Chapter 3
Immunotoxicological Tests

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INTRODUCTION

This study defines immunotoxicity as an adverse or inappropriate change in the structure or function of the immune system after exposure to a foreign substance. An overreactive or a suppressed immune system can lead to certain health effects commonly associated with immune dysfunction. Skin and respiratory allergies indicate that the immune system has become overreactive–hypersensitive-to a foreign substance. Increased rates of certain types of infection or incidence of certain tumors indicate that parts of the immune system have become suppressed. For example, researchers received an important clue regarding human immunodeficiency virus when physicians reported more frequent diagnoses of a rare infection, *Pneumocystis carinii*, and a rare tumor, Kaposi's sarcoma, in the same individual. Similarly, certain fungal or viral infections point to a suppressed immune system, for these ubiquitous infectious agents rarely surmount the body's defense systems.

Evidence accumulated over time indicates that some chemicals encountered in commerce can alter the structure or function of the immune system. Occupational and consumer experiences have identified several allergens. Clinical experience with certain drugs and studies of accidental exposure to certain industrial substances indicate that some chemicals can lead to disease by suppressing various immune functions or can cause autoimmune reactions.

Rather than wait for adverse effects to manifest themselves, chemical manufacturers and regulators now seek means to predict immunotoxicity prior to human exposure. immunotoxicologists have developed in vivo and in vitro tests, often used in combination, to analyze the effects of substances on various components and processes of the immune system. This chapter describes methods for evaluating chemical immunotoxicity. It also provides a brief explanation of the difficulties entailed in interpreting currently available tests and using their results to predict immunotoxicity in humans. Finally, it reports on some known or suspected immunotoxicants.

TEST METHODS

The purpose of toxicity testing is to ascertain the potential for a substance to adversely affect the structure or function of an organ system. Toxicological tests must be valid, i.e., they must actually measure the effect of interest. They must also be reliable, i.e., it must be possible to duplicate the test in different laboratories with a minimal number of inaccurate results. Importantly, since the test results may be used as the basis of regulation, it must be possible to extrapolate the likely results of human exposure from data garnered from toxicological tests on experimental animals.

An immunotoxicity assessment measures quantitative and functional changes in the immune system following exposure to the test substance. It measures whether a chemical alters lymphoid organ weights or histology, causes qualitative or quantitative changes in humoral, cell-mediated, and nonspecific immunity, or modifies susceptibility to infectious agents or tumors. (Ch. 2 details the various functions of the immune system and its components.) Most tests used in immunotoxicological assessments are performed on experimental animals, usually rodents. In the best experiments, animals are exposed to a putative toxicant in a manner that mimics human exposure conditions as closely as possible. In many of the tests, tissue or fluids removed from an animal that has been killed (sacrificed) are examined in vitro to detect evidence of toxicity. Some tests, primarily the host resistance assays, are performed in vivo, but have disease or death as the endpoint measured.

Because of the harm that most current tests can do to the test subject, predictive testing for immunotoxicity using human subjects is quite rare. It is possible to perform some of the common predictive tests on human peripheral blood, bronchoalveolar lavage fluid, or nasal

lavage fluid since removal of these fluids does no harm. Tests similar to those used to predict hypersensitivity are used on humans in a clinical setting-to determine the cause of allergic symptoms—but clinical testing is beyond the scope of this report.

A basic list of immunotoxicity tests and what they measure is provided in the following sections. The list is illustrative rather than comprehensive. Descriptions of basic tests and specific methodology can be found in several sources (3$8,75).

Pathology

Pathology– the science of disease– is a basic tool of toxicology. Examination of the organs and cells of the immune system using routine pathologic tests can yield evidence of immunotoxicity. Many of the following tests are applied routinely to new chemicals, and can be interpreted to suggest the need for further study of immune system effects.

Hematology--A complete blood cell count measures the total number of blood cells. A differential blood cell count discriminates between the red blood cells and the types of white cells (e.g., lymphocytes, monocytes, neutrophils, basophils, and eosinophils) in the blood. Alterations in cell counts can indicate potential immunotoxic effects.

Histology-The lymphoid organs – the thymus, spleen, lymph nodes, and bone marrow — provide information about possible immune alterations when examined at the cellular level. Changes in the thymus may indicate T cell alterations; B and T cell accumulations in specific areas of the spleen and the lymph nodes suggest a potential change in either humoral immunity (B cells) or cell-mediated immunity (T cells). Bone marrow evaluation can yield information about pluripotential stem cells (immune cell progenitors).

Organ weights –T cells complete their maturation in the thymus. The spleen contains B and T cells. Thymus or spleen weights outside normal reference ranges are viewed as important indicators of potential immunotoxicity.

Cellularity– Counts of certain cells in the tissues of the thymus, spleen, bone marrow, and peripheral lymph nodes can be used to determine potential immune alterations, since a change in cell number suggests an immunotoxic effect on that organ.

Quantitation of splenic B and T cells–B and T *cells* cannot be distinguished by size or shape, but they do have distinct surface markers. A variety of specialized markers can be used to identify cell types and subsets within type. Marked cells can be counted manually or by using automated cell counters. Alterations in cell counts suggest possible immunotoxic effects.

humoral Immunity

humoral immunity involves the production of specific antibodies by B cells following exposure and sensitization to a specific antigen (see ch. 2). The following tests of humoral immunity call for quantitation in vitro of cell types following exposure in vivo to a test agent. The tests are performed on organ cells (which come only from experimental animals) or on peripheral blood (which can come from animals or humans).

Antibody plaque forming cell (PFC) response–This test measures the number of B cells capable of producing antibodies following exposure to an antigen. The test commonly employs a T-cdl-dependent antigen, such as sheep red blood cells (SRBC), and can reveal whether B cells, T helper cells, and macrophages are functioning properly. This test cannot identify the cell type (or types) responsible for the abnormal result. This assay can also be performed following immunization with T-cell-independent antigens to exclude the possibility that altera-

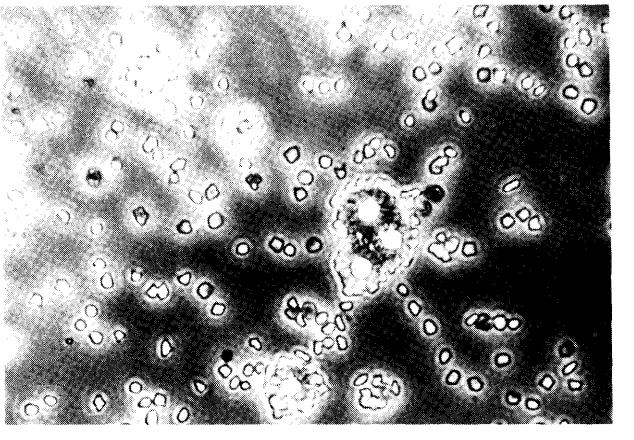

Photo credit: American Type Culture Collection, Rockville, MD

Human T cells surrounded by sheep red blood cells in a PFC assay.

tions in antibody production to T-dependent antigens are due to T helper cell dysfunction.

