Positive predictive value (% of persons with markers present who were actually exposed): 10/60 = 16.7%

Positive predictive value 100/145 = 69%

Positive predictive value 100/280 = 35.7%

Source: Adapted from Ozonoff (1987).

poorer tests (markers) give higher positive predictive values, as shown in Example 2. However, in Example 3, the predictive value is less, despite high prevalence, because of the less accurate test applied. In all three examples, the negative predictive value is 100%, because the sensitivity is 100%, meaning that "all negatives" are true negatives.

Population sensitivity and specificity are determined by both intrinsic and extrinsic factors. Intrinsic factors include the differences in the distribution of the marker in the unexposed population and in the exposed population. An ideal biologic marker is absent in all unexposed persons and easily detectable in all those who have been exposed to the toxicant. Unfortunately, no such marker is known to exist for any organ system, and, because of the variability of immune-system responses within and between individuals, the distributions of most markers are likely to show considerable overlap in unexposed and exposed individuals.

Extrinsic factors also influence population specificity and sensitivity. People who have not been exposed to a toxicant could have other conditions that cause the appearance of the same biologic marker seen in people who have been exposed. Errors in measurement also influence population specificity and sensitivity. Analytic imprecision blurs the intrinsic distinction in distribution of a marker between exposed and unexposed populations, and analytic inaccuracy can lead to misclassification of individuals as positive or negative for the marker.

The particular decision rules or criteria used to determine the event or condition status (especially for health effects) can also have a profound influence on determining population sensitivity and specificity. If, for example, the event or condition is a disease, the "case definition" is often a subjectively determined collection of signs and symptoms. Adding or excluding a particular symptom can result in the inclusion in the group under study of a larger or smaller number of persons with the disease. This will in turn have an influence on estimates of sensitivity and specificity.

For a given test, where the definition of a positive on the test is a value above (or below) some index value, or threshold, the sensitivity of the test can sometimes be increased by adjusting the cutoff or threshold value. However, because sensitivity and specificity are linked, for a given test increasing one will inevitably result in decreasing the other. There are circumstances, however, in which adding another test (or tests) can lead to increasing both the sensitivity and specificity. There the cost is not decreased sensitivity (overspecificity), but rather the actual dollar cost in conducting the additional test(s). A simple inexpensive test with poor specificity can sometimes be followed by a more complex and more expensive test with greater specificity that will weed out many (if not all) false positives.

In developing a test, an estimate is usually made of sensitivity and specificity as a result of a trial on a known or (often) an easily acquired population, such as medical students or nurses. The measures of the test (i.e., sensitivity and specificity) may often shift with the population tested, so that persons using the test (or marker or system of markers) need to be aware that the first published sensitivities and specificities are almost certain to be different from the same measures computed from a different population. The interaction of prevalence, specificity, and sensitivity are illustrated in Table 8-2.

AUTHENTICATION OF THE EVENT STATUS

Accurately assigning the presence or absence of a specified event in reference populations used to estimate the sensitivity and specificity of a biologic marker is critically important to the overall validation process. Unless the status of the event itself can be accurately determined in each member

TABLE 8-2 The Interrelationship Among Prevalence, Sensitivity, and Specificity

Positive predictive values in a test with 95% *specificity* and varying *sensitivity* for four possible exposure prevalences					
Specificity = 95%					
		Prevalence			
		0.0001%	0.001%	0.01%	0.1%
Sensitivity	95%	0.19	1.9	16.1	68
	90%	0.18	1.8	15.4	67
	85%	0.17	1.7	14.7	65
	80%	0.16	1.6	13.9	64
	75%	0.15	1.5	13.2	63
	70%	0.14	1.4	12.4	61
	65%	0.13	1.3	11.6	59
	60%	0.12	1.2	10.8	57
Positive predictive values in a test with 95% *sensitivity* and varying *specificities* for four possible exposure prevalences					
Sensitivity = 95%					
		Prevalence			
		0.0001%	0.001%	0.01%	0.1%
Specificity	95%	0.19	1.9	16.1	68
	90%	0.09	0.9	8.8	51
	85%	0.06	0.6	6.0	41
	80%	0.05	0.5	4.6	35
	75%	0.04	0.4	3.7	30
	70%	0.03	0.3	3.1	26
	65%	0.03	0.3	2.7	23
	60%	0.02	0.2	0.2	21

Source: Ozonoff (1987). Reprinted with permission; copyright 1987, Gordon and Breach Science Publishers.

of the population, the predictive value of any biologic marker is likely to be less than estimated.

Before an event or condition can be confirmed, however, it must be clearly defined. This is often the most crucial factor in estimating the predictive value of any biologic-marker result (Radford, 1981). Event definitions should be considered separately for biologic markers of exposure, effect, and susceptibility. For markers of exposure, the definition of an event may depend on the detection limits of analytic techniques. As these limits become lower and lower, the possibility exists that nearly all persons in industrialized societies will be shown to have some exposure to many, if not most, environmental pollutants. Thus, an arbitrary level of "excessive" exposure might have to be used to define "exposed" and "unexposed" populations. For biologic markers of susceptibility, the condition must be defined in terms of the increased risk of a specified health effect (or of any step in the continuum between exposure and disease) for some defined exposure. For biologic markers of effect, the effect must be clearly defined within the wide spectrum of potential immune-related effects, ranging from those associated with overt disease to those which have no known or apparent health consequences.

Once specific events or conditions have been defined, the definitions must be tested and confirmed. Instances of exposure as defined by biologic markers should be confirmed by physical or chemical measurements of toxicant levels. In some workplaces, epidemiologic exposure indexes could provide legitimate surrogate measures of exposure (Fingerhut et al., 1989). However, verification of exposure status, especially when the researcher is seeking dose-response relationships, usually demands actual measurement of toxicant levels. Where intensity of biologic markers can be measured, these should correlate positively with other accepted measures of exposure. Susceptibility can be validated by the evidence (usually epidemiologic) of an increased relative risk for a specified health event. Prospective studies are often informative for separating the contributions of normal biologic variation, low-level toxicant effects, and truly increased susceptibility attributable to exposure at a specific time to a given toxicant, but other epidemiologic techniques have a useful place. Effects or disease status should be confirmed similarly to susceptibility. For well-defined effects, including death and diagnosable diseases, confirmation through medical records and epidemiologic studies should be possible. For subclinical, immune-related effects, confirmation is more difficult and could depend solely on supporting evidence from other biologic markers. For vague, nonspecific self-reported complaints, confirmation is usually not possible; these present a very difficult problem for epidemiologic studies.

STUDY DESIGN

Immunologic markers can be used as dependent (outcome) or independent (risk-factor) variables in exploratory epidemiologic studies of the relationship between exposure and effect. Much of the literature on the immunotoxic effects of occupational and environmental exposure to toxic chemicals consists of use of cross-sectional studies, in which the outcome variables are markers of effect. The clinical significance of these markers is often unclear, particularly for markers that indicate immunosuppression (Trizio et al., 1988). This unknown clinical significance comes about as a product of the one-time assessment of effect in a cross-sectional study. A single assessment of a marker of immunotoxicity will usually not provide definitive information as a true indication of exposure. Multiple measurements of a marker over time should provide a more accurate representation.

Investigators have used immunologic

markers to define disease in only a few studies. The usefulness of markers should extend to studies of preclinical disease. Immunologic markers, such as human leukocyte antigen (HLA), have been used as the risk factors or independent variables, as for example in case-control designs (Masi, 1979). Because such immunologic markers as the HLA system have been linked to numerous diseases, they could have potential for use in field studies as markers of susceptibility.

There is strong support for the use of immunologic and immunotoxicity markers in prospective studies as early indicators of effect of xenobiotic exposure. In the classical prospective design as indicated earlier, two disease-free groups, distinguished by exposure, are followed for a given period, the incidence of disease is assessed, and the disease rates of the groups are compared. One or more (usually different) immunologic markers can be used to identify the resulting disease or dysfunction. In prospective studies, researchers must confirm that both groups are free of the disease(s) of interest at the inception of the study. If disease is defined as deviation from the norm of some condition identified by or correlated with an immunologic marker, the level of the marker at the study's beginning must be measured (Schulte, 1987).

In prospective studies, the research is more likely to be productive if the researchers know the natural history of the disease and the marker, because "the effects of a chemical may be directly related to the temporal relationship between the chemical exposure and antigenic challenge" (Bick, 1985). This knowledge of disease progression and immune-marker expression will aid researchers in determining when to evaluate the groups in a study. Evaluation too soon after exposure could lead to false-negative results. Periodic assessments that yield time-series data are preferable to a single evaluation. These data often need to be analyzed by procedures not well known to epidemiologists and immunologists. It is not sufficient to analyze such data by using repeated cross-sectional analyses at each observation point. A more suitable approach is to use a model in which the dependent variable in a linear regression is compared with earlier dependent-variable values and an attempt is made to see whether the relationship changes with time. Generally, however, in prospective studies the appearance of an (immunologic marker of) effect can be estimated by the standard hazard rate function and analyzed by the relative-risk regressions method.

REFERENCE POPULATIONS

Occupational exposures often present the best instances in which to test markers of immunotoxicity in chronically exposed individuals, because exposure and health status are often documented. Such populations are ideal for coordinated, cost-effective prospective studies. There are several sources of groups who have been exposed in the workplace. Some companies have established inhouse medical monitoring or worker registries (Tamburro and Liss, 1986). The National Institute for Occupational Safety and Health periodically conducts health-assessment studies that involve immune-system markers (Shopp et al., 1989). Occupational-health and environmental-health clinics can at times be a good source of groups of exposed persons. Many of the clinics are members of the Association of Occupational and Environmental Clinics (Vogt et al., 1990).

Superfund sites offer researchers both an opportunity and a mandate for using immune-system markers in health-effects and exposure studies. The 1987 Superfund Amendment and Reauthorization Act requires the Agency for Toxic and Substances and Disease Registry (ATSDR) to conduct health-assessment studies at the toxic-waste sites on the National Priority List. Studies are to include the use of immune-system

markers to assess baseline immune function, toxicant exposure, and possible susceptibility. The toxicants of greatest concern—which include heavy metals, volatile organic compounds, pesticides, and polyaromatic hydrocarbons—have been listed by ATSDR and the Environmental Protection Agency (EPA, 1987).

Registries of persons exposed to certain toxicants are being established through Superfund by ATSDR. The toxicants studied are chosen on the basis of their anticipated public-health importance. The first such subregistry is for trichloroethylene (Burg, 1990), which has been implicated as a cause of immune dysregulation (Byers et al., 1988) and leukemogenesis (Lagakos et al., 1986). Registries could provide researchers an opportunity to conduct prospective studies in which they could test the predictive value of some immune-system markers.

Studies conducted by state public-health agencies of nonoccupational toxicant exposure have included tests for immune-system markers (Bekesi et al., 1987; Fiore et al., 1986; Stehr-Green et al., 1988). Exposure registries have been established to permit longitudinal studies. The third National Health and Nutritional Evaluation Survey began in 1989; the first phase will run through 1992; the second phase will go through 1995. About 40,000 persons constituting a representative sample of the U.S. population will receive medical examinations and a battery of laboratory tests. This sampling should provide normative reference data on immune-system markers and perhaps allow researchers to relate the data to toxicant exposures. A subset (perhaps 4,000) will be tested for evidence of exposure to volatile organic compounds, phenolic compounds, and pesticides.

CASE STUDIES

Four field studies have been selected to illustrate the use of biologic markers, especially as related to immunotoxicology. The summaries presented are not meant to be comprehensive; they are intended to provide information on how a study was or could be designed to use markers of immunotoxicity. The fact, however, is that epidemiologic studies that have made full use of the marker concepts have not been completed.

CASE 1:

Incidence of immune hyper-sensitization in workers using hexamethylene diisocyanate (HDI) and its trimer (THDI) (Grammer et al., 1988).

Background

Isocyanates can induce symptoms of respiratory disease through immune mechanisms (inducing antibody products) or as nonimmune irritants. A group of 150 workers who used HDI and THDI were evaluated to determine the prevalence of immune sensitization and its relationship to work-related respiratory disease.

Study Design

An 18-month prospective study was conducted on 150 current workers in a factory where truck cabs had been sprayed with paint that contained HDI and THDI. The researchers administered a medical history and symptom questionnaire to workers with a minimum of 2 months of employment. On the basis of the questionnaire responses, two physicians (who did not know any subject's work history) identified workers with asthma, allergic rhinitis, allergic conjunctivitis, and hypersensitivity pneumonitis. They then distinguished symptoms of these conditions from those attributable to HDI and THDI. Exposures to HDI and THDI were measured several times in each work area and were expressed as time-weighted averages.

Workers were classified according to job class, tobacco use, and other behavioral and demographic characteristics. The immunologic markers used included IgE and IgG antibodies to HDI-HSA (human serum albumin) and THDI-HSA determined by enzyme-linked immunosorbent assay.

Results

Approximately 21% of the workers had a positive antibody result (generally low-level IgG). One worker had symptoms that appeared to be caused by exposure to HDI. Generally, there was no difference among job classifications or between smokers and nonsmokers for antibody levels. The number of workers whose antibody levels increased roughly equaled the number whose antibody levels decreased. The investigators concluded that the low-level presence of antibodies in the absence of clinically observed disease could indicate exposure but not current clinical disease.

Strengths

A prospective design was used to determine whether exposure to HDI or THDI resulted in the formation of a biologic marker of exposure and whether the marker was correlated with clinical disease.

Limitations

The lack of measurements of the toxicants in the breathing zone (i.e., personal sampling) limited the researcher's ability to make individual exposure-response determinations. Researchers were unable to distinguish job categories on the basis of immunologic markers.

CASE 2:

Workers exposed to 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD) (Sweeney et al., 1989).