B cell mitogen response –This test measures the ability of B cells to proliferate (undergo mitogenesis) after stimulation by a bacterial mitogen. Analysts measure the amount of DNA synthesis by B cells from the spleen or peripheral blood after exposure to the suspect chemical. Reference values are determined in unexposed cells, and a decrease in synthesis or proliferation below those values may indicate that the B cells did not respond to stimulation.

Immunoglobulin (Ig) levels in serum –Several methods exist to measure serum or body fluid Ig levels. Changes in a given Ig level are proportional to the number of B cells secreting that particular class of antibody. The most common techniques for measuring serum Ig can identify class (e.g., IgG, IgA, or IgE) and subclass (e.g., IgG has four major subclasses, each of which can be associated with specific disorders). In the case of IgE, the radioallergosorbent test (RAST) can be used to identify antigen specific antibody.

Cell-Mediated Immunity

Cell-mediated immunity describes any immune response in which antibody plays a subordinate, rather than a dominant, role (see ch. 2). Most immune responses involve both humoral and cell-mediated immunity, but the responses can be measured separately.

The test for delayed hypersensitivity response is carried out in vivo. Other tests for cell-mediated immunity are evaluated in vitro after exposure in vivo. Human testing is possible if peripheral blood is to be evaluated, and in some cases human skin testing can be done.

T cell mitogen response –This test measures the ability of T cells to proliferate after stimulation by mitogens such as plant lectins. As with the B cell mitogen assay, analysts measure the amount of DNA synthesis in T cells from the spleen or peripheral blood. A decrease in synthesis or proliferation (below reference values) may indicate that the T cells did not respond to stimulation.

Cytotoxic T lymphocyte (CTL) assay–This assay measures the ability of certain T cells, those induced to differentiate into cytotoxic T cells, to lyse (destroy) cells of the type with which they were immunized. The cytolytic activity of these activated cells is assessed by measuring the amount of radioactivity released from radiolabeled target cells of the same type used to immunize the animal. Cytolytic activity below reference values established in the test indicates a possible immunotoxic effect.

Delayed hypersensitivity response– This assay measures the ability of the immune system to mount a delayed hypersensitivity response after injection with an antigen. Measurement of swelling and redness at the site of antigen injection can be used to evaluate the response, as can other types of assays using radioisotopic procedures that measure the influx of macrophages or serum albumin.

Mixed leukocyte response (MLR)—The MLR is a general test of cell-mediated immunocompetence. The test measures T cells' ability to recognize foreign lymphocytes, to transform and proliferate into various effector T cells, and to release cytokines.

Nonspecific immunity

Immunity is considered nonspecific when it develops without prior contact with antigens. Tests for nonspecific immunity are most often performed on experimental animals. However, tests of natural killer (NK) cell activity can be performed using peripheral blood, and the tests measuring microphage numbers and functions can be done using human lavage fluids.

Natural killer (NK) cell activity – NK cells are thought to lyse (kill) several types of tumor cells and virus-infected cells. The cytotoxic activity of NK cells can be measured by the amount of radioactivity released from labeled NK-sensitive targets when they are incubated together with the NK cells. The release of radioactivity is an indication that the tumor cells have been attacked and damaged in a way that would ultimately lead to their death.

Macrophages– Microphage functions commonly measured include antigen presentation and cytokine production (mechanisms used by the microphage for cell-to-cell communication), phagocytosis (consumption of debris and invading organisms), intracellular production of oxygen free radicals (substances to kill the or-

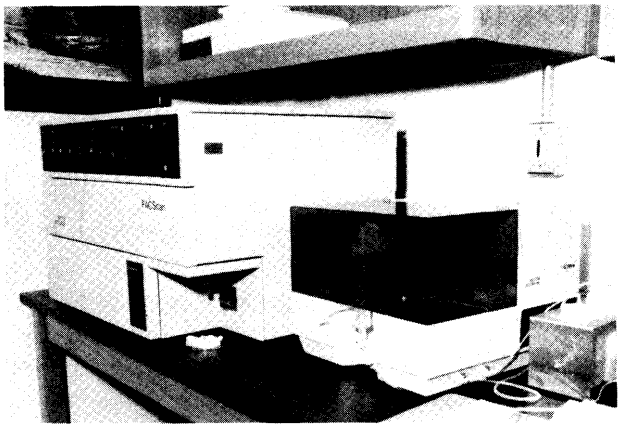

Photo credit: Medical College of Virginia, Richmond, VA

Flow cytometers are used to count cells in various immunotoxicity assays.

ganisms the cell consumes), and direct tumor-killing activities. Macrophages can also be counted in the manner described for B and T cells.

Host Resistance

Assays of host resistance – ability to fight infections or tumors–are performed on experimental animals. They test overall immunocompetence. In host resistance assays, animals are first exposed to a potential immunotoxicant and then exposed to — challenged with – a tumor, bacterium, virus, or parasite. Different components of the immune system can be evaluated by selecting specific challenge agents.

Challenge with one type of tumor cell, a PYB6 sarcoma, assesses the cytolytic activity of T cells (cell-mediated immunity) and NK cells (nonspecific immunity); challenge with cells from another tumor, the B16F10 melanoma, mainly permits evaluation of the nonspecific immunity afforded by NK cells and macrophages. Influenza virus and Streptococcus bacteria provoke antibody production and are used generally to test humoral immunity, though some scientists believe that no adequate host resistance assay of humoral immunity has been developed. Antibody apparently plays no role in resistance to Listeria bacteria, which depends on T cells and macrophages, thus Listeria challenge measures cell-mediated immunity. These challenge agents are illustrative and do not represent a complete list of tests in use.

Hypersensitivity

The assays described above generally test for a suppressed immune system. Tests for hypersensitivity can also be performed on laboratory animals to screen for a substance's allergic potential (or in an allergist's office to discern the reasons for an individual's allergic symptoms). Several types of tests have been developed to measure whether chemicals can produce an allergic response directly or by bonding with proteins in the body. Tests of skin and respiratory reactions can be performed in experimental animals and humans.

Skin reactions: Guinea pig sensitization tests-Tests to determine a substance's potential to induce delayed-type hypersensitivity – usually manifest as allergic contact dermatitis–are conducted most often in guinea pigs. The tests measure erythema and edema (redness and swelling). Common tests include the Draize test, the open epicutaneous test, the Buehler test, the Freund's complete adjuvant test, the optimization test, the split adjuvant test, and the guinea pig maximization test. The essential difference among these tests is the manner in which the animal is exposed to the test substance (3,64).

Skin reactions: Mouse tests—In addition to the guinea pig tests, a mouse ear swelling test (MEST) has recently been developed and validated. Some researchers believe the MEST is a sensitive, efficient, and cost-effective alternative to the guinea pig tests (26). More recently, a murine (mouse) local lymph node assay has been developed to identify chemicals that are contact allergens. This test assesses proliferation of T cells in the

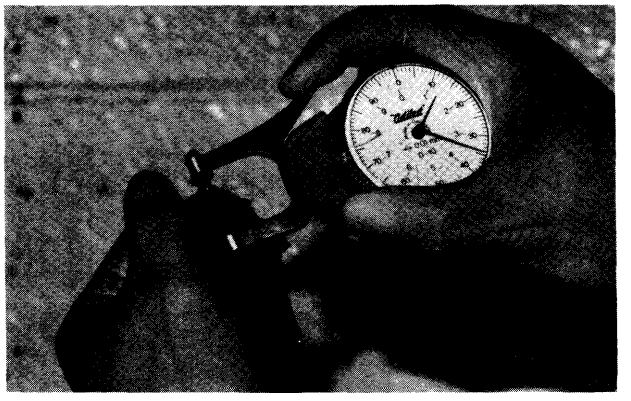

Photo credit: Medical College of Virginia, Richmond, VA

The mouse ear swelling test.

draining lymph node of the ear after application of the chemical to the ear. The test developers find the results of the lymph node assay to be as reliable as results from guinea pig tests (47,79).

Skin reactions: Human sensitization assays-Skin tests can also be conducted in humans– either experimentally, to predict sensitization potential (on informed volunteers), or clinically, to determine the cause of an allergic reaction. Four basic types of predictive tests are used: a single induction/single challenge patch test; a repeated insult patch test; a repeated insult patch test with continuous exposure; and a maximization test. As with the animal tests, erythema and edema are measured (64). [Clinical test methods are beyond the scope of this study.]

Respiratory reactions—Experimental animals, often guinea pigs, can be tested for pulmonary hypersensitivity by measuring certain types of pulmonary function after inhalation exposure (43). Carefully controlled, clinical studies of human respiratory responses have also been undertaken (63,67,68,78).

Serum IgE levels--Total IgE levels and IgE levels specific for a particular antigen can be measured in the peripheral blood of both humans and experimental animals.

Test Selection

The time and resources available for testing new and existing chemicals generally preclude application of all tests to all chemicals. Therefore researchers try to develop screening processes that reserve the most comprehensive tests for the chemicals considered most likely to engender problems. Toxicologists often examine a chemical's structure-activity relationship to known toxicants as a preliminary step. They compare the test chemical's molecular structure to previously studied substances. If a chemically analogous substance is identified, its effects on the body– its activity-will be presumed for the test substance, to the extent the substances are similar. An evaluation of the structure-activity relationship of a new chemical can indicate whether it might affect the immune system and whether an immunotoxicity assessment is desirable. Determinations based on structure-activity relationships are not fool-proof, however, since structurally similar compounds can have quite different levels of toxicity.

Pathologic tests of immune system organs are fairly standard in general toxicologic testing. These tests can reveal structural changes in immune system organs, which can be used to determine whether additional immunotoxicity testing is warranted. The main drawback to these tests is that they assess only structure, not immune function.

Interest in screening chemicals for possible immunotoxic effects has led some chemical manufacturers and regulatory agencies to develop standardized testing tiers. Testing regimes for possible immunosuppressants are generally divided into two or more tiers. The first tier is limited to a screening-type effort intended to assess, as efficiently as possible, the integrity of the major components of the immune system. Subsequent tiers represent more in depth evaluation of those responses and also include assays that evaluate host 'resistance to challenge with infectious agents or transplantable tumors.

There is also some interest in developing a tier testing method for hypersensitivity assessment. One approach to assessing the potential for low molecular weight compounds to act as respiratory allergens calls for a literature search on the compound, followed by an evaluation of its ability to bind with serum proteins (required for allergenicity), followed in turn by skin tests, and, if warranted, respiratory tests (77).

Tier testing is not appropriate for all needs. FDA, for instance, eschews standardized tests in favor of a case-specific evaluation of substances intended for human consumption.

immunotoxicology is still a developing field. Selection of immunotoxicological tests for chemicals remains discretionary. The tests applied to a particular substance are those considered most efficacious in a particular instance by the manufacturer and the regulator.

EPIDEMIOLOGIC EVALUATION OF IMMUNOTOXICITY

Environmental epidemiology, which attempts to associate disease or other adverse outcomes with an en-

vironmental exposure, measures health effects in humans at exposure conditions that are by definition realistic (4). Epidemiologic studies can identify possible associations that should be tested in laboratory environments and can be used to evaluate human health risks suggested by laboratory exposures (17).

Some epidemiologic studies of suspected immunotoxicants have been performed. Contact hypersensitivity and asthma are known hazards of many occupations because of the experience of exposed workers (29,42,57). Accidental exposure to industrial chemicals outside the workplace has occasionally yielded opportunities to study health effects in humans, as with polybrominated biphenyls (PBBs) (see box 3-A), polychlorinated biphenyls (PCBs) (see box 3-B), and dioxin (36). Some of the studies on PBBs, PCBs, and dioxin have shown evidence of quantitative immunosuppression, but none has shown evidence of actual health effects, and followup studies have sometimes contradicted the original studies. A recent study of the effects of passive smoking *on* children did reveal an immune-mediated effect on lung function corresponding to elevated prevalence of asthma and chest colds (12). Current epidemiologic research on immunotoxicity includes examination of the correlations between asbestos exposure and the development of immune system dysfunction (30,54,97). Some experts believe that increased immunological evaluation of asbestos workers may eventually explain the long latency between asbestos exposure and disease (14).