Background

TCDD is a potent toxicant in some small mammals and produces selective immunotoxicity in mice (Luster et al., 1987). Human exposure to TCDD can cause chloracne. There are no reported cases of death or serious illness (Filippini et al., 1981; Suskind and Hertzberg, 1984). Some reports suggest human immunotoxic effects (Hoffman et al., 1986; Knutsen et al., 1987; Jennings et al., 1988), but no convincing evidence has emerged from these studies. Some of the data have been challenged (Evans et al., 1988).

Study Design

A cross-sectional study was conducted on production workers previously exposed to TCDD-contaminated chemicals. Some workers were exposed to high doses over several years (Sweeney et al., 1990). A total of 541 persons (281 exposed and 260 control subjects) were brought to a single, central clinical research facility for evaluation. Control subjects were matched to study subjects by age, gender, and community of residence. The study included an extensive history and medical examination, a large array of immune tests (Shopp et al., 1989), and measurement of serum TCDD levels (D.G. Patterson, Jr., et al., 1989).

Results

Results for immune-system markers from the study are now under analysis (Sweeney et al., 1989). Measurements of serum TCDD levels, a marker of exposure, and a

good estimate of the dose in terms of body burden could be used to determine whether there is a dose-response relationship for immune-system markers of effect (Fingerhut et al., 1989).

Strengths

The size and chronic high-level exposure of the study population will allow researchers to make valid conclusions about whether TCDD affects the immune system. Serum TCDD has a half-life of several years and can be detected long after exposure has ceased, allowing researchers to determine long-past exposure by measuring TCDD levels (Pirkle et al., 1989). The duration of employment in TCDD-contaminated areas has been found to be highly correlated with serum TCDD levels (Sweeney et al., 1990). The well-matched control population will allow the researchers to determine valid reference ranges for this population of older, mostly white males. The array of immune assays was comprehensive and well controlled; it was tested repeatedly on a small group of laboratory volunteers (Shopp et al., 1989). The full extent of variability throughout the immunologic assessments can be determined from this control population. Detailed medical histories and medical examinations will allow researchers to explore correlates of exposure, markers of immunotoxicity, and health status. Because members of this study population are approaching an age at which immune-system function declines, a fairly susceptible group is available for study. Participation rates were high in both the exposed and the control populations (Sweeney et al., 1989).

Limitations

The length of the study might permit seasonal variations to affect the distribution of results. The required long-distance travel for some participants could have affected immune response because of stress or changes in circadian rhythm. A study of this kind is very costly. Unfortunately, this well-designed study is not yet completed.

CASE 3:

Effect of solvent-contaminated well water on the immune system (Byers et al., 1988).

Background

In this study, four kinds of markers were used to assess the immunologic effect of drinking solvent-contaminated water. The study took place because of a statistically significant excess of leukemia in a census tract that received some of its potable water from two contaminated wells.

Study Design

A modified case-control design with repeated evaluations was used to determine whether persons exposed to contaminated water exhibited immunologic effects different from effects found in those who were not exposed. The case subjects were family members (those with leukemia probands) and were residents from the census tracts receiving water from the contaminated wells. Control subjects were selected by probability sampling techniques and were matched to case subjects on age, sex, and social habits. The groups were compared for absolute numbers of T cells (CD3), CD4 cells, and CD8-positive cells; the ratio of CD4 to CD8; the presence of autoantibodies; and the incidence of infections and rashes.

Results

Changes in the immunologic system were

manifested by altered ratios of T-lymphocyte subpopulations and by an increased incidence of autoantibodies, infections, and rashes.

Strengths

The findings were consistent with earlier reports in the scientific literature. They occurred about 5 years after exposure, possibly demonstrating a persistent effect. The findings were consistent with the use of multiple markers over multiple periods. The analysis used appropriate sophisticated statistical techniques.

Limitations

The study design did not allow researchers to discern whether the illnesses found resulted from genetic or environmental factors. The exposure information was on an area basis, rather than an individual basis, and required that the researchers assume that all case subjects were exposed and that all control subjects were not. Because the study was done several years after the exposure occurred, intervening factors, such as unrelated exposures, could have affected the results.

CASE 4:

Tight-building syndrome (Levin and Byers, 1987), study of environmental illness.

Background

Immunologic effects have not been the primary focus of investigations of the "sick-building" syndrome. Immune-system responses have been determined to be consistent with the range of symptoms reported, and they are believed to be confined to immunologically mediated respiratory diseases, occasionally involving the skin or mucous membranes (Samet et al., 1988). Immune-system markers have rarely been used, except to assess antibody responses to infectious organisms, for example, in Legionnaires' disease, or to assess specific allergies. The work of Levin and Byers (1987), however, describes the use of immunologic markers in studying several tight-building situations in which people were exposed to airborne pollutants. Levin and Byers suggest that immunologic responses resulting from chronic low levels of exposure to toxic chemicals can be assessed by evaluating a constellation of signs, symptoms, and laboratory findings. In this case study, they reviewed the use of T-helper/suppressor-cell (CD4:CD8) ratios to evaluate exposed and control populations.

Study Design

Levin and Byers (1987) cite data from four studies with different exposure scenarios in which the helper/suppressor ratios were plotted according to the percentage of the population in each category. The comparison was between exposed patients and asymptomatic, apparently unexposed control subjects.

Results

The ratios of T-helper to suppressor cells were found to be statistically lower in exposed than in control subjects in the four studies cited. These changes were supported by a similarity in symptomatology of the patients evaluated.

Strengths

This approach allows researchers to quantitate the immunologic markers of a

series of diverse conditions to characterize better the potential health effects of unknown origin. It also allows researchers to quantitate immunologic changes and validate the markers by review of reported symptoms.

Limitations

In each of the reports of tight-building syndrome reviewed by Levin and Byers (1987), each study included only a small number of exposed subjects, and the analytic methods were not published for detailed review. With small numbers of subjects, differences in the way cases and controls are assessed can contribute to an apparent effect in the small exposed group. The possibility of bias in the selection of the exposed cases cannot be ruled out. The findings have not been replicated and published in the scientific literature.

RECOMMENDATIONS

Immunotoxic markers can be used to identify exposure, effect, or susceptibility. For epidemiologic studies, markers should be selected for use on the basis of their predictive value in human populations, validated animal models, and the underlying biology of the markers.

Biologic markers of immunotoxicity, to be used in epidemiologic studies, are subject to several limitations: The predictive value of the markers should be known, and it should be high. Study populations must be large enough to yield firm estimates. The comparison of results from exposed populations with those from unexposed populations should be controlled for known confounders. Standardized assays should be used to allow for the confirmation and interpretation of markers. Multiple immune markers should be assessed, and multiple periods should be considered for specimen collection.

Biologic markers of immunotoxicity can best be tested in occupationally exposed populations. The use of biologic markers of immunotoxicity in environmental epidemiologic research requires interdisciplinary collaboration among laboratory scientists, field scientists, and clinicians in planning, implementing, and interpreting studies.

Cooperative interchange among laboratories that use markers of immunotoxicity to monitor other kinds of exposure to immune-related disease (such as HIV infection) should be encouraged, so as to standardize some of the more promising new assays. The possibility of banking specimens from persons with documented exposures to chemicals should be explored, so that new assays can be investigated quickly. Attention should be given to how the results of uncertain assays of immunotoxicity markers will be interpreted and communicated to study subjects. The legal and ethical implications of labeling groups or individuals on the basis of altered marker frequencies should be considered.

Surveys of the prevalence of immunotoxicity and immunologic markers should be conducted to provide baseline and reference values. Pilot studies should be performed before large-scale epidemiologic studies are begun.

9

Use of Biologic Markers In Controversial Areas Of Environmental Health

In recent years a number of concerns have been voiced about the effects of chemical contaminants of the air, water, and food supply on human health. Clinical syndromes or disease entities have been described in which there is an alleged susceptibility to these chemicals at concentrations generally present in the environment and tolerated by most of the population. Terms such as sick-building syndrome (SBS), multiple-chemical-sensitivity (MCS) syndrome, and environmental illness (EI) have come into use to describe illness in groups of patients whose reactions to environmental toxicants are either induced by or exacerbated by exposure to the so-called chemical environment. A related syndrome, reactive-airways-dysfunction syndrome, also has been described. Some authors have claimed an immune pathogenesis for chemical sensitivities; others have dismissed the entire area as a somatization disorder or mass hysteria.

The controversies in this area could not be resolved by the Subcommittee on Immunotoxicology. Members of the group believe that there is insufficient evidence that MCS is an immunologic problem and that this report is not the proper place to provide a definitive discussion of these issues. Nonetheless, the group's members decided to devote a chapter to these issues, because it will be necessary to use immune-system biologic markers to determine whether the immune system is involved with these disorders; this is an area of increasing national concern, with significant but uncounted patient populations suffering morbidity and disability, no matter what the cause; and the work of this subcommittee can be a stepping stone to a more definitive discussion by another group. The emphasis in this chapter is on health effects of exposure to airborne chemicals. This chapter addresses six questions:

- Does the alleged chemical environment exist as a measurable entity?
- What is known about the exposure of the population to the substances that have been alleged to produce symptoms in susceptible people?
- What are the potential health effects of these compounds?
- What evidence is there that some individuals are susceptible to toxicants at levels present in the ambient air that are tolerated by most of the population?
- What is the nature of SBS and MCS, and what case definitions have been given?
- What steps should be taken to resolve

problems in definition of such disease states and the frequency of diseases or syndromes related to xenobiotic exposure and the mechanisms underlying these syndromes?

EVIDENCE OF EXPOSURE TO ORGANIC CHEMICALS

The toxicants implicated in chemical-sensitivity syndromes are predominantly low-molecular-weight hydrocarbons that often have additional atoms of nitrogen, oxygen, and the halogens. Some of these organic compounds occur naturally—they are found in edible and toxic plants. Some are synthetics used as pesticides, pharmaceutical agents, perfumes, disinfectants, and food additives. Others, including the volatile organic compounds (VOCs), are products of combustion, including vehicle exhaust, tobacco smoke, and emissions from heating units. Outgassing from synthetic materials also can contribute to the presence of VOCs in the air. Table 9-1 lists some of the sources of compounds that allegedly cause problems for sensitive individuals (Randolph, 1962).

The Total Exposure Assessment Methodology (TEAM) study was designed to measure VOCs in drinking water, indoor air, and outdoor air and to quantify the demographics of a group of people in Elizabeth and Bayonne, New Jersey. Eleven of 19 compounds studied (chloroform, 1,1,1-trichloroethane, benzene, styrene, o-xylene, carbon tetrachloride, *m/p*-xylene, *m/p*-dichlorobenzene, ethylbenzene, trichloroethylene, and tetrachloroethylene) were consistently present in personal air samples taken in the breathing zone of the subjects and in exhaled air at higher concentrations than found in the outdoor air of the two cities (Wallace et al., 1985). The correlation between personal air samples and exhaled air measurements was better than that between outdoor air and exhaled air concentrations. It was concluded that these toxicants are found more frequently indoors and that the population gets a stronger dose from indoor sources than from outdoor sources. The early phases of the TEAM study compared personal air in homes to outdoor air. Later work was extended to include public buildings (Sheldon et al., 1988a,b). Eight compounds were found at greater concentrations in the indoor air of new buildings than in outdoor air. Concentrations were greater in the indoor air by factors of as much as 100.

The TEAM study is an important contribution to environmental medicine. It verifies the existence of the "chemical environment," points out that indoor air quality is a major determinant of body burden of VOCs, and provides a list of such chemicals that must be assessed for chronic toxicity and for eliciting hypersensitivity responses in susceptible persons. Other studies of indoor air have focused on radon, asbestos and other fibers, tobacco smoke, formaldehyde, indoor combustion, moisture, and microorganisms and allergens from biologic sources (NRC, 1981). The microorganisms and biologically derived allergens may play an important part in indoor air pollution, but are not within the scope of this project.

HEALTH EFFECTS OF INDOOR AIR CONTAMINANTS

In discussing the health effects caused by exposure to VOCs, it is helpful to distinguish two classes of health effects that have been described. Class A syndromes have one distinct, quantifiable clinical response, such as bronchospasm, depression, or cardiac arrythmia, resulting from an exposure to a single well-defined airborne chemical. A number of studies ranging from scientifically conducted double-blind studies to case reports and anecdotal data have discussed class A syndromes, and it is accepted that airborne chemicals can cause a variety of clinical responses in susceptible individuals at doses tolerated by most of the population. The responses range from occupational

asthma caused by toluene diisocyantate (TDI) (Chapter 3) to a lupus-like syndrome caused by laboratory exposure to hydrazine (Chapter 4).

TABLE 9-1 Agents Reported to Cause Symptoms in Chemically Sensitive Individuals

Indoor air contaminants
Utility gas
Combustion products of gas, oil, or coal
Coal smoke
Fumes from fresh paint, turpentine, mineral spirits, detergents
Fragrances from toiletries and perfumes
Cleaning products, such as bleach, ammonia, disinfectants
Insect sprays and repellents
Odorous synthetic carpets, pads, adhesives, and building materials
Outdoor air contaminants
Automobile and diesel exhaust
Industrial air pollutants, especially from refineries and storage tanks
Paint-manufacturing and sulfur-processing fumes
Fumes from roof tar and roads
Chemical additives and contaminants of food and water
Insecticide and fumigant residues
Some chemical preservatives
Sulfur residues
Chemical flavoring and sweetening agents
Plastic containers and lined tins
Chlorine in water
Synthetic drugs, cosmetics, and miscellaneous chemicals
Medications, including aspirin, sulfonamides, synthetic vitamins
Allergic extracts or other biologic materials that contain phenol
Cosmetics
Synthetic textiles
Bed linens washed with detergents, dried in gas driers, or impregnated with synthetic starch or sizing

Source: Randolph (1962).

Class B syndromes are those in which there is a variety of symptoms, sometimes highly subjective and difficult to quantitate, that result from exposure to several odorous chemicals. SBS and MCS fall into this category. These illnesses are highly controversial, and some researchers consider them to be hysterical in origin or a form of somatization (Terr, 1986); others consider them important, distinct clinical syndromes worthy of case definitions and serious study (Cullen, 1987).