Epidemiologic research on any substance is complicated by the lack of good exposure data (4). Efforts to improve this situation are underway, however. In May 1990, the Board on Environmental Studies and Toxicology of the NAS and the Agency for Toxic Substances and Disease Registry cosponsored a meeting on Frontiers in Assessing Human Exposures to Environmental Toxicants. This meeting signaled increased emphasis on developing improved epidemiologic evaluation measures and increased coordination of Federal, academic, and industrial research efforts in this area. Confounding factors, such as diet and exposure to chemicals other than the one under study, make epidemiology a challenging field for researchers.

Idiosyncrasies of the immune system compound the difficulties for epidemiologic studies of immunotoxicants. The range of "normal" immune responses and "normal" conditions of immune system components varies greatly within the population, thus it is hard to define a "suppressed" human immune system in the absence of disease (18). Since epidemiologic studies do not permit challenging the study population with infectious agents, satisfactory evidence of immunosuppression may not be available during the study period, particularly if immunosuppression is transient.

Epidemiologic investigations of immunotoxicity are complex undertakings that require much time and many resources. Few epidemiologic studies have specifically examined environmental or occupational exposure to immunotoxic substances (33,49). They are likely, however, to be the only means of gaining realistic data on the human health effects of nontherapeutic substances.

PROBLEMS WITH PREDICTING AND INTERPRETING IMMUNOTOXICITY

The immune system is a complex toxicological target. As with other organ systems, its response to chemical insults has features that complicate the interpretation of experimental findings. For instance, test agents commonly show variations in impact on test subjects that can be attributed to species, strain, or sex differences (e.g., hexachlorobenzene stimulates the immune system in rats but suppresses the immune system in mice (103); chloral hydrate in drinking water significantly depressed humoral immune function in female mice, but male mice showed no alterations (45,108)).

immunotoxicological studies often reveal complex dose-response relationships as well. A specific test agent can stimulate the immune system at one dose and suppress the immune system at another. Problems with interpreting dose-response relationships also manifest as problems with evaluating the immune system's reserve or redundant capacity. Scientists cannot accurately predict whether exposure to a test agent that reduces one cell type will impair immune function or whether that same test agent will stimulate another component of the immune system, which will then compensate for the irnpaired component (e.g., dimethylnitrosamine (DMN) reduced B and T cell function in mice, but susceptibility to Listeria was reduced because DMN stimulated macrophage function (37,108)).

Experimental conditions also influence immune response. Reaction to exposure in vivo often differs significantly from reaction to exposure in vitro (e.g., estradiol benzoate decreased NK cell activity in mice in vivo, but not in vitro (39)). Acute and chronic doses can render different results (e.g., acute doses of dichloroethane

Box 3-A– Polybrominated Biphenyls: The Michigan Case

Industrial incidents allow scientists to examine human immunotoxicity in "real-life" situations. One incident involving polybrominated biphenyls (PBBs) in Michigan "frustrates some of the problems in assessing immunotoxicity in humans.

In the 1970s, **a** commercial preparation of PBB was accidentally used in place of an inorganic ingredient in preparing a feed supplement for lactating cows, and the supplement was subsequently used throughout Michigan in 1973 and 1974. Toxic manifestations in the cattle included reduced milk production, joint swelling, hyperkeratosis, persistent mastitis, cutaneous and subcutaneous infections, abscess formation on back legs and udder-, and various reproductive anomalies, but did not involve indications necessarily related to infection. It took 9 months from initial contamination to identify the toxicant.

Many people consumed the contaminated products-– milk, milk products, meat, eggs, and poultry— and PBB was found in serum and adipose tissues of individuals at least through **1980,** Clinical symptoms included fatigue, a striking decrease in the ability to do physical or mental work, and an unusual requirement for sleep. Reduced memory and energy were also noted. Arthritic changes and other symptoms affecting the liver, neurological, and musculoskeletal systems were detailed. Increased susceptibility to infections or tumors, however, was not described.

Two studies, in 1977 and 1980, of farm residents exposed to PBB examined immunological parameters. These individuals were compared to age-matched controls: dairy farm residents living in nearby Wisconsin, who had not been exposed to the contaminated products, and New York City residents. Total B and T cell counts, functional mitogenic assays, immunoglobulin content, and NK cell abnormalities were studied. For at least 18 of 45 Michigan residents examined, a statistically significant decrease in the absolute number of T cells (about 60 percent of the amount in nonexposed control subjects) and a decreased mitogenic response to both T and B cell mitogens. The absolute number of B cells was unchanged. In a follow-up study, the researchers concluded that the immunological dysfunction reported in the first study had persisted, lasting at least 5 years. Elevated levels of IgG, IgA and IgM were also reported.

Two other studies of a different, larger cohort of exposed Michigan residents, however, found no significant differences in an array of immune parameters. The first found significantly higher levels of circulating lymphocytes in the PBB-exposed group when tested in 1976 to 1977, but found no decrease in the absolute numbers of T or B cells. The second study reported no significant differences in B or T cells among exposed Michigan residents, PBB workers, and unexposed individuals. The researchers reported depressed mitogenic responses to certain mitogens in PBB-exposed individuals v. unexposed persons, but considered the values within the normal range of their laboratory, and thus did not attach any significance to the finding.

In all studies, the individuals exposed had not exhibited an unusual increase in infections or tumors, two widely accepted and applied indicators of suppression-type immunotoxicity. Although the lack of clinical manifestations might indicate that it was and is too early to predict or extrapolate immunotoxicit y of PBB based on the Michigan accident, the conflicting laboratory values and conclusions of these studies, derived with different methods on different populations, underscore the problems associated with evaluating human epidemiologic studies, Clearly PBB affected many organ systems, but its *immunotoxicological* significance *is* difficult to discern.

SOURCE-S: Office of Technology Assessment, 1990 based on J.G. Bekesi, J.F. Holland, H.A. Anderson, et al., "Lymphocyte Function of Michigan Dairy Farmers Exposed to Polybrominated Biphenyls," *Science* 199:1207-1209, 1978; J.G. Bekesi, J. Roboz, A. Fischbein, et al., "Immunological, Biochemical, and Clinical Consequences of Exposure to Polybrominated Biphenyls," *Immunotoxicology and Immunopharmacology*, J. H. Dean, M.I. Luster, A.E. Munson, et al., (eds.) (NewYork, NY: Raven Press, 1985); J.G. Bekesi, J.P. Roboz, S. Solomon, et al., "Altered Immune Function in Michigan Residents Exposed to Polybrominated Biphenyls," *Immunotoxicology*, G.G. Gibson, R. Hubbard, and D.V. Parke (eds.) (New York, NY: Academic Press, 1983); R. Burrell, "Identifying and Controlling Immunotoxic Substances," contract paper prepared for the Office of Technology Assessment, U.S. Congress, April 1990; PJ. Landrigan, K.R. Wilcox, Jr., J. Silva, Jr., et al., "Cohort Study of Michigan Residents Exposed to Polybrominated Biphenyls: Epidemiologic and Immunologic Findings," *Annals of the New York Academy of Sciences* 320:284-294, *1979;* J. Roboz, J. Greaves, and J.G. Bekesi, "Polybrominated Biphenyls in Model and Environmentally Contaminated Human Blood: Protein Binding and immunotoxicological Studies," *Environmental Health Perspectives* 60:107-1 13, 1985; and J.K. St ross, LA. Smokier, J. Isbister, et al., "The I Iuman Health Effects of Exposure to Polybrominated Biphenyls," *Toxicology and Applied Pharmacology* 58:145-150, 1981.

Box 3-B – Polychlorinated Biphenyls: The Taiwan Case

In February 1979, rice bran oil poisoned over 2,000 people in Taiwan. The oil had been accidentally contaminated by polychlorinated biphenyls (PCBs), which were detectable in oil samples at concentrations of from 4.8 to 204.9 ppm, and in the blood of poisoned patients from 3 to 1,156 ppb. Subsequent analyses convinced most scientists that the toxic effects were associated with the chlorinated dibenzofurans (chemicals closely related to dioxin) that contaminated the PCBs. Patients experienced headaches, ocular disturbances, diarrhea, mylagia, arthritis, and general malaise.

The syndrome, which was first seen in Japan in 1%8, where it is called the “Yusho Syndrome;" is often referred to as "Yu-Cheng,” literally “oil disease.” Yu-Cheng is characterized mostly by distinctive acneiform eruptions and pigmentation, as well as other symptoms involving nonimmune organ systems. An increase in infections per se was not detailed, and infections that were reported seemed to be largely confined to superficial skin infections not usually associated with immunodeficiency.

For the most part, in vitro lymphocyte mitogen assays of patient lymphocytes showed enhanced stimulation. T cell loss was reported, particularly helper cells, and increased skin reactivity (delayed-type hypersensitivity) to tuberculin and streptococcal enzymes was also documented. Tuberculin reactivity seemed to persist into the fourth year. These people were probably tuberculin sensitive before the accident and carried the bacteria in an inactive state. One possible future consequence of the PCB and furan exposure could be reactivation of tuberculosis, although to date this has not been reported.

SOURCE: Office of Technology Assessment, 1991; based on R. Burrell, “Identifying and Controlling immunotoxic Substances,” contract paper prepared for the Office of Technology Assessment, U.S. Congress, June 1990; Y.-C. Lu, Y.-C. Wu, “Clinical Findings and Immunologic Abnormalities in Yu-Cheng Patients,” *Environmental Health Perspectives* 59:17-29, *1985;* and Wilson, J. D., "A Dose-Response Curve for Yusho Syndrome,” *Regulatory Toxicology and Pharmacology* 7:364-369, *1987.*

suppressed the mouse immune system, but chronic administration in drinking water had no effect (62)). An oral dose can have no effect while injection of the same substance provokes a response. Thus the route of exposure can affect the tests results (e.g., as with chlordimeform exposure in mice (81)). The age or maturational status of the test animal may also affect results. with prenatal or neonatal exposure to immunotoxicants often provoking a much stronger reaction than adult exposure (e.g., as with DES (38)). Nonspecific environmental factors can also affect the immune system. For instance, even moderate dietary restrictions in mice can reduce spleen cellularity. However, the effects of cell reduction are unpredictable (e.g., the reduction in spleen cellularity was accompanied by an increase in PFC response (66,108)).

Even when toxicologists overcome the difficulties in interpreting results from tests on laboratory animals, the question remains as to whether animal responses correspond to human responses. Experience with therapeutic drugs provides evidence to support a conclusion that many results can be extrapolated from laboratory animals to humans. However, little direct evidence exists with regard to industrial or environmental exposures to immunotoxicants. Purposely exposing humans to suspected toxicants generally is considered unethical clinical practice. Epidemiologic studies have posed serious problems for researchers since reliable exposure data have usually been lacking, though there has been some improvement in this area (102).

Perhaps the greatest problem with extrapolating test results from animals to humans is evaluating the clinical significance of altered immune responses, particularly the significance of suppressing humoral, cell-mediated, or nonspecific immunity. Observational experience is based mainly on severe and long-lasting immunosuppression resulting from therapeutic drug treatments, and scientists do not know the clinical relevance of moderate and transient perturbations of the immune system. Conversely, changes in the immune system may not be immediately apparent, i.e., biologically significant changes in immune function could occur with few morphological

correlates and remain subclinical until the animal or human is subjected to a particular stress or insult.

Comprehensive and reproducible testing schemes exist to evaluate immunotoxicological potential of chemicals in experimental animals. However, few chemicals have been tested in this manner, and the immunotoxicological findings in experimental animals can only serve as indicators of concern in humans. Historically, the immune system has received little attention as a target organ for toxicity, and immunology has not been an integral part of the toxicology curriculum (61). Increased education and research integrating immunology and toxicology would benefit scientists and policymakers interested in identifying and controlling immunotoxicants.

EXISTING DATA ON immunotoxicITY

Few of the chemical substances now marketed have undergone immunotoxicological testing. This section describes some of the research that has been done on substances or classes of substances to determine whether they can suppress the immune system or cause hypersensitivity or autoimmune reactions. Most of the referenced studies have been performed on laboratory animals since human studies on nontherapeutic substances are notoriously difficult. Specific note is made of the origin — animal or human— of the data.