It is important to distinguish the two classes. Although there are solid scientific

data to support the existence of class A syndromes, complaints of chemical susceptibility are sometimes dismissed as hysterical, even when there is a well-defined, quantifiable clinical response that is amenable to scientific study. Furthermore, there could be a relationship between emotional state and physiologic response. For example, a patient with occupational asthma who has had severe reactions to chemicals, such as TDI, might develop hysterical symptoms from exposure to other noxious odors.

There have been several reports of chemical exposures that cause well-characterized class A illnesses in patients at dose levels normally tolerated by the population at large. It is well documented that low-molecular-weight organic compounds can cause allergic and autoimmune diseases, as in the case of a laboratory worker who developed a disease similar to systemic lupus erythematosus after exposure to hydrazine in the workplace (Reidenberg et al., 1983). The disease went into remission when the patient avoided contact, and then resumed—with symptoms of rash, fatigue, and arthralgias—within 2 days of the patient's resuming work with the material. Peripheral-blood lymphocytes from the patient and her twin sister, but not from three normal control subjects, showed a dose-dependent inhibition of the mitogenic response to concanavalin A after in vitro exposure to hydrazine. Pokeweed-mitogen-stimulated IgG production decreased in the patient and her twin sister after daily in vivo exposures to hydrazine.

Provocative challenges have been used to document that inhalants, such as tobacco smoke and perfumes, can induce bronchospasm in some patients with asthma (Shim and Williams, 1986). Bronchoconstriction occurred after exposure to perfumes and colognes in four patients, with forced expiratory volume in 1 second declining 58% in the most severely affected. Shim and Williams's survey of 60 asthma patients found that more than half were sensitive to tobacco smoke, perfumes, or other inhalants; these patients made lifestyle changes to avoid contact with these substances. Substantial declines in pulmonary function after provocative challenges also have been documented in asthma patients with a history of sensitivity to tobacco smoke (Stankus et al., 1988).

The onset of asthma in previously healthy individuals following a single high-level exposure to an irritating vapor, fume, or smoke has been termed reactive-airways-dysfunction syndrome (RADS) (Brooks et al., 1985a,b). Symptoms develop from minutes to hours after exposure, which is most often associated with an industrial accident. Respiratory symptoms and bronchial hyperreactivity persist for months to years, and chronic airways disease that is difficult to treat can result.

An increasing incidence of depression in the United States has been documented (for a review, see Klerman and Weissman, 1989). In this tragic epidemic, the role of environmental chemicals or the stress imposed by our more complicated lifestyle has not been clarified. Psychiatric disorders that arise from exposure to specific organic compounds at levels tolerated by most persons are best documented for pharmaceutical agents. Depression and psychiatric disorders are associated with use of tricyclic antidepressants, scopolamine, amphetamines, phenylcyclidine, phenylpropanolamine, and other pharmaceuticals (Ellenhorn and Barceloux, 1988). Case reports of depression caused by the inhalation of home furnace fumes have been reported (Randolph, 1955). Cases of psychosis resulting from exposure to air contaminants at concentrations tolerated by the normal population have been described (Randolph, 1962). Toxic psychosis can occur from exposure to a number of chemicals, including organophosphate pesticides (Gershon and Shaw, 1961).

Nasal disorders have been associated with exposure to some chemical toxicants. Vasomotor rhinitis, a condition of nasal congestion, and chronic rhinosinusitis, which is well recognized by allergists and otolaryngologists, can be exacerbated by exposure to

fumes from solvents, newsprint, and vehicle exhaust at levels tolerated by the general population. A vasomotor lability is postulated for these intractable disorders, and patients report various degrees of relief to be achieved by avoiding contact with the irritants. In addition, some persons who have vasomotor rhinitis are sensitive to temperature changes, and some have symptoms that are exacerbated by drinking alcoholic beverages.

Some workers with documented occupational exposure to various solvents are known to develop aversion reactions to odorous chemicals at levels tolerated by most people. Cacosmia has been defined as "nausea, headaches, and subjective distress in individuals exposed to neutral odors" (Ryan et al., 1988). Citing the "rich neural interconnections between the olfactory system and the temporal regions of the brain," Emmett (1976) hypothesized that complaints of cacosmia could correlate with decreased performance on neurobehavioral tests that are sensitive to dysfunction of the temporal lobe. Workers exposed to solvents, a group known to suffer from cacosmia, were targeted. Relative to a 17-member control group, solvent-exposed workers performed considerably worse on tests of learning and memory, spatial skills, attention and mental flexibility, and psychomotor speed and manual dexterity. There were no differences in tests of general intelligence, picture completion, or similarities subtests. Complaints of cacosmia correlated with poor performance on oral learning and immediate visual reproduction tests. Other investigators have documented complaints of fatigue, tension, irritability, mood changes, and difficulty with concentration and memory in solvent-exposed workers relative to controls (Husman, 1980; Juntunen et al., 1980; Struwe and Wennberg, 1983). Both cacosmia and neurobehavioral abnormalities in solvent-exposed workers can persist for long periods after the exposure ends.

A study of 12 patients with nonarterio-sclerotic cardiac arrhythmias or chest pain had their arrhythmias reproduced by challenge with low airborne concentrations of chemicals that they inhaled (Rea, 1978). The objective measurement was cardiac rhythm. The exposures were double-blinded, but odor masking was not reported. Such cardiac reactions to challenges have been described (Harkavy, 1963; Taylor and Harris, 1970) and could represent cardiac anaphylaxis (Booth and Patterson, 1970; Levi, 1972).

LaMarte et al. (1988) evaluated two patients who claimed that occupational exposure to carbonless copy paper caused hoarseness, cough, rash, and flushing. These patients also met the criteria for a scientifically verifiable chemical sensitivity. Patch testing was used to identify the causative chemical as alkylphenol novolac resin. A patient exposed in a blind study developed laryngeal edema that was verified by direct examination of the vocal cords by video endoscopy of the larynx. Plasma histamine was measured before and after challenge, and it rose by a factor of 6. Class B syndromes are much more difficult to understand conceptually and are more difficult to study scientifically than are class A syndromes, because of methodologic difficulties. SBS occurs when there is an outbreak of well-defined symptoms in occupants of a building (World Health Organization, 1983). Several outbreaks, with the most common symptoms being irritation of the eyes and mucous membranes of the respiratory system, headache, fatigue, and mental confusion, have been reported in association with tightly sealed, energy-efficient buildings. Questionnaires administered by physicians documented significantly greater numbers of complaints of headache, lethargy, dry skin, irritation of eyes, running noses, and dry throats in air-conditioned buildings than were found in naturally ventilated buildings (Finnegan et al., 1984). Differences in globe temperature, dry-buld temperature, relative humidity, moisture content, and air velocity, as well as concentrations of positive and negative ions, carbon monoxide, ozone, and

formaldehyde have been eliminated in a comparative study as the cause of SBS (Robertson et al., 1985). Although complex factors could play a role in the syndrome, attention has focused on the presence of VOCs in poorly ventilated air; concentrations of VOCs inside office buildings greatly exceed outdoor levels (Hollowell and Miksch, 1981). Some authorities suggest that SBS is of psychologic origin or that it represents an anxiety state, mass hysteria, or a conditioned reflex. Investigators at the Karolinska Institute in Sweden have demonstrated, however, that blinded passers-by exposed to a mobile breathing chamber linked to the air supply of a "sick building" experienced the same reactions to building air as had the building's inhabitants (Berglund et al., 1984). Chemically related syndromes are distinct from syndromes associated with airborne microbiologic antigens associated with IgE-mediated outbreaks of respiratory illness in buildings (Solomon, 1990).

A relationship between SBS and MCS was postulated by Hirzy and Morison (1991), after some workers exposed to new carpeting at the Environmental Protection Agency's Waterside Mall office in Washington, D.C., exhibited induction of MCS. After analyzing the data on temporal and geographic concentrations of 4-phenylcyclohexene in the mall, Hirzy and Morison (1991) suggested that this compound can produce symptoms characteristic of SBS and induce MCS.

Controlled studies of humans exposed to mixtures of VOCs found in new homes are being undertaken and show promise in resolving controversy in this area. A Swedish study exposed chemically sensitive individuals to a representative mixture of VOCs and found subjective reactions of discomfort and reduced digit span, a measure of concentration (Molhave et al., 1986)

Volunteers with no history of chemical sensitivity complained of increased headache, general discomfort, and unpleasant odor, but not cognitive dysfunction, when exposed to a similar mixture (Otto et al., 1990). Nasallavage data have been presented that suggest that polymorphonuclear cells migrate into the upper airway of humans 18 hours after a 4-hour exposure to VOCs (Koren et al., 1990).

The concept of the chemical environment as an entity that causes disease was extensively discussed by allergist Theron Randolph in a series of papers culminating in his 1962 book *Human Ecology and Susceptibility to the Chemical Environment* (Randolph, 1962, and references therein). Randolph described a group of patients who had adverse reactions that resulted from apparent individual susceptibility to chemical compounds inhaled at doses far below normally toxic levels. Symptoms were different from those of allergic reactions and included chronic illnesses that he reported would go into remission when the subjects avoided the compounds that caused the difficulty. Table 9-1 is a list of the chemicals that Randolph reported to cause reactions in his patients. Table 9-2 lists the characteristics of the illness discussed by Randolph. The symptoms he described include a variety of physical complaints (headache, arthralgia, myalgia, palpitation, bronchospasm, and seizure) and mental complaints (loss of concentration, confusion, depression, irritability, inappropriate anger, hallucinations, and manic states). His work has been highly criticized, primarily by the academic community of allergists, who cite problems with his methods and with the lack of scientific evidence of the existence of the specific adaptation syndrome (Executive Committee of the American Academy of Allergy and Immunology, 1986).

CASE DEFINITIONS OF MULTIPLE-CHEMICAL-SENSITIVITY SYNDROME

On the basis of patients he and others had seen at occupational-medicine clinics

around the country, Cullen (1987) formulated a case definition of MCS. It has seven diagnostic features, which are listed in Table 9-3.

TABLE 9-2 Randolph's Characterization of MCS

An acquired disorder, often following an exposure to unusually high levels of an organic chemical.
Symptoms related to chemical exposures both psychiatric (depression, mania, hallucinations, anxiety) and physical (arthritis, bronchospasm, rhinitis).
Stimulatory syndromes triggered by acute exposures (for example, mania) followed by withdrawal symptoms hours to days after removal from an exposure (for example, headache, depression).
Adaptative phenomena: chemically sensitive individuals do not have acute reactions to chemical exposures while living in the chemical environment; during this phase, chronically ill.
Spreading phenomena: the illness progressive, with the patient becoming susceptible to larger numbers of chemicals and having more serious symptoms as the illness progresses.
Avoidance: by avoiding the chemical environment, the chemically sensitive individual has resolution of symptoms and remains well.

Cullen's definition is highly restrictive and does not apply to many groups of patients with chemical sensitivities, such as asthma patients who react to odors; persons with SBS who complain only of headaches, mucous-membrane irritation, and lethargy; and individuals with xenobiotic-induced autoimmunity. Cullen proposed his definition to distinguish a specific population of patients from the many who claim chemical sensitivities, and although a population that fits this definition exists, further refinements will probably be made as academic physicians become more involved with research in this area.

An operational definition has been proposed by Ashford and Miller (1989):

> The patient with multiple chemical sensitivities can be discovered by removal from the suspected offending agents and by re-challenge, after an appropriate interval, under strictly controlled environmental conditions. Causality is inferred by the clearing of symptoms with removal from the offending environment and recurrence of symptoms with specific challenge.

A study that found objective abnormalities in a group of patients who claimed to have MCS was performed at the University of Pennsylvania's Smell and Taste Center. Eighteen subjects with a history of MCS were studied for abnormalities in olfactory threshold (Doty et al., 1988). Although the olfactory thresholds to phenyl ethyl alcohol and methyl ethyl ketone were found not to be elevated relative to a control group, nasal airway resistance measured with a rhinomanometer was higher both before and after challenges in the chemically sensitive group. Under some conditions, nasal airway resistance was increased by exposure to the chemicals. Before testing, patients in the control group and in the chemically sensitive group completed a survey to determine depression (the Beck depression inventory). Members of the group with MCS were scored as more depressed than were members

of the control group. This study—which could not be blinded, because one of its objectives was to measure olfactory threshold—illustrates that there are circumstances in which odor masking or blinding is not feasible or desirable. This study did not rely on patient assessment or symptom scores.

TABLE 9-3 Cullen's MCS Case Definition

The disorder is acquired as a result of some environmental exposure, insult, or illness that can be documented.
Symptoms involve more than one organ system.
Symptoms recur and abate in response to predictable stimuli.
Symptoms are elicited by exposures to chemicals of diverse structural classes and toxicologic modes of action.
Symptoms are elicited by demonstrable exposures. This criterion is to exclude delusional patients.
Exposures that elicit symptoms must be very low—many standard deviations below the "average" exposures known to cause adverse human responses.
No single widely available test of organ-system function can explain the symptoms.

Another study used structured diagnostic interviews and self-reported measures of somatization and psychopathology to determine that a group of MCS patients had a greater incidence of anxiety and depression than did a control group (Simon et al., 1990). The patients in the report of Simon et al. were workers from one plant. While many workers had complaints, only those with lingering problems were in the patient group. Psychiatric illness has been reported in chemically sensitive patients since the original description (Randolph, 1962), and many feel that chemical exposures lead to psychiatric illnesses. Others believe that psychiatric illness is causative, that is, chemically sensitive patients have a primary psychiatric illness that leads to an inappropriate aversion to odorous chemicals. Clinical research should be able to resolve this controversy. It will be interesting to see whether the techniques of Simon and collaborators can be used to establish or disprove this connection.