Immune Suppression

Several substances clearly exhibit a toxic effect on the immune system by suppressing normal immune responsiveness. Table 3-1 lists several substances and classes of substances thought to be immunosuppressive toxicants. This subsection further analyzes the effects of some of these substances.

Therapeutic Drugs

Therapeutic drugs designed to prevent transplant rejection or to treat autoimmune disorders and cancer are the most thoroughly studied immunosuppressive substances. They are listed here as immunotoxicants because of the clearly demonstrated association between the therapeutic use of immunosuppressants and the increased incidence of infections and cancer (59). For instance, 50 percent of transplant patients get cancer within 10 years (65).

Table 3-I—Known or Suspected Immunosuppressants

Halogenated aromatic hydrocarbons (HAHs)
PCBs
PBBs
Dioxins
Immunosuppressive drugs
Azathioprine
Glucocorticosteroids
Cyclophosphamide
Cyclosporin A
Pesticides
Organophosphates (e.g., malathion)
Organochlorides (e.g., DDT)
Carbamates (e.g., aldicarb)
Polycyclic aromatic hydrocarbons (PAHs)
3-methylcholanthracene
Benzo[a]pyrenes
Benzene
Illegal drugs
Cannibinoids (e.g., marijuana)
Phencyclidine (PCP)
Opiates (e.g., heroin)
Heavy metals
Lead
Nickel
Cadmium
Mercury
Organotins
Air pollutants
Nitrogen dioxide
Ozone
Cigarette smoke

SOURCE: Office of Technology Assessment, 1991.

The most frequently used immunosuppressive drugs fall into four basic categories –alkylating agents, glucocorticosteroids, antimetabolites, and natural products. Research remains to be done on exactly how these drugs produce immunosuppression, but each appears to act through a different mechanism. Alkylating agents disrupt cell functions, particularly mitosis. They are, therefore, highly toxic to rapidly proliferating cells, such as lymphoid cells. Cyclophosphamide is representative of this type of drug, and is used as a pretreatment in bone marrow transplant recipients to prevent graft rejection and is also used as a cancer treatment and to reduce symptoms of certain autoimmune diseases (91). Treatment with cyclophosphamide, while very effective, carries with it an increased risk of certain cancers (95).

Glucocorticosteroids alter phagocytosis and depress T and B lymphocyte function, though the exact mechanisms for these immunosuppressive effects remain unknown. Prednisolone and methylprednisolone, glucocorticosteroids, are therapeutic for transplant recipients and for individuals suffering from extreme allergic reactions (91). Glucocorticosteroids are as-

sociated with enhanced susceptibility to infection (95). Azathioprine is a widely used antimetabolite and acts chiefly by inhibiting protein synthesis (71). It is used clinically in transplant patients and also as an anti-inflammatory agent. A common side effect of azathioprine treatment is bone marrow suppression (95). Cyclosporin A, a natural product derived from fermentation products of two fungi, appears to act through modulation of mechanisms regulating immune responsiveness. Cyclosporin A suppresses cell function but spares B cell function, and has proved successful with transplant patients, though long-term use has been shown to lead to higher infection rates and incidence of non-Hodgkins lymphoma (55).

Benzene

Benzene, a basic industrial chemical, serves as a solvent or feedstock in the synthetic chemical, printing, lithograph, rubber cement, rubber fabricating, paint, varnish, stain remover, adhesive, and petroleum industries. Benzene has been causally linked to several health problems, including immune dysfunction, and it is subject to stringent workplace regulations because of its proven carcinogenicity (1 ppm TWA; 5 ppm STEL (29 CFR 1910)). High dose exposure to benzene results in decreased immune cell function and increased lethality of infections. Acute exposure studies in rats have demonstrated bone marrow toxicity and depressed immune function. Benzene metabolizes remain in bone marrow after exposure ceases, and may lead to long-term immunotoxic effects.

Workers chronically exposed to benzene (>100 ppm) experienced increased rates of agranulocytosis and myelogenous leukemia, accompanied by an increased risk of infection (22). Depressed levels of some immunoglobulins in humans exposed to benzene have been reported (21), but a 1988 study showed no change in lymphocyte function in workers exposed for long periods to benzene concentrations of 1 to 5 ppm with peaks up to 100 ppm (107). There have been major disparities between studies of individuals that experienced short-term, high dose exposure, and studies of individuals subjected to long-term, low dose exposure. Benzene is, therefore, considered immunotoxic, but the magnitude of effect and the exposure threshold remain to be established.

Pesticides

Pesticides have been the subject of numerous animal studies of immunosuppression in recent years. Rodent studies of organophosphates, such as malathion; organochlorides, such as DDT; and carbamates, such as carbofuran, showed evidence of immunosuppression (93). However, none of these pesticides has been submitted to a systematic evaluation for immunotoxicity. In addition, it appears that intentionally added inert ingredients or other contaminants maybe responsible for the observed suppressive effects (83,93). Many experts consider the animal studies indicative of potential immunotoxicity in humans, though certainly not conclusive evidence, and recommend prudence when dealing with pesticides, particularly the organochlorides, which remain stable in the environment and become concentrated in the food chain.

Most studies of the immunosuppressive effects of pesticides in workers indicate no decrease in resistance to disease even where changes in immune system components were measurable (43,48,106). However, one study of workers handling organophosphate pesticides did find an increase in upper respiratory tract infection (35). Epidemiologic evidence of the effects of pesticide exposure outside the workplace is quite sparse (23). A study of women who drank aldicarb-contaminated groundwater showed altered numbers of T cells, but the biological significance of the alteration was not demonstrated (25,93).

Halogenated Aromatic Hydrocarbons

Among the most infamous halogenated aromatic hydrocarbons (HAHs) are polybrominated biphenyls (PBB), polychlorinated biphenyl (PCBs), and dioxins. PCBs (now banned) has been used as plasticizers and as a heat transfer medium; PBB as a fire retardant. Dioxin appears as a contaminant in some commercial substances. Studies of the effects of HAHs on experimental animals indicate that they can have adverse effects on the immune system (94). Findings in laboratory animals exposed to PCBs or PBB at levels higher than most human exposures include severe atrophy of primary and secondary lymphoid organs, lower circulating immunoglobulin levels, and decreased specific antibody response (85,%). Evidence from epidemiologic studies is inconclusive regarding immunotoxicity of these substances in humans. Studies of human exposure have shown abnormal laboratory values for immune parameters, but no conclusive clinical evidence of immune aberration (see boxes 3-A and 3-B).