IMMUNE-SYSTEM DYSFUNCTION IN MCS PATIENTS

Three studies of immune-system markers in patients alleged to have MCS have offered conflicting results. Decreased absolute suppressor-cell counts and increased CD4:CD8 ratios relative to controls were found in one study (Rea et al., 1982), a decreased CD4:CD8 ratio was found in another (Levin and Byers, 1987), and no consistent abnormality was detected in a third (Terr, 1986). The study by Rea and his collaborators evaluated seven patients with rheumatoid arthritis, 70 patients with "vascular dysfunction," and 27 asthma patients. Chemical sensitivity was established by "inhaled challenge under rigid environmentally controlled conditions in a glass and steel testing booth. Challenges were double-blinded with three placebos acting as controls." Vascular dysfunction results in such symptoms as headache and mental

confusion attributed to environmentally induced vasospasm.

The study by Rea and co-workers (1982) grouped patients by diagnosis, and the abnormal values could result from the underlying disorder, rather than chemical sensitivity. Rheumatoid arthritis or other specific conditions are induced by or exacerbated by exposure to xenobiotic substances according to these authors. A different study of 43 patients with rheumatoid arthritis undergoing water fasting in a controlled environment reported statistically significant improvement of seven indicators of arthritis (Kroker et al., 1984). A long-term study to see whether interventions of this nature can modify chronic diseases seems warranted, given the inadequacy of current therapies for severe progressive rheumatoid arthritis. Rea et al. (1982) proposed that chemical exposure can induce vascular dysfunction that leads to symptoms that involve several organ systems. This could prove to be a unifying concept in explaining how symptoms can arise in more than one organ system. Further work in this area by other investigators is necessary to substantiate the hypothesis. Certainly, if odors or chemical inhalants were to induce localized vascular spasm in susceptible individuals, perhaps through an olfactory-limbic-autonomic link, a large number of clinical manifestations, from headache to myalgia, could result. The fact that the "vascular dysfunction" group of Rea et al. had a variety of unspecified T-cell abnormalities could be significant and warrants confirmation by independent groups.

Levin and Byers (1987) have presented an elaborate theory of intolerance to the chemical environment that results from immune dysregulation. The evidence of this hypothesis involves a generalization supported by data from patients in Woburn, Massachusetts, exposed to trichloroethylene; individuals in rural Wisconsin exposed to a variety of industrial solvents, dyes, and pesticides; New Mexico workers exposed to industrial chemicals in a computer-chip factory; and residents of Catachee, South Carolina, exposed to polychlorinated biphenyls. These patients allegedly developed chemical sensitivities and had decreased CD4:CD8 ratios, which is opposite to the increased CD4:CD8 ratios found by Rea et al. Levin and Byers do not give scientific support or documentation that these patients are indeed chemically sensitive.

Terr (1986) evaluated 50 patients with a diagnosis of EI for the California Worker's Compensation Appeals Board and found no evidence of immune-system dysfunction. He saw a group of patients with alleged chemical sensitivities who had histories of other disorders (such as asthma or depression). He compiled results of immunologic tests found in the medical records of these patients and performed by different physicians or laboratories, and he found no evidence of immune dysfunction. He concluded that the presence of other disease and the lack of evidence of immune dysfunction suggested that these patients did not have EI. His patients had a variety of complaints and diagnoses, and laboratory data were not standardized. He described no challenge procedures or other methods to determine whether the patients had exacerbations of their illnesses from exposure to the chemicals, even though the population included patients with such diagnoses as asthma and dermatitis, which clearly can result from occupational exposure. Terr's method for determining that there was no chemical associated with complaints was to argue that, because he could give other diagnoses to the patients' illnesses, they could not be diagnosed as having EI. This argument is inadequate for patients who are given other diagnoses that can be caused by or exacerbated by environmental exposures. Terr's conclusions are a poorly supported opinion expressed by one who has evaluated patients on behalf of a workers' compensation appeals board. The study of markers of effect on the immune system would have served as a more reliable basis for such evaluations.

One group has studied antibodies to low-molecular-weight hydrocarbons complexed

with albumin in chemically sensitive individuals (Thrasher et al., 1988). In a preliminary study, six patients with multiple subjective health complaints historically related to chronic formaldehyde exposure had antibodies to formaldehyde-human serum albumin (f-HSA) as follows: IgE (two patients), IgM (three of four tested patients), and IgG (five patients). All six patients had elevated levels of antigen memory cells (Ta-1). A flulike illness developed in these patients after exposure to formaldehyde. In a study of 67 patients with common complaints that included a lingering flulike illness, sensitivity to airborne chemicals, symptoms of emotional and cognitive distress, and upper and lower airway disease, 65 had antibodies to f-HSA (Broughton and Thrasher, 1988). The low titers in most cases (1:4 was the most prevalent) and inadequate data on titers in unaffected populations make these observations meaningless.

In contrast, R. Patterson and collaborators (1989) have analyzed IgG antibody against f-HSA in 35 patients who either had symptoms attributed to formaldehyde exposure or had a history of intravenous exposure to formaldehyde from formaldehyde-sterilized renal-dialysis membranes. In no case was there a correlation between serum antibody titers to formaldehyde and disease. Only one of 16 patients with symptoms attributed to formaldehyde exhibited antibody, and the dialysis patients with high levels of specific IgG against formaldehyde had no sensitivity to airborne exposures.

None of the studies discussed here can be considered conclusive. The hypothesis that there is something called "environmental illness" characterized by immune dysregulation and hypersensitivity to chemicals has not been rigorously supported. However, there is strong evidence that groups of patients with specific immunopathologic diseases can have their illnesses exacerbated by or even caused by xenobiotic exposure. Well-established examples include xenobiotic-induced lupus and asthma induced after exposure to airborne chemicals.

A crucial step in resolving the intense controversy in the area of chemical sensitivities will be the development of refined terminology. To this end, members of the subcommittee believe that whenever possible the term "multiple chemical sensitivity" should be replaced with a specific diagnosis to avoid the confusion between diagnosis and etiology that is inherent in the term. The application of a specific diagnosis should not exclude the possibility that the illness could be related to chemical exposures. If a patient has emphysema secondary to cigarette smoking, it is clear that the diagnosis is emphysema, and the etiology of the disease is cigarette smoking. The MCS label implies that chemical exposures make the person ill, but many different clinicopathologic entities can arise from adverse reactions to chemicals. Hence, MCS has been used by various observers to describe a broad range of problems, ranging from cardiac arrythmia or vasculitis reproducible by challenge to somatization disorders with reactions to sham challenges. An analogous case applied to a patient with asthma who is allergic to pollens, molds, danders, house dust, and mites would be to give a diagnosis of multiple aeroallergen sensitivity. In this case, the diagnosis of extrinsic asthma is one appreciated by all physicians and patients.

BIOLOGIC MARKERS OF SENSITIVITY TO CHEMICALS

Provocative Challenge

Provocative challenge, in which individuals with alleged hypersensitivity are exposed to incriminated chemicals in a blinded fashion, is the standard in the field of human immunotoxicology. Ashford and Miller (1989) found agreement on this point in their interviews of allergists and clinical ecologists. However, provocative challenge is a research tool only, and it must be refined. One problem involves odor masking for testing individuals who might suffer from cacosmia,

that is, who could react nonspecifically to any strong odor.

Skin Tests

Skin testing with automobile exhaust, formaldehyde, and synthetic alcohol, for example, has been used as a diagnostic test for chemical hypersensitivity. For skin tests to be accepted, several large, independent studies of patients must demonstrate that persons with verified MCS have positive skin tests, and that an equal number of individuals without MCS have negative skin tests to these substances. Until these studies are done, skin tests must be considered experimental and a research tool.

ANTIBODIES TO FORMALDEHYDE-HUMAN SERUM ALBUMIN ADDUCTS

One group has advocated using the presence in serum of f-HSA antibodies as a biologic marker for MCS. For these tests to be accepted, it must be demonstrated in several large series of patients by independent investigators that persons with verified chemical susceptibilities have positive tests and that an equal number of individuals without unique problems associated with chemical exposure have negative titers to these antibodies. Until these studies are done, these tests must be considered experimental and a research tool.

Avoidance Regimens

Isolation from the chemical environment, with resolution of chronic symptoms, has been advocated as a diagnostic tool for MCS. The ubiquitous presence of VOCs and the claim that patients can acclimate—not react acutely to chemical exposures, but have chronic symptoms while continuously exposed to low levels—make this an attractive method for diagnosis. This approach is applied by clinicians of all persuasions in diagnosing drug intolerance and should be a valuable research and clinical tool, whether the chemical exposure associated with the problem is airborne or dietary.

T-cell Helper-to-Suppressor Ratios

Ratios of CD4 to CD8 cells, measured by fluorescence-activated cell sorters or other instruments, have been proposed as biologic markers of chemical sensitivity. Rea et al. (1982) suggest that this ratio is elevated for patients with some, but not all, diagnoses. Because there is considerable overlap between normal and affected subjects, this test is of no use in the clinical evaluation of individual patients. Levin and Byers (1987), on the other hand, claim a decrease in this ratio; Terr (1986) finds no abnormality of the ratio. However, the exposure in the workplaces may not be equivalent. The CD4:CD8 ratio cannot be recommended as a biologic marker for chemical sensitivity, although it could have some use in comparing groups of patients in clinical research settings.

CONCLUSIONS

There are several distinct clinicopathologic entities that in selected cases are either caused by or exacerbated by exposure to xenobiotic substances, particularly low-molecular-weight organic chemicals. These include asthma and rhinitis, cacosmia as defined by Ryan et al. (1988), autoimmune diseases (including systemic lupus erythematosus), laryngeal edema, depression, psychosis, and other neurobehavioral disorders. Although the associations between these disorders and xenobiotics have best been demonstrated for pharmaceuticals, the extent to which inadvertent exposure to environmental toxins, particularly the VOCs found in indoor air and the natural and additive

chemicals found in foods, can induce or exacerbate physical disorders remains an important public-health question.

The sick-building syndrome is a real phenomenon, in which susceptible occupants of closed buildings have symptoms of headache, eye and nasal irritation, mucous-membrane irritation, lethargy, and difficulty with concentration. A role for VOCs in the etiology of this syndrome is suggested, and the hypothesis that this syndrome is solely of psychologic origin is not consistent with existing data.

The reported evidence is inadequate to define a distinct clinicopathologic entity, MCS syndrome, that is different from the associations between the chemicals and illnesses discussed above. However, there is a significant patient population—poorly enumerated, but growing—that claims dysfunction and disability due to an intolerance to xenobiotics (mostly VOCs) at levels commonly encountered in industrialized societies and apparently tolerated by most people. There is insufficient evidence to ascribe an immune etiology to this disorder.

The medical profession has not developed consistent or adequate approaches to the evaluation, treatment, and reimbursement of such patients. Social agencies, insurance companies, and other societal groups cannot deal effectively with this patient population until the medical questions are resolved. Even with a concerted effort, it will be some time before this can happen, and humane and uniform interim measures must be instituted to help affected persons.

The use of anecdotal reports without standardized case definitions and attention to alternative explanations will not resolve the current controversies surrounding this issue. A series of well-designed studies, including studies in controlled-exposure facilities and multiple epidemiologic and psychosocial studies, is required to address this problem.

RECOMMENDATIONS

Because sick-building syndrome appears to be a real phenomenon caused by contamination of indoor air with VOCs that cause discomfort to a substantial number of persons, programs should be developed to establish indoor air pollution standards for homes, schools, and workplaces. These standards should restrict VOCs or other chemicals involved in indoor air pollution to levels below those at which significant numbers of occupants suffer headaches, mucous-membrane irritation, eye and nose irritation, lethargy, and difficulty with concentration.

Well-designed clinical and epidemiologic studies that use appropriate immune-system biologic markers should be performed to investigate the relationship between environmental chemicals and specific syndromes of uncertain origin, namely MCS.

A workshop was convened with experts from environmental and occupational medicine, allergy and clinical immunology, epidemiology, public health, immunology, psychiatry, and other disciplines to advise the Subcommittee on Immunotoxicology on the MCS syndrome. The proceedings of this workshop will be published later. The recommendations of this workshop were:

1. A case-comparison study of patients seen in occupational and environmental medicine should enroll patients who claim to respond to low levels of environmental chemicals. The information from this multi-center study should be used to study the prevalence of MCS in the general population. Populations with well-defined exposures, such as victims of a toxic spill or workers with uniform occupational exposure, could be studied longitudinally for the development of chemical sensitivities. Patients with multiple chemical sensitivity will be selected because of symptoms or signs related to chemical exposures at levels tolerated by the population at large. The chemicals in

question are different from the well-recognized allergens, such as dust, molds, pollens, and danders. Symptoms must wax and wane with chemical exposures and may occur in one or more organ systems. Although many patients describe the onset of this syndrome with an acute toxic chemical exposure, such an initiating exposure is not required for inclusion. Patients with pre-existent or concurrent diseases such as asthma, arthritis, and psychiatric illnesses are not to be excluded from study, because many believe that chemical exposures play a role in inducing or exacerbating these conditions.

2. Research units or environmental control units in which MCS patients will be housed in a chemical-free environment are needed. Challenges will then be conducted in a double-blinded fashion with attention to adaptation and de-adaptation phenomena. Responses of patients to controlled exposure should be monitored with immunologic, neurologic, endocrinologic, psychologic, and social markers and measures. Dose-response relationships should be studied.

10

Summary and Recommendations

The field of immunotoxicology has undergone considerable growth and expansion since its inception in the early 1970s. The discipline was founded by combining knowledge from the areas of immunology and toxicology to study the effects that xenobiotics exert on the immune system. It has progressed from the initial identification of immunotoxic chemicals to the development of sensitive, quantitative assays that assess chemically induced alterations of the immune system and that determine the mechanisms by which xenobiotic substances compromise immune function. These immunoassays and other more conventional tests may well serve as the key to identifying biologic markers of immunotoxicity. Immunotoxicology now plays a role in developing health standards and permissible levels of human exposure to xenobiotics.