The name dioxin is assigned to 75 chemicals with similar composition. The most widely studied dioxin,

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), affects young animals more severely than adults and leads to severe thymic atrophy and moderate atrophy of the peripheral lymph nodes at doses that cause other toxic effects as well (94). Recent animal research indicates that genetic predisposition plays a major role in susceptibility to the effects of dioxin (76,82). A study of mobile home park residents exposed to TCDD yielded abnormal in vitro test results in about 25 percent of the exposed population, but uncovered no reports of clinical disease due to demonstrated cellular immunity (36). These effects were not detectable upon reexamination of the same population, and the authors of the second paper consider the possibility that the first paper was in error (24). Some data on children accidentally exposed to TCDD show normal immunoglobulin levels and elevated lymphocyte responsiveness, but the only clinical evidence of disease is chloracne (70). The position of the majority of scientists who have examined human health effects of dioxin is that little or no harm has been done by its dissemination in the environment (31,73).

Polycyclic Aromatic Hydrocarbons

Fossil fuel combustion produces polycyclic aromatic hydrocarbons (PAHs), and distillates from these products are widespread in the chemical industry. Exposure occurs in the workplace and in the environment generally. Many PAHs are carcinogenic, and a growing body of evidence indicates that they may also be immunotoxic. A 1980 study showed that mice exposed in utero to benzo[a]pyrene (BaP), a PAH, experienced significant, persistent suppression of humoral immunity(99). A later study by the same author showed inhibition of the PFC response and the MLR when spleen cells were exposed to concentrations of BaP (100). Another PAH, 7,12-dimethylbenz[a]anthracene (DMBA), significantly increased susceptibility to murine cytomegalovirus (80). Noncarcinogenic PAHs do not appear to produce immune alterations (104). The immunotoxic effects of PAHs have not been widely studied in humans.

Oxidant Gases

The transportation system and many industrial processes produce airborne pollutants. Two air pollutants that have been studied for their immunotoxic effects are ozone and nitrogen dioxide, oxidant gases regulated under the Clean Air Act (CAA). Studies on experimental animals reveal increased susceptibility to infection after exposure to low levels of ozone and nitrogen dioxide. Pulmonary NK cell activity in rats was significantly suppressed following continuous exposure to ozone, but after 10 days, NK cell activity returned to normal, even in the continued presence of ozone (13). Dutch researchers found that ozone suppressed or enhanced pulmonary NK cell activity in the rat depending on the exposure level (101). Mice exposed to 2.5 to 5.0 ppm of nitrogen dioxide showed increased susceptibility to bacterial infection (40).

Ozone has been shown to cause immediate short-term changes in lung function and increased respiratory symptoms among healthy adults and children who exercise moderately or heavily during periods of elevated ozone concentrations. Some studies suggest that there may be some persistent effects associated with long-term exposure to ozone, although understanding of such effects, including whether they are immune-mediated, is currently limited (98). Government researchers recently summarized the results of several epidemiologic studies of nitrogen dioxide exposure (32). The studies reveal an increased prevalence of respiratory infections among individuals, particularly children, exposed to nitrogen dioxide, but small sample sizes and poor exposure data tend to lessen the significance of the findings. EPA has set national primary ambient air quality standards for ozone (0.12 ppm) and nitrogen dioxide (0.053 ppm) based on health effects other than immunotoxicity (40 CFR 50).

Indoor Air Pollutants

Some air pollutants become particularly concentrated indoors. Some of the epidemiologic evidence of the health effects of nitrogen dioxide comes from studies of individuals in homes with gas stoves. Sidestream cigarette smoke, in one study of children, increased the incidence of respiratory infections through immune-mediated mechanisms (12). Concerns have been raised about off-gassing from products containing formaldehyde, but animal (20) and human (69) studies of immune functions following inhalation of formaldehyde indicate that it is not immunosuppressive.

Metals

Heavy metals are ubiquitous – in the air and water, at work and at home — and a few of them have been studied for immunotoxicity. A 1982 study of the effects of lead on macrophage function in mice indicated a significant immunosuppressive effect (46). A more recent study of lead's effects on host resistance in mice yielded conflicting results depending on time and amount of exposure,

making it difficult to draw any conclusions about lead's immunotoxicity (50). The latter study also evaluated the effects of nickel and selenium. Nickel's effects on host resistance varied significantly with time and amount of exposure; selenium uniformly increased resistance to infection. Other studies of nickel indicate that it suppresses NK activity in mice and rats (86,87,89) but that nickel chloride does not adversely affect the immune system of the developing mouse (88). One study of cadmium showed that it significantly depressed NK cell activity in mice but had no effect on mortality due to infection (19). A study of cadmium in aged mice showed no immunosuppressive effects (10). The evidence for immunosuppressive effects of mercury on animals is conflicting (52). Organotins, used as heat stabilizers, catalytic agents, and antifungal/antimicrobial compounds, have been widely studied, but show highly variable effects depending on the species, times. and amount of exposure involved in the test (11,84,85,90).

Hypersensitivity

This report defines immunologic hypersensitivity as allergic response (see ch. 2). Delayed-type hypersensitivity manifests as allergic contact dermatitis and is the result of a T cell mediated inflammatory response. Immediate hypersensitivity is an antibody-mediated response that manifests as allergic rhinitis, asthma, or anaphylaxis. This section describes some problems with hypersensitivity resulting from environmental exposures.

Contact Sensitivity and Skin Disorders

The skin is an excellent route for toxic agents to enter the body. Workers engaged in leather tanning and finishing poultry/egg processing, manufacturing sealants, adhesives, or abrasives, fish packing, boat building and repairing, and landscaping are at risk for skin manifestations of immunotoxicity called occupational dermatoses. Cell-mediated hypersensitivity causes 20 to 30 percent of occupational dermatoses (14).

Another skin disease of immunologic significance is atopic dermatitis (eczema). This condition is a chronic, periodic skin disorder, primarily of infants and children, that depends on a complex interrelationship of genetic predisposition, and an imbalance of immunologic and pharmacologic mediators in the skin (53). Environmental agents, including jewelry and cosmetics, may provoke initial attacks or recurrences (14).