This document was prepared by scientists with diverse backgrounds in and knowledge of immunology, toxicology, immunotoxicology, risk analysis, and other disciplines. Its purpose is to assess the past and current status of immunotoxicology and to identify areas for future research. The field of immunotoxicology is expanding rapidly, and there is interest not only in the traditional immunotoxic effects of drugs and environmentally dispersed chemicals on hypersensitivity, autoimmunity, systemic immunity, and carcinogenesis, but also investigations related to risk assessment, food safety, water quality, and indoor air. Some of these features have been addressed in this document.

Immunotoxicology is a scientific discipline that explores the effects of physical, biologic and chemical agents on the immune system, which is extremely sophisticated and is self-regulated as well as being influenced by other systems. Immunocytes, orchestrated by their receptors and secretory products, act as a network. Although the immune system was long considered autonomous in both regulation and action, recent data strongly suggest that a significant reciprocal interaction occurs between the nervous, endocrine, and immune systems to maintain homeostasis. These interregulatory patterns confirm the existence of a nervous-endocrine-immune axis, which complicates attempts to study and model whole-body responses in vitro. Although sophisticated in vitro systems have specific applications, intact animal systems are essential for accurate investigations of the immunotoxic potential of xenobiotics.

This document presents a brief history and review of immunology, immunotoxicology,

and biologic markers (Chapters 1 and 2). These chapters provide supportive information for the remainder of the document. The immune system can be expressed bidirectionally; excessive stimulation can result in hypersensitivity, autoimmunity, or both. Suppression can increase the susceptibility of the host to infectious and neoplastic agents. Hypersensitivity (Chapter 3) has become an important human health problem in industrialized societies. Inhalation of a variety of chemicals can cause individuals to develop asthma, rhinitis, pneumonitis, or chronic granulomatous pulmonary disorders, among others. Hypersensitivity is an immunologically based host response to a compound or its metabolic products (as distinguished from the purported "hypersensitivity syndrome," which has not been shown to have an immunologic basis (Chapter 9). Hypersensitivity reactions, including autoimmunity, are frequently influenced by heredity. IgE is an important mediator and biologic marker of hypersensitivity, as this immunoglobulin binds to basophils and mast cells and initiates release of the inflammatory mediators responsible for the symptoms on re-exposure to the allergen. Validated tests are available to assess hypersensitivity in animals and humans.

Autoimmune disease occurs when an individual's immune system attacks the body's own tissues or organs, resulting in functional impairment, inflammation, and occasionally permanent tissue damage (Chapter 4). Autoimmune disease is an infrequent occurrence in humans that can result in considerable discomfort and even death. Certain xenobiotics are known to induce autoimmune disorder, but there is a paucity of information concerning the relationship of autoimmunity to environmental exposure.

The immune system provides the primary defense against invasion by pathogens and neoplastic agents. Exposure to drugs and chemicals can impair this natural host defense mechanism, which can lead to an increased incidence of infectious disease or cancer. Xenobiotic-induced immune dysfunction has been well established in animals for several chemicals. In some cases, the immune system has been identified as the most sensitive target organ for the minimum toxic dose of a xenobiotic. Although one or more of the many compartments of the immune system can be suppressed significantly, this suppression might not be expressed as an immune-mediated disease. Rather, suppression can be viewed as a potential risk because of the reduced ability of the host to resist natural and acquired diseases.

There is limited information to suggest that humans exposed to environmental pollutants are immunologically compromised. However, it has been well established that treatment of humans with immunosuppressive therapeutic agents can result in an increased incidence of infectious disease and neoplasia. Animal immunologic bioassays are useful to identify possible hazards associated with human exposure to xenobiotics. It is accepted that the immune systems of many animals and humans are comparable, that animal models are available to assess immune dysfunction objectively, that positive immunosuppressants, such as cyclophosphamide and cyclosporin A, are used to validate assays, and that data obtained from animal studies can sometimes be verified in humans. Although the principles and phenomena in humans and animals are basically similar and comparable, it is recognized that different responses can occur.

Animal models have been used to identify immunotoxic agents, to develop immune-system profiles, to identify mechanisms of action, and to alert researchers and regulators to potential health risks associated with exposure to specific xenobiotics, either consumed as drugs or through environmental exposure (Chapter 6). Several immunotoxicity bioassays have been validated in animals to detect drug-and chemical-induced immunomodulation. Some of these bioassays are sensitive and predictive of immune dysfunction. Many others that either are in development or have not been used extensively will have application in

immunotoxicology, once they have been validated. Although many tests are available to screen for immunotoxicants, the choice of an initial test that evaluates the T-cell-dependent antibody response permits assessment of several compartments of the immune system simultaneously. These procedures are easily performed, quantitative, sensitive, and economical, and they detect a large percentage of known immunosuppressive agents.

In animal models, data are obtained from cells, tissues, and organs that are collected on death at the end of an experiment; this practice obviously cannot be duplicated in humans. However, use of animal data, coupled with information gained from limited human biologic and epidemiologic studies, has proved of value in human risk assessment. Several tests assess humoral and cellular immunity, as well as nonspecific resistance in humans (Chapter 7). This report suggests a series of tests to assess immune-system competence in persons who have been exposed to a known or suspected immunotoxicant. Some of these procedures parallel animal studies and require prospective validation in exposed populations and in control groups to ascertain their predictive value. This aggressive approach will permit use of sensitive procedures for detection of immunomodulation in humans.

The limits on experimentation in humans predicate the use of epidemiologic methods to obtain health information after accidental or occupational exposure. Epidemiologic research can involve an experimental study in which the conditions are controlled and the effects are subsequently observed in a test population, or it can use cohorts or cases in which the test population is observed without altering the circumstances (Chapter 8). Epidemiologic procedures frequently permit long-term monitoring of the health effects in large numbers of persons exposed to defined levels of a given environmental xenobiotic. Data obtained in such investigations can provide valuable and relevant information on the immunotoxic properties of numerous xenobiotics in humans that supports or disputes animal data that cannot be obtained otherwise (experimentally) in normal human populations.

Are some individuals genetically predisposed or innately hypersensitive to harm caused by given xenobiotics? It is well known that some individuals experience skin or pulmonary disorders in reaction to contact allergens. However, although it has been suggested, it is not known whether the systemic arm of the immune system in a given individual is uniquely sensitive to immunomodulation by several chemicals (Chapter 9). Current evidence does not indicate that "sick-building syndrome" originates in or involves the immune system. Many other factors could be involved in the etiology of such conditions and, should the immune system be involved, it could be in secondary or indirect responses to conditions, rather than as a directly contributing factor in the etiology of these syndromes.

The survival of humans and animals in their natural environment depends on a functional immune system. It is clear that drugs and other chemicals can induce serious immune dysfunction in animals and humans. Although the discipline of immunotoxicology is relatively young, there has been considerable progress in demonstrating that some xenobiotics can in fact modulate immunity and that, in some instances, the immune system is a primary target organ. Nevertheless, progress has been slow and the level of knowledge needs to be expanded, both in depth and in breadth, to elucidate fully the effect of xenobiotics on the functioning immune system and human health. This document has reviewed the past and evaluated the current state of immunotoxicology.

CHEMICAL-INDUCED IMMUNOSUPPRESSION IN HUMANS

Conclusions

There is increasing awareness and concern

within the scientific and public communities that chemical pollutants can suppress immune processes and thus result in an increase of neoplastic and infectious diseases. Adverse effects in humans treated with immunosuppressive drugs, numerous studies employing experimental animals, and, to a lesser extent, isolated cases of altered immune function in humans inadvertently or occupationally exposed to xenobiotic substances support these concerns. Evidence is weak that individuals who are putatively exposed because they live in the vicinity of contaminated sites or near chemical manufacturing plants have been immunologically compromised to the extent that they have an increased risk of disease. Nevertheless, examples of chemically induced disease and our knowledge of the pathogenesis of disease support the likelihood that damage to the human immune system is associated with adverse health effects, some of which could become apparent only after a long latency. Furthermore, exposure to immunotoxic xenobiotics can present additional risk to individuals whose immune systems are already compromised, for example, because of malnutrition, infancy, or old age. Finally, the value of incorporating immunologic data for toxicologic assessment of drugs, chemicals, and biologics for human hazard evaluation is increasingly recognized. However, it is often difficult to extrapolate changes in a given area of immune function in experimental animals to the incidence of clinical or pathologic effects in humans. Some biologic markers have been useful in identifying increased susceptibility to immune-related diseases or susceptibility to some substances. In general, however, the use of markers of immunotoxicity to identify increased susceptibility to environmental hazards has received little attention. Although immune-system markers offer promise in assessing the effect of environmental toxicants on human health, many of these markers are inadequately characterized with respect to their association with toxicant exposure, long-term health effects, and individual susceptibility to chemical injury.

Recommendations

Clinical studies in humans are needed to determine the relationship between chemical exposures and immune-mediated diseases. Of particular concern are the contributions of xenobiotic exposure to the increased frequency or severity of infectious or neoplastic diseases. Does the known ability of ultraviolet light to suppress immune function play a role in the increased incidence of skin cancer detected after prolonged exposure to sunlight? Is there a causal relationship between chemical-induced immunosuppression and non-Hodgkin's lymphoma? Carefully designed clinical and epidemiologic studies should be undertaken with well-defined populations. Consideration should be given to groups for which exposure levels to immunotoxicants, including duration and dose, can be confirmed. Clinical examinations should include the most advanced immunodiagnostic techniques. The use of these techniques should be preceded by validation of the assays such that they are known to be sensitive to modulation by immunotoxicants and the normal ranges are established. Clinical evaluations should include the use of sensitive, validated immunodiagnostic techniques, standardized case definitions, and information derived from the detection of validated biologic markers of immune-system changes. In addition, factors that contribute additional risk, such as age, genetic predisposition, stress, and malnutrition should be examined. One or more environmental health centers that focus on immunologic disorders should be developed and staffed with experts in immunotoxicology, clinical immunology, occupational medicine, and epidemiology. Within such centers, response plans would

be developed and a team of scientists would be available to collect appropriate specimens promptly when accidental exposures occur.

ROLE OF ENVIRONMENTAL CHEMICAL EXPOSURE IN HYPERSENSITIVITY AND AUTOIMMUNE DISEASES

Conclusions

There is evidence that environmental exposure to toxic chemicals plays a role in the induction of autoimmune diseases and hypersensitivity responses, either by acting as direct causative agents or by increasing the severity of pre-existing hypersensitivity disease. For example, 2-5% of all cases of asthma in the United States are induced by workplace exposure to toxicants. Prospective studies have suggested that about 6% of research-animal handlers become sensitized to animal serum, urine, or dander; that 10-20% of bakers develop asthma associated with flour dust; and that almost all workers in the platinum-salt industry develop at least mild allergic respiratory symptoms. In the United States alone, approximately 5% of persons exposed to toluene diisocyanate fumes in the polyurethane foam and plastic manufacturing industries develop severe asthmatic symptoms. Evidence also exists that asthma, the incidence of which has increased by 58% since 1970, can be exacerbated by air pollutants, such as ozone and nitrogen dioxide. Likewise, the influence of environmental chemical exposures on autoimmune diseases, such as rheumatoid arthritis, which afflicts thousands of persons, has never been systematically examined.

Recommendations

Studies of human markers of immunotoxicology should be designed and tested to document their analytic accuracy and precision and to evaluate their predictive value for identifying toxicant exposure and predicting adverse health outcomes. The use of immunotoxicity markers in longitudinal studies of short duration (months) and long duration (years) will be required. Additional studies could be warranted to determine the prevalence of hypersensitivity and autoimmune diseases influenced by exposure to environmental chemicals. Because instances of autoimmune disease are not reportable to local public-health departments, some effort should be made to establish a national registry for persons with such diseases. Until this is done, no reliable estimates of prevalence can be established.

Immune and genetic biologic markers, particularly those which would be practical for wide-scale use, also should be explored for use in identifying individuals who are highly susceptible to environmental hazards. Special attention should be focused on markers of susceptibility that aid in diagnosis and reveal the mechanistic aspects of environmental disease. Moreover, factors that predispose individuals to disease caused by immunotoxic chemicals should be determined. This research should address the roles of age, genetics, nutrition, and concurrent disease in the pathogenesis of chemical-induced immune-system dysfunction.

ANIMAL AND IN VITRO MODELS

Conclusions

The development and use of animal models and in vitro models to find markers of immunotoxicity are essential for the continued growth of this discipline. Results from the model systems are needed to engage appropriate decision-making processes on chemical use and to predict the risk to human health as it pertains to immune-mediated diseases. Such studies should

establish immune-system profiles for xenobiotics in animals and provide the foundation for understanding structure-activity relationships, mechanisms of chemical-induced injury, and, if required, means of therapeutic intervention.

Recommendations

The National Toxicology Program, the Environmental Protection Agency, other federal agencies, and groups in the private sector have developed protocols for evaluating the potential of chemical toxins to suppress the immune systems of rodents. Abnormalities in these tests often precede overt evidence of immunopathology. Such tests should continue and be developed further to allow accurate assessment and prediction of adverse effects on the immune and host defense systems of humans. Of special importance could be the temporal relationship between suppression of the immune system and the development of clinical disease.

Sequences of standard tests for natural and acquired immunity should be used to screen for chemicals that alter immune function. More specific and sensitive tests should be adopted after they are validated and compared with established but less desirable tests. The application of newer biotechnical methods could provide better insight into the action of immunotoxicants and their significance in the development of diseases of immune dysfunction. Immune-system profiles developed for chemicals in animals could provide information of predictive value for humans.

The ability of chemicals to depress immune responsiveness should be compared with their corresponding ability to depress the capacity of experimental animals to resist infectious agents or neoplasia. It is anticipated that there will not always be a detectable positive correlation, and in-depth analyses will be required to identify the molecular basis of decreased immune responsiveness and the underlying mechanisms (including intermediate stages) responsible for altered host resistance to disease. A positive correlation between chemical exposure and altered immune function must be established.

Animal studies usually involve short-term, high-dose exposure; human exposure is generally chronic and at low levels. Methods need to be developed to provide more accurate extrapolation from animals to humans, particularly as it pertains to development of clinical diseases.

Procedures have been established to predict chemical-induced hypersensitivity and autoimmunity in laboratory animals. These methods need to be applied to ascertain their predictive value in humans. In some cases, such as hypersensitivity induced by inhalation and ingestion of allergens, additional tests that more closely reflect human experiences might need to be developed. The use of such predictive tests should be encouraged to provide sensitive markers for assessing immunotoxicity of xenobiotics and for setting acceptable levels of exposure. Markers of susceptibility that could reveal the diagnostic and mechanistic aspects of environmental disease should be studied in laboratory models. Biologic markers from noninvasive techniques in humans should be an emphasis of these studies.

Because autoimmune diseases appear to develop from immune dysregulation, the ability of xenobiotics to interfere with normal immune regulation that could predispose persons to autoimmune and chronic inflammatory diseases should be studied. Better animal models should be developed to evaluate the influence of xenobiotics on autoimmune disease and to describe the underlying mechanisms of regulatory dysfunction, with the ultimate goals of prevention and therapy.

There also is a need to ascertain the extent to which chemicals modify inflammatory responses, as measured by increases or decreases in the number and function of leukocytes at sites of inflammation.

Experimental guidelines are not available

to investigate the multiple-chemical-sensitivity syndrome adequately. Development of such guidelines should be developed for the investigation of the etiology, symptomatology, and pathogenesis of this syndrome. This putative disorder must be defined and carefully characterized, because there is no definite evidence to associate it with immune-system dysfunction.

MARKERS OF SKIN AND MUCOSAL RESPONSES

Conclusions

The skin and mucosal surfaces of the respiratory and gastrointestinal tracts are generally the first tissues of contact by environmental agents. The immune components of these surface tissues are especially adapted for their recognition and response functions. Although the contribution of these tissues to host defense mechanisms is still unclear, evidence exists that some environmental chemicals can enhance neoplastic diseases after exposure to the skin, the gastrointestinal tract, and particularly the lung. It is known, for example, that many inhaled gases (such as nitrogen dioxide and ozone) and particles (such as asbestos and silica) alter local immunity and thus result in increased susceptibility to lung infection. In addition, exposure of the skin to ultraviolet light suppresses immune-system responses that could prevent the development of skin tumors caused by exposure to sunlight. More recently, such chemicals as dimethyl-benzanthracene and cyclosporin A have been shown to inhibit immune-system responses in the skin and thus to lead to systemic immunosuppression.

Recommendations

Studies need to be conducted to determine whether enhanced susceptibility to or severity of viral, bacterial, and neoplastic diseases is related to, or a direct consequence of, impaired local immune function. Biologic markers need to be identified in humans and animals to detect potentially dangerous changes in local immune function. Markers chosen for development should meet several criteria. They should be sensitive; be derived from noninvasive techniques; be meaningful with respect to susceptibility, severity, and recovery from disease; detect injury to specific and nonspecific immunity of the humoral and cell-mediated immune systems; evaluate local versus systemic immunity; and provide data that can be used to compare adverse effects across species.

EDUCATION AND TRAINING

Conclusions

Education of scientists to investigate the many environmental problems must have high priority. There are few opportunities for students to pursue graduate education and training in immunotoxicology. Physicians and scientists trained in immunotoxicology and environmental health research are needed in the private and academic sectors to help develop expertise in this area. The public needs to be better informed of the risk to the immune system associated with chemical exposure.

Recommendations

More information about immunotoxicology needs to be incorporated into graduate and postgraduate medical education. New postdoctoral fellowships should be made available to cross-train scientists from other disciplines.

Public understanding and perception of risk associated with chemical exposure, especially as related to the immune system, should be fostered through various mechanisms, including the mass media.

The Subcommittee on Immunotoxicology supports establishment of federally funded research centers, program grants, competitive grant programs, and training grants in immunotoxicology. The subcommittee's members advocate a fostering of communication and dissemination of information within and between government agencies, academe, and industry.

The availability of data, both published and proprietary, should be improved to permit realistic assessment of adverse health effects of environmental chemicals.

ENVIRONMENTAL EXPOSURES AND SENSITIVITY SYNDROMES

Conclusions

There has arisen considerable public concern regarding "sick-building syndrome" (SBS) and multiple-chemical-sensitivity (MCS) syndrome. SBS is a better-defined entity, consisting mainly of irritation of mucous membranes that occurs in occupants of new, airtight buildings. These effects are believed to be associated with exposure to irritants, chemical or biologic immunogens, or mixtures of chemical pollutants.

Individuals who develop adverse reactions to numerous chemicals at doses below presumed toxic levels are said to have MCS. The agents said to cause these symptoms include many environmental contaminants, chemical additives, synthetic drugs, and cosmetics. The existence of MCS has been challenged in the scientific and medical communities. Although all areas of environmental medicine have issues to resolve (such as extrapolation of animal data to humans and development of more predictive or clinically relevant tests), there are numerous basic scientific facts and data gaps that clearly distinguish MCS from other areas of immunology and toxicology. The causative environmental or other agents are unknown (they are assumed to be almost any synthetic or natural environmental contaminant, rather than a chemical of specific structure or a class of chemicals). Rather than having a specific disease symptomatology, MCS often is characterized by diverse subjective symptoms that potentially affect one or several organ systems. The etiology, pathogenesis, and prevalences of MCS have not been established, although many conflicting theories and various clinical presentations have been reported. This paucity of solid scientific data has severely clouded objective scientific understanding of this syndrome, including its clinical diagnosis and effective treatment.

Recommendations

A case-comparison study of patients claiming unique susceptibility to chemicals in the environmental workplace should be undertaken. Because sick building syndrome appears to be a real phenomenon caused by contamination of indoor air that cause discomfort to a substantial number of workers, indoor air pollution standards for homes, schools, and workplaces should be established. These standards should restrict offending agents including volatile organic compounds to levels below those at which significant numbers of occupants develop symptoms.

There is a need to establish a multidisciplinary team of experts in lung physiology, immunotoxicology, clinical immunology, psychiatry, toxicology, occupational medicine, and industrial hygiene to study patients with these purported syndromes. A standard, comprehensive panel of clinical procedures should be applied to aid their diagnosis. Blind-challenge studies, using well-defined cohorts with established exposures, might need to be conducted.

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Glossary[1]

Adaptive immune response A response to foreign material characterized by specificity and memory that can be promoted by antibody-or antigen-committed lymphocytes.

Allergen An antigen that provokes an allergic reaction.

Allergy A clinical manifestation of hypersensitivity; classically defined as antigen-specific altered reactivity of host to antigen.

Alloantigen An antigen that occurs in some, but not all, members of a species.

Allotype Any of the alternative characters controlled by allelic genes.

Anaphylaxis Any life-threatening allergic reaction.

Anergy Absence of immune reaction to a specific antigen.

Angioedema Angioneurotic edema. Recurring attacks of transient edema suddenly appearing in areas of the skin or mucous membranes and occasionally of the viscera, often associated with dermatographism, urticaria, erythema, and purpura. In the hereditary form, transmitted as an autosomal dominant trait, it tends to involve more visceral lesions than the sporadic form, especially of the respiratory and gastrointestinal tract. Two types of the familial form have been identified: one involves failure of synthesis of the inhibitor of complement component C1, the

[1] Sources used to compile this glossary include the following: *Dorland's Illustrated Medical Dictionary*, 26th ed., W. B. Sanders Co., Philadelphia, 1,485 pp., 1981. *Immunology*, I. Roitt, J. Brostoff, and D. Male, Gower Medical Publishing, London, 316 pp., 1985. *International Dictionary of Medicine and Biology*, vols. 1 and 2, John Wiley & Sons, New York, 3,200 pp., 1986. *Advanced Immunology*, D. Male, B. Champion, and A. Cooke, J.B. Lippincott Co., Philadelphia, 222 pp., 1987.

	other involves the synthesis of an abnormal protein.
Antibody	A protein molecule belonging to the immunoglobulin class of proteins. Antibodies are found in serum and secretions, they are produced by B cells in response to an antigen, and they combine specifically with the antigen.
Antibody-dependent, cell-mediated cytotoxicity (ADCC)	A phenomenon in which target cells coated with antibody are destroyed by specialized killer cells (K cells), which bearFc receptors.
Antigen	Foreign material that can induce an immune response mediated by antibodies or lymphocytes.
Antigenic determinant	A single antigenic site on a complex antigenic molecule or particle; epitope.
Ataxia-telangiectasia	Severe progressive cerebellar ataxia, associated with oculocutaneous telangiectasia, sinopulmonary disease with frequent respiratory infections, and abnormal eye movements; it is a hereditary disorder transmitted as an autosomal recessive trait, usually appearing when the child attempts to learn to walk. It is also associated with immunodeficiency (IgA and IgE) and sometimes associated with cell-mediated dysfunction.
Atopy	A genetic predisposition toward the development of IgE-mediated immediate hypersensitivity reactions against common environmental antigens.
Autoantibodies	Antibodies produced by a host to its own tissues.
Autoimmune disease	A disease involving a humoral or cell-mediated immunity to the tissues of one's own body. A failure of the immune system to discriminate between self and nonself.
Basophils	Circulating polymorphonuclear leukocytes that have a small number of prominent purple or black cytoplasmic granules when stained with dyes that indicate a base pH. The granules contain histamine and chondroitin sulfate. In tissues they are called mast cells.
B lymphocytes (B cells)	Cells that originate in bone marrow that migrate into blood, lymph, and lymphoid tissue. They express specific surface antibody, which can bind antigen and results in B-cell differentiation into plasma cells. B cells carry immunoglobulin and class II MHC (major histocompatibility complex) antigens on their surfaces.
Candidiasis	Infection with a fungus of the genus *Candida*. It is usually a superficial infection of the moist cutaneous areas of the body and is generally caused by *C. albicans;* it most commonly involves the skin (dermatocandidiasis), oral mucous membranes (thrush, def. 1), respiratory tract (bronchocandidiasis), and vagina (vaginitis). Rarely there is a systemic infection or endocarditis.
Carrier	A large immunogenic molecule or particle to which an antigenic determinant is attached, allowing the determinant to become immunogenic.
CD4	Helper T cells. A cell-surface antigen on T cells having specificity for class II major histocompatibility complex.
CD8	Suppressor T cells. A cell-surface antigen on T cells having specificity for class I major histocompatibility complex antigens.

Cell-mediated cytotoxicity Killing (lysis) of a target cell by a lymphocyte.

Cell-mediated immunity Immune reaction mediated by T cells; in contrast with humoral immunity, which is antibody-mediated. (See also delayed-type hypersensitivity.)

Chemotaxis The movement of an organism or an individual cell, such as a leukocyte, in response to a chemical gradient.

Chlamydia A genus of the family Chlamydiaceae, order Chlamydiales, occurring as two species that cause a wide variety of diseases in humans and animals.

Class I MHC antigens Antigens encoded by the major histocompatibility complex (MHC) of genes that are found on all nucleated cells and are composed of two polypeptide chains of 45 and 12 kilodaltons.

Class II MHC antigens Antigens found on antigen-presenting cells and composed of two polypeptide chains of 28 and 32 kilodaltons. Also known as Ia antigens,

Complement A family of at least nine normal serum proteins designated C1, C2, etc., that is activated by antigen-antibody complexes, which results in lysis of erythrocytes and bacteria, enhanced phagocytosis, and immune adherence.

Contact sensitivity An epidermal reaction characterized by eczema caused when an antigen is applied to previously sensitized skin. Also called contact hypersensitivity.

Cross-reactivity The ability of an antibody, specific for one antigen, to react with a second antigen; a measure of relatedness between two antigenic substances.

Cytokine A nonantibody protein secreted by one cell of the immune system that mediates a response in other cells. A lymphokine is a type of cytokine.

Cytomegalovirus One of a group of highly host-specific herpes viruses that infect humans, monkeys, or rodents, with the production of unique large cells bearing intranuclear inclusions. The virus specific for humans causes cytomegalic inclusion disease, and it has been associated with a syndrome resembling infectious mononucleosis. Also termed salivary gland virus.

Cytotoxic T cell A thymus-derived lymphocyte that circulates in search of a target cell, displaying a determinant recognized by its receptor. It must recognize target-cell antigen in association with major histocompatibility complex determinants.

Delayed-type hypersensitivity A T-cell-mediated reaction to antigen, which takes 24-48 hours to develop fully and which involves release of lymphokines and recruitment of monocytes and macrophages. Previous exposure is required. Examples include response to *Mycobacterium tuberculosis* (tuberculin test) and contact dermatitis (poison ivy). Also called cell-mediated immunity.

Dendritic A cell type found in spleen and lymph nodes that is involved in antigen presentation and the stimulation of lymphocytes.

Eczema A superficial inflammatory process involving primarily the epidermis, characterized early by redness, itching, minute papules and vesicles, weeping, oozing, and crusting, and later by scaling lichenification and often pigmentation. It is not a disease entity or an acceptable diagnosis.

Edema The presence of abnormally large

	amounts of fluid in the intercellular tissue spaces of the body; usually applied to demonstrable accumulation of excessive fluid in the subcutaneous tissues. Edema may be localized and due to venous or lymphatic obstruction or to increased vascular permeability, or it may be systemic and due to heart failure or renal disease. Collections of edema fluid are designated according to the site, e.g., ascites (peritoneal cavity), hydrothorax (pleural cavity), and hydropericardium (pericardial sac).
Endocytosis	The uptake by a cell of material from the environment by invagination of its plasma membrane; it includes both phagocytosis and pinocytosis.
Enzyme-linked immunosorbent assay (ELISA)	An assay in which an enzyme is linked to an antibody and a colored substrate is used to measure the activity of bound enzyme and, hence, the amount of bound antibody.
Eosinophil	A granular leukocyte with a nucleus that usually has two lobes connected by a slender thread of chromatin, and cytoplasm containing coarse, round granules that are uniform in size and stained by eosin. Also called acidocyte, eosinocyte, eosinophilic leukocyte, and Rindfleisch's cell.
Epitope	Antigenic determinant.
Epstein-Barr virus	A herpes-like virus that causes infectious mononucleosis and is associated with Burkitt's lymphoma and nasopharyngeal carcinoma.
Erythema	A name applied to redness of the skin produced by congestion of the capillaries, which may result from a variety of causes, the etiology or a specific type of lesion often being indicated by a modifying term.
Fc	Fragment of antibody without antigen-binding sites, generated by cleavage with papain; contains the C-terminal domains of the heavy immunoglobulin chains.
Fc receptor	A receptor on a cell surface with specific binding affinity for the Fc portion of an antibody (immunoglobulin) molecule. Fc receptors are found on many types of cells.
Fibrinolytic	Pertaining to, characterized by, or causing fibrinolysis, causing breakdown of fiber.
Fistula	An abnormal passage of communication, usually between two internal organs or leading from an internal organ to the surface of the body; frequently designated according to the organs or parts in communication, as anovaginal, bronchocutaneous, hepatopleural, pulmonoperitoneal, rectovaginal, urethrovaginal, and the like. Such passages are frequently created experimentally for the purpose of obtaining body secretions for physiologic study.
Gastroenteritis	Inflammation of the stomach and intestines.
Gastroenteropathy	Any disease of the stomach or intestines.
Glomerulonephritis	A variety of nephritis characterized by inflammation of the capillary loops in the glomeruli of the kidney. It occurs in acute, subacute, and chronic forms and may be secondary to hemolytic streptococcal infection. Evidence also supports possible immune or autoimmune mechanisms.
Granuloma	A tumor-like mass or nodule of granulation tissue, with actively growing fibroblasts and capillary buds, consisting of a collection of modified macrophage resembling epithelial cells (epithelioid cells), surrounded by a rim of giant multinucleate

	cells, either of the Langerhans' or foreign body type; it is due to a chronic inflammatory process associated with infectious disease, such as tuberculosis, syphilis, sarcoidosis, leprosy, lymphogranulma, etc., or with invasion by a foreign body.
HLA complex	Human leukocyte antigen complex, the major human histocompatibility complex situated on chromosome 6; contains several subregions, called A, B, C, etc.
Haplotype	The group of alleles of linked genes contributed by either parent; the haploid genetic constitution contributed by either parent.
Hapten	A compound, usually of low molecular weight, that is not itself immunogenic, but that, after conjugation to a carrier protein or cells, becomes immunogenic and induces production of antibody, which can bind the hapten alone in the absence of carrier.
Helper T cells	A functional subclass of T cells that help generate cytotoxic T cells and cooperate with B cells in the production of an antibody response. Helper T cells usually recognize antigen in association with class II major histocompatibility complex molecules and bear CD4 markers.
Hemolytic anemia	Decreased hemoglobin in the blood with a decrease in red blood cells and in the volume of packed red blood cells resulting from abnormal destruction of red blood cells in the body.
Herpes simplex	An acute viral disease marked by groups of vesicles, each vesicle about 3-6 mm in diameter, on the skin, often on the border of the lips or the nares (*h. labialis*, cold sores), or on the genitals (*h. genitalis*). It often accompanies fever (*h. febrilis*, fever blisters), although there are other precipitating factors, such as the common cold, sunburn, skin abrasions, and emotional disturbances.
Histamine	4-(2-Aminoethyl)-imidazole, $C_5H_9N_3$, an amine occurring as a decomposition product of histidine that stimulates visceral muscles, dilates capillaries, and stimulates salivary, pancreatic, and gastric secretions. It is found in the granules of the basophils and mast cells responsible for anaphylactic reactions.
Humoral immunity	An immune reaction that can be transferred with immune serum (as opposed to cell-mediated immunity). In general, refers to resistance that results from the presence of a specific antibody.
Hypersensitivity	An adaptative immune response against an antigen that occurs in an exaggerated or inappropriate form and that can lead to tissue damage. Four types of hypersensitivity are recognized. (See allergy.)
Hypersensitivity pneumonitis	Allergic alveolitis.
Ia antigen	Immune-associated surface antigen of mouse cells, such as B lymphocytes and macrophages, that is determined by the major histocompatibility complex II system.
IL-1 (interleukin-1)	An acute-phase reactant synthesized by many cell types, including monocytes and lymphocytes. This hormone has many effects, including the activation of resting T cells, the promotion of synthesis of other lymphokines, and the activation of macrophages and endothelial cells. Also an endogenous inducer of fever.
IL-2 (interleukin-2)	Soluble substance released

	by T cells through the synthesis of other lymphokines to promote proliferation of other T cells (also called TCGF, T-cell growth factor).
IL-3 (interleukin-3)	A multilineage colony-stimulating factor released by T cells that appears to act synergistically with granulocyte-macrophage colony-stimulating factor to stimulate hematopoiesis. It is also a growth factor for mast cells.
IL-4 (interleukin-4)	A growth factor for B cells that induces expression of class II major histocompatibility complex antigen on their surfaces. This hormone also enhances the cytolytic activity of cytotoxic T cells and is a mast cell growth factor. Also known as B-cell-stimulating factor type 1.
IL-6 (interleukin-6)	Also known as B-cell-stimulating factor type 2 or B-cell differentiation factor, it induces the differentiation of activated B cells into immunoglobulin-secreting plasma cells.
Immune complex	The product of an antigen-antibody reaction that also can contain components of the complement system.
Immunodeficiency	A defect or absence of some component of the immune system, e.g. immunoglobulin or T cells, that may result in the inability of the host to eliminate or neutralize foreign substances.
Immunofluorescence	A method of determining the location of antigen (or antibody) in tissue by the pattern of fluorescence resulting when the tissue is exposed to the specific antibody (or antigen) labeled with a fluorochrome.
Immunogen	A substance that can induce an immune response and react with the products of an immune response. Compare with antigen.
Immunoglobulin (Ig)	The protein classes that contain antibody. Each Ig moleculeis made up of two or more heavy chains and two or more light chains and has two or more antigen-binding sites.
Immunologic memory	A phenomenon characterized by the presence in the body of an expanded set of clonally derived antigen-specific lymphocytes that can be rapidly recruited to produce an augmented immune response on subsequent exposure to the specific antigen.
Immunopotentiation	An increase in the functional capacity of the immune response.
Immunosuppression	A reduction in the functional capacity of the immune response caused by drugs prepared with the express purpose of suppressing the immune system.
Immunosurveillance	The mechanisms by which the immune system recognizes and destroys malignant cells before the formation of an overt tumor.
Interferon	Proteins that are formed by animal cells in the presence of a virus, that prevent viral reproduction, and that can induce resistance to a variety of viruses in fresh cells of the same animal species. There are three classes: alpha, beta, and gamma. Alpha interferon (IFNα) is made by lymphocytes and macrophages. Beta interferon (INFβ) is synthesized by fibroblasts and epithelial cells. Alpha and beta interferons were once called type 1 interferon. Gamma interferon (IFNγ), also called type 2 interferon, is synthesized by lymphocytes. All three interferons can be induced during viral infection. They have antiviral and antiproliferative effects, and all induce expression of major histocompatibility complex I antigens.

K cell	A lymphocyte with Fc receptors that allow it to bind to and kill antibody-coated target cells.
Killer T cell	A T cell with a particular immune specificity and an endogenously produced receptor for antigen that can kill its target cell after attachment to the target cell by this receptor. Also called cytotoxic cell.
Kinin	Any of a group of endogenous peptides that cause vasodilation, increase vascular permeability, cause hypotension, and induce contraction of smooth muscle.
Langerhans cells	Stellate, dendritic cells of the mammalian skin thought to be of the melanocyte series. They are strongly positive for class II MHC antigen.
Lymphocyte	Small cell with virtually no cytoplasm, found in blood, in all tissue, and in lymphoid organs, such as lymph nodes, spleen, and Peyer's patches.
Lymphocyte-activating factor	Interleukin-1.
Lymphokines	Soluble substances secreted by lymphocytes that have a variety of specific and nonspecific effects on other cells. A generic term for molecules other than antibodies that are involved in signaling between cells of the immune system and are produced by lymphocytes (cf. interleukins).
Lymphoma	Any neoplasm, usually malignant, of the lymphatic tissues.
Lymphopenia	A reduction in the number of circulating lymphocytes. Also called lymphocytopenia.
Lymphopoiesis	Production of lymphocytes.
Lyse	To cause or produce disintegration of a compound, substance, or cell.
Macrophage	Mononuclear cell derived from bone marrow and found in blood (where it is known as the monocyte), lymph, and many organs. Two main functions are recognized: phagocytosis and antigen presentation.
Major histocompatibility complex (MHC)	A cluster of genes encoding cell-surface antigens that are polymorphic within a species and that code for antigens that lead to rapid graft rejection between members of a single species that differ at these loci. They also function in signaling between lymphocytes and cells expressing antigen. Several major classes of protein, such as MHC class I and II proteins, are encoded in this region.
Mast cell	A small cell similar in appearance to a basophil and found associated with mucosal epithelial cells. These cells depend on T cells for proliferation, and they contain cytoplasmic granules laden with heparin, slow-reactive substance of anaphylaxis, and eosinophil chemotactic factor of anaphylaxis, which are released when antigen binds to membrane-bound IgE.
Mitogen	A substance that nonspecifically stimulates the proliferation of lymphocytes and other cells.
Monoclonal	Literally, coming from a single clone. A clone is the progeny of a single cell. In immunology, "monoclonal" generally describes a preparation of antibody that is homogeneous, or cells of a single specificity.
Monocyte	A mononuclear phagocytic leukocyte, 13-25 µm in diameter, with an ovoid or kidney-shaped nucleus, containing lacy, linear, chromatin, and abundant gray-blue cytoplasm filler with fine, reddish and azurophilic granules. Formed in the bone marrow from the promonocyte, monocytes are transported to tissues, as of the

	lung and liver, where they develop into macrophages. Formerly called large mononuclear leukocyte and hyaline or transitional leukocyte.
Mucocutaneous	Pertaining to or affecting the mucous membrane and the skin.
Myelopoiesis	The process of formation and development of blood cells in the bone marrow.
Natural killer cell (NK cell)	A lymphocyte that can destroy nonspecifically certain virally infected and tumor cells.
Neoantigen	A tumor-specific antigen.
Neutropenia	A decrease in the number of neutrophilic leukocytes in the blood.
Neutrophils	Granular leukocytes having a nucleus with three to five lobes connected by slender threads of chromatin and cytoplasm containing fine inconspicuous granules. Neutrophils have the properties of chemotaxis, adherence to immune complexes, and phagocytosis. Called also polymorphonuclear, polynuclear, or neutrophilic leukocytes. Their counterparts in nonhuman mammals are heterophils. Any cell, structure, or histologic element readily stainable by neutral dyes.
Null cells	A population of lymphocytes bearing neither T-cell nor B-cell differentiation antigens.
Opsonizing	The process by which bacteria are altered by the attachment of antibody so that they are more readily and more efficiently engulfed by phagocytes.
Periodontitis	Inflammatory reaction of the tissues surrounding a tooth (periodontium), usually resulting from the extension of gingival inflammation (gingivitis) into the periodontium.
PFC (plaque-forming cell)	An antibody-secreting B cell that can be recognized by the production of a hemolytic plaque.
Phagocytosis	The ingestion of foreign material, for example, by a macrophage, into a cell.
Plasma cell	A terminally differentiated B lymphocyte that can synthesize and secrete antibody.
Pneumonitis	Inflammation of the lungs.
Polyarteritis nodosa	Inflammation of several arteries with the formation of numerous nodules within the walls of the arteries. (Also referred to as periarteritis or panarteritis.)
Polymorph	A phagocytic polymorphonuclear leukocyte.
Polymorphonuclear leukocyte	The mature neutrophil leukocyte, so-called because of its segmented and irregularly shaped nucleus.
Primary response	The immune response to a first encounter with antigen. The primary response is generally small, has a long induction phase or lag period, consists primarily of the release of IgM antibodies, and generates immunologic memory.
Properdin	A relatively heat-labile, normal serum protein (a euglobulin) that, in the presence of complement component C3 and magnesium ions, acts nonspecifically against gram-negative bacteria and viruses and plays a role in lysis of erythrocytes. It migrates as a β-globulin and, although not an antibody, may act in conjunction with complement-fixing antibody.
Prostaglandin E-2	An unsaturated fatty acid, 20 carbons long, with an internal

	cyclopentane ring. It causes vasodilation, inhibits gastric secretion, induces labor and abortion, and is immunosuppressive. A derivative of arachidonic acid.
Radioallergosorbent test (RAST)	A solidphase radioimmunoassay for detecting IgE antibody specific for a particular allergen.
Raynaud's phenomenon	Intermittent bilateral attacks of ischemia of the fingers or toes and sometimes of the ears and nose, marked by a severe pallor and often accompanied by paresthesia and pain; it is brought on characteristically by cold or emotional stimuli and relieved by heat and is due to an underlying disease or anatomic abnormality. When the condition is idiopathic or primary, it is termed Raynaud's disease.
Recall antigen	Material recognized by "memory" cells that stimulates rapid (secondary) immune responses.
Rhinitis	Inflammation of the mucous membrane of the nose.
Sarcoidosis	A chronic, progressive, generalized granulomatous reticulosis of unknown etiology, involving almost any organ or tissue, including the skin, lungs, lymph nodes, liver, spleen, eyes, and small bones of the hands and feet. It is characterized histologically by the presence in all affected organs or tissues of noncaseating epithelioid cell tubercles. Laboratory findings may include hypercalcemia and hypergammaglobinemia; there is usually diminished or absent reactivity to tuberculin, and in most active cases, a positive Kveim reaction. The acute form has an abrupt onset and a high spontaneous remission rate, whereas the chronic form is insidious in onset and progressive.
SDS gel electrophoresis	A form of polyacrylamide gel electrophoresis that uses sodium dodecyl sulfate (SDS) in the buffer.
Self antigen	Antigenic component of an individual's tissues. Normally self surface markers are recognized by the immune system neonatally and immunologic tolerance develops.
Serotonin	5-Hydroxytryptamine which is present in many tissues, especially blood and nervous tissue. It stimulates a variety of smooth muscles and nerves and is postulated to function as a neurotransmitter.
Sjögren's syndrome	A symptom complex of unknown etiology, usually occurring in middle-aged or older women, marked by keratoconjunctivitis sicca, xerostomia, and enlargement of the parotid glands; it is often associated with rheumatoid arthritis and sometimes with systemic lupus erythematosus, scleroderma, or polymyositis. An abnormal immune response has been implicated.
SLE (systemic lupus erythematosus)	An autoimmune disease of humans usually involving antinuclear antibodies and characterized by skin rash, hematologic alterations, and glomerulonephritis.
SRBC (sheep red blood cell)	A T-cell-dependent target antigen often used in hemolytic plaque assays of immune responsiveness.
Stem cell	Pluripotent cell that can serve as a progenitor cell for the lymphoid lineage or the myeloid lineage or both (hemopoietic stem cell).
Suppressor T cell	A subpopulation of T lymphocytes that act to reduce the immune responses of other T or B cells. Suppression can be antigen-specific, idiotype-specific,

	or nonspecific under different circumstances. Cells with this function cannot be identified with one marker, although many appear to carry the CD8 molecule.
Thrombocytopenia	Decrease in the number of blood platelets.
Thymus	An organ in the thoracic or cervical region of mammals, composed of lymphatic tissue in which minute concentric bodies, the remnants of epithelial structures, or thymic corpuscles, are found. This organ is necessary for the development of thymus-derived lymphocytes and is the source of several hormones involved in T-cell maturation, for example, thymosin, thymopoietin, thymulin, and thymocyte humoral factor.
Thymus-dependent antigen	An antigen that requires an immune response from thymus-derived lymphocytes to elicit an immune response from B lymphocytes.
Thymus-independent antigen	An antigen that does not require the participation of T lymphocytes to elicit an immune response in B cells.
T lymphocytes (T cells)	Cells that originate in bone marrow, mature in the thymus, and then migrate into blood, lymph, and lymphoid tissue. They express nonimmunoglobulin antigen receptors and are derived functionally into helper, suppressor, and cytotoxic subpopulations.
Transferrin	Serum β-globulin that binds and transports iron. Several types (e.g., C, B, and D) have been distinguished on the basis of electrophoretic mobility and related as the products of corresponding dominant somatic genes, Tf^C, Tf^B, and Tf^D.
Urticaria	A vascular reaction of the skin marked by the transient appearance of smooth, slightly elevated patches (wheals), which are redder or paler than the surrounding skin and often attended by severe itching. The eruption rarely lasts longer than 2 days, but may exist in a chronic form. Certain foods (e.g., shellfish), drugs (e.g., penicillin), infections, or emotional stress may be the exciting cause.
Varicella zoster	Chickenpox.
Wiskott-Aldrich syndrome	A condition characterized by chronic eczema, chronic suppurative otitis media, anemia, and thrombocytopenic purpura; it is an immunodeficiency syndrome transmitted as an X-linked recessive trait, in which there is poor antibody response to polysac-charide antigens and dysfunction of cell-medicated immunity.
Xenobiotic	A chemical from a nonbiologic source.

BIOGRAPHIES

DAVID WILSON TALMAGE is chairman of the Subcommittee on Immunotoxicology and a distinguished professor at the University of Colorado. He was formerly dean of the Medical School and then director of the Webb Waring Lung Institute. Dr. Talmage received his M.D. degree from Washington University and has honorary degrees from Buena Vista College and Colorado State University. He was a Markle scholar of medical science, a consultant for the Veterans Administration Hospital, and an editor of the *Journal of Allergy*. His memberships include the National Academy of Sciences, the Institute of Medicine, the American Society for Clinical Investigation, the American Association of Immunologists, for which he served as president for 1 year, and the American Academy of Allergy, for which he also served as president for 1 year. His research concerns the effect of oxygen during culture on survival of mouse thyroid allografts and immunologic tolerance in animals bearing cultured allografts.

DAVID E. BICE is a senior scientist at the Inhalation Toxicology Research Institute in Albuquerque, New Mexico. He is the education coordinator at the Inhalation Toxicology Research Institute and holds joint appointments in the Departments of Medicine and Pharmacology at the University of New Mexico and the Department of Pathology at Colorado State University. His primary research interests are to determine the mechanisms responsible for the induction of pulmonary immunity and how abnormal immune responses cause pulmonary hypersensitivity. He also studies pulmonary defenses to pathogens and how immune responses in the lung may be altered by inhalation of pollutants. Dr. Bice has served on numerous committees including those of the National Research Council and the National Institutes of Health.

JOHN C. BLOOM holds a B.S. degree in biology from the University of Pittsburgh and doctorates in veterinary medicine and comparative hematology from the University of Pennsylvania. He completed his postdoctoral training at Lankenau Hospital (Jefferson Medical College) in hematology/oncology and served on the faculty of the University of Pennsylvania School of Veterinary Medicine for 5 years before joining Smith Kline and French Laboratories

as associate director of pathology, where he worked for 8 years. He currently heads clinical pathology at Lilly Research Laboratories and holds faculty appointments at the University of Pennsylvania and Purdue University.

LOREN D. KOLLER, D.V.M., Ph.D., has been the dean of the College of Veterinary Medicine at Oregon State University since July of 1985. Prior to this appointment as dean, he served as associate dean of veterinary medicine at the University of Idaho, Moscow, Idaho, from 1978 to 1985. Previous appointments were at the U.S. Army Medical Unit at Fort Detrick, Maryland, at the National Institute of Environmental Health Sciences, and at the School of Veterinary Medicine at Oregon State University. Dr. Koller received his D.V.M. degree from Washington State University in 1965, and a Ph.D. in pathology from the University of Wisconsin in 1971. Dr. Koller has been actively engaged as a pathologist and researcher in the areas of pathology, toxicology, immunology, carcinogenesis, and nutrition. He has pioneered the area of immunotoxicology since the early 1970's. He is a member of several scientific and professional organizations, and has served as president of the Immunotoxicology Specialty Section of the Society of Toxicology. Dr. Koller has published extensively in numerous refereed journals, served on editorial boards and grant review panels, and has served as a consultant on numerous occasions. He is currently chairman of the Animals in Research Committee for the Society of Toxicology.

MICHAEL EMANUEL LAMM is a professor and chairman of pathology at Case Western Reserve University. He received his M.D. from the University of Rochester, his M.S. from Western University and has earned a diploma from the American Board of Pathology. He advanced from an intern to resident pathologist at University Hospital in Cleveland and from assistant professor to professor of pathology at the School of Medicine for New York University. He also was a research associate in chemistry for the National Institute of Health. Dr. Lamm's memberships include New York Academy of Sciences, the American Society of Biological Chemists, the Society of Experimental Biology and Medicine, the American Association of Immunology, and the American Association of Pathologists. Dr. Lamm's research is focused on mucosal immunity and immunopathology.

MICHAEL I. LUSTER is head of the Immunotoxicology Group at the National Institute of Environmental Health Sciences and adjunct professor in toxicology at Duke University Medical Center. His research interest, as reflected in his numerous publications, include immunology and toxicology. He has served on committees for the U.S. Environmental Protection Agency, Food and Drug Administration, Centers for Disease Control, and the AIDS Executive Committee for the National Institutes of Health. Dr. Luster also serves on the editorial board of *Environmental Health Perspectives*, and *International Journal of Immunopharmacology*.

WILLIAM J. MEGGS is an assistant professor with the Department of Medicine, East Carolina University School of Medicine. He earned his B.S. from Clemson University, his Ph.D. from Syracuse University, and his M.D. from University of Miami. The fellowships and awards Dr. Meggs has received include the Woodrow Wilson Honorary Fellowship, the Clemson University Faculty Medal, and The National Science Foundation Post-Doctoral Fellowship. Dr. Meggs has

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ALBERT E. MUNSON is with the Medical College of Virginia. Previously he was associated with the Memorial Institute as a cancer research scientist. Dr. Muson's memberships are extensive and include the American Association for Cancer Research, the American Association for Microbiology, the American Association of Immunologists, the American Cancer Society, the American College of Toxicology, the American Society of Pharmaceutical and Experimental Therapeutics, the Archives of Toxicology, the International Society of Immunopharmaceuticals, the National Capital Area Chapter of the Society of Toxicology, the Reticuloendothelial Society, Sigma Xi, and the Society of Risk Assessment.

KATHLEEN E. RODGERS is a faculty member in the Department of Obstetrics and Gynecology, School of Medicine at the University of Southern California. Her research interests include the assessment of the immunotoxicologic potential of organophosphate pesticides, the immunopharmacology of nonsteroidal anti-inflammatory drugs, peritoneal wound healing, and development of immunoassays for ovarian proteins. Dr. Rodgers received a B.A. degree in biology for the University of California, Irvine, and Ph.D. degree from the University of California, Riverside. She is a member of the Society of Toxicology (national and Southern California chapters) and the American Association of Immunotoxicologists. She is currently secretary-treasurer for the Immunotoxicology Specialty Section for the Society of Toxicology.

NOEL R. ROSE is professor and chairman of the Department of Immunology and Infectious Diseases at The Johns Hopkins School of Hygiene and Public Health. He holds joint appointments in the Departments of Medicine and of Environmental Health Sciences. He also directs the World Health Organization collaborating laboratory for immunologic disorders. His major research interests relate to questions of immunologic self-non-self discrimination and the problem of autoimmune disease. He and his colleagues have published extensively on the genetic basis of autoimmunity and on the actions of environmental and infectious agents in promoting autoimmune diseases in susceptible humans and in experimental animals. He is currently editor-in-chief of *Clinical Immunology and Immunopathology*, secretary-treasurer of the Clinical Immunology Society, steering committee member of the Immunotoxicology

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PAUL A. SCHULTE is chief of the Screening and Notification Section of the National Institute for Occupational Safety and Health, and adjunct faculty of the Department of Environmental Health at the University of Cincinnati. Dr. Schulte received a B.A. in zoology and geography from the University of Toronto, a M.S. in health planning from the University of Cincinnati, and a Ph.D. in epidemiology from the University of Cincinnati. He served on advisory panels of the U.S. Environmental Protection Agency, Agency for Toxic Exposure and Disease Registry, Pennsylvania Occupational Disease Study, the Council on Environmental Quality, and the National Research Council. He has served as a consultant to the World Health Organization teaching occupational epidemiology in Beijing. He has served as guest editor for the *Journal of Occupational Medicine*.

CURTIS C. TRAVIS, Ph.D., is director of the Office of Risk Analysis in the Health and Safety Research Division at Oak Ridge National Laboratory (ORNL), Oak Ridge, Tenn. Dr. Travis obtained his training at the University of California, Davis, receiving his Ph.D. in applied mathematics in 1971. He served as research engineer at the Jet Propulsion Laboratory of the California Institute of Technology 1966-68, and taught engineering and applied mathematics at Vanderbilt University, 1971-74, and the University of Tennessee, 1974-76. He has been at ORNL since 1976. Dr. Travis is chairman of the Office of Science and Technology Policy Task Force on Risk Analysis in Federal Agencies, a member of the Science Advisory Board for the National Center for Toxicological Research (NCTR), and a member of the Scientific Advisory Panel on the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) for the U.S. Environmental Protection Agency. He collaborated with the Agency for Toxic Substances and Disease Registry in production of their Guidance Document for Health Assessments at Superfund Sites. Dr. Travis is also president-elect of the National Society for Risk Analysis (1990), and is editor-in-chief of *Risk Analysis: An International Journal*.

ERNEST S. TUCKER III, M.D., is chairman of pathology at California Pacific Medical Center in San Francisco. He was formerly director of the Immunology Reference Laboratory at Scripps Clinic and Research Foundation in La Jolla, Calif. His medical training and pathology residency were at the University of Alabama in Birmingham, and later he held a research fellowship in immunopathology at Scripps Clinic and Research Foundation. He has served in the military at the Armed Forces Institute of Pathology as a specialist in pulmonary pathology. He has held faculty positions at the University of Alabama, University of Wisconsin, University of California, San Diego and currently at the University of California, San Francisco. In 1977, he was instrumental in developing the Immunology Reference Laboratory at Scripps Clinic to transfer diagnostic medical technology from research studies at Scripps Clinic. During his tenure at Scripps Clinic he directed the assessment of immunologic parameters in the health status evaluations of subjects in the U.S. Air Force RANCH HAND study of the long-term effects of exposure to Agent Orange (dioxin). He has published extensively in the areas of immunology and immunopathology with special emphasis on the role of biochemical and cellular factors in immunologic diseases. He is past commissioner of continuing education and vice president of the American Society of Clinical Pathologists and is currently president-elect of that organization.

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GARY R. BURLESON is in the Pulmonary Toxicology Branch, Environmental Toxicology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, N.C., where he is involved in the development of methods to assess pulmonary immunocompetence following inhalation of air pollutants. Dr. Burleson serves on the advisory board of *Modern Methods in Toxicology*, the Steering Committee of the Immunotoxicology Discussion Group, and on various committees of the immunotoxicology specialty section of the Society of Toxicology. He holds adjunct faculty positions at North Carolina State University, North Carolina Central University, and New York University Medical Center. His laboratory has developed models for the study of pulmonary immunology, pulmonary viral disease, asthma, inflammation, and pulmonary oncology.

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