A number of therapeutic and cosmetic materials provoke various types of skin reactions. Oils used as bases in many ointments for either medical or cosmetic purposes can induce contact sensitivity. If substances (e.g., perfumes) are dissolved in solvents, cutaneous absorption is greatly facilitated. One type of immunologic hypersensitivity, "underarm anti-perspirant granuloma," occurred when compounds containing the element zirconium were introduced. Although excellent antiperspirants they induced cell-mediated hypersensitivity, which over along period of application led to pathological manifestations of granuloma formation. Once the key ingredient was identified, the compounds were either eliminated or modified to avoid the condition.

Some common metals also cause hypersensitivity responses. The nickel in costume jewelry can cause contact sensitivity 5 percent of all contact dermatitis can be attributed to nickel-containing compounds (14). Exposure to platinum, chromium, mercury, and gold can also lead to skin sensitization. Table 3-2 lists a number of agents known to induce types of contact sensitivity.

Respiratory Disorders

Numerous inhalants cause immune-mediated respiratory disorders, including some types (but not all) of bronchial asthma, hypersensitivity pneumonitis, allergic rhinitis, bronchopulmonary aspergillosis, silicosis, asbestosis, coalworkers' pneumoconiosis, and possibly byssinosis (60). Some of these conditions result from a humoral immune response, as in the case of IgE-mediated, bronchial asthma, while others have cell-mediated immune involvement, as with the mineral pneumoconioses.

Hypersensitivity pneumonitis is a respiratory disease of immune origin. The exact mechanism of its pathogenesis is controversial, but the involvement of several immune components, including nonspecific complement activa-

Table 3-2—Common Contact Sensitizers

Plant	Minerals
Poison ivy	Beryllium
European primrose	Nickel
Synthetic compounds	Cadmium
Benzocaine	Chromates
Epoxy resins	Silver
Mercaptan	Zirconium
Picric acid derivatives	Cutting oils
C-1 Hydrocarbons	
Ethylenediamine	
Paraphenylenediamine	
Thimerosol	

SOURCE: Office of Technology Assessment, 1991.

tion, T and B lymphocyte stimulation, and microphage activation, has been reported (15,72). Hypersensitivity pneumonitis is caused by allergy to specific microbial or organic substances. However, the dusts that cause the condition are sometimes mixtures of many potentially inflammatory or bio-active agents.

Asthma is the leading chronic disease of childhood (27). Occupational asthma is the most common occupational respiratory ailments in the Western world (16). Numerous agents can induce asthma, and they are generally divided into two groups: large molecular weight substances, usually proteins, that cause classical, IgE-mediated asthma; and low molecular weight materials that cause non-IgE-mediated, longer lasting types of asthma. Occupational asthma is often of the non-IgE-mediated type; table 3-3 presents a list of the some common incitants for occupational asthma.

Table 3-3—industrial Chemicals Associated With Occupational Asthma

Platinum salts	Ethylenediamine
Nickel salts	Phthalic anhydrides
Pyrethrum	Colophony resins
Diisocyanates	Exotic wood dusts

SOURCE: Office of Technology Assessment, 1991.

Substances That Are Allergenic

Some commonly used drugs cause allergic reactions in users. Penicillin produces an allergic reaction in 1 to 10 percent of users (less than 1 percent are of the life-threatening anaphylactic variety) (l). Some drugs used to treat hypertension are also suspected of producing allergic reactions in patients using the drug for extended periods (l), although the exact mechanism of the reaction remains unknown. Over-the-counter medications are also allergenic for some users and bear warnings to that effect. It should be noted that not all adverse drug reactions are immune-mediated though the reaction may be called "allergic" (see box 2-B). For instance, aspirin produces an allergy-like reaction in some users, but the weight of the evidence now indicates a non-immunologic basis for aspirin intolerance (21).

Formaldehyde has historically been used to increase wrinkle resistance and fabric durability, and many garment industry workers were believed to develop an allergic reaction to free formaldehyde (21). However, recent challenge studies on patients with asthma yielded no evidence that formaldehyde could cause or aggravate symptoms, and attempts to measure serum antibody and skin reactions yielded no adverse reactions (69). OSHA regulates formaldehyde as a carcinogen.

Toluene diisocyanate (TDI) is used in plastics manufacture and reportedly has induced asthma and contact dermatitis in occupationally exposed individuals. Some patients with 'I"DI-induced asthma remain symptomatic even years after cessation of exposure, and some observers believe that TDI may cause airways to become hyperreactive to agents such as smoke or air pollutants. Most individuals with TDI-induced asthma react similarly to other diisocyanates (42). OSHA now regulates TDI at levels below those demonstrated to induce hypersensitivity in humans (0.005 ppm TWA; 0.002 ppm STEL).

Definitive data demonstrate **that** Occupational exposures to some pesticides (e.g., some carbamate and organophosphorous esters) can induce contact hypersensitivity. While animal studies indicate that antibody response to pesticide derivatives is possible (93), no reports of IgE sensitization of humans to pesticides have been confirmed. Some scientists believe reported reactions may be irritative rather than allergic in nature.

Autoimmunity

Autoimmune diseases are aberrations of the immune system resulting in an organism attacking a part of itself as a foreign substance. Certain toxic **substances** that are biological in nature can increase the risk of certain autoimmune conditions. For example, it is well known that certain streptococcal infections, when left untreated, may lead to rheumatic fever and post-streptococcal glomerulonephritis. However, the evidence for chemical-induced autoimmune disorders is ambiguous (2,34,41).

A growing list of pharmaceuticals have been shown to induce autoantibody formation (antibodies against self antigens) or actual autoimmune pathologies (table 3-4; box 3-C; 9,28,41). Genetic susceptibility also plays an important role in immunotoxicology and autoimmunity. Because of the strong genetic component and a generally poorer understanding of autoimmunity compared to other immune responses, deciphering the exact role of toxic chemicals in the induction of autoimmunity is difficult.

SUMMARY AND CONCLUSIONS

Scientists have developed a number of tests that assess the various components and processes of the immune system. Pathologic evaluations and certain assays of

Table 3-4-Substances Associated With Autoimmune Responses

Antihypertensive drugs	**Metals**
Hydralazine	**Lithium**
Methyldopa	**Gold**
Anti-arrhythmia drugs	**Mercury**
Procainamide	**Cadmium**
Practolol	**Other substances**
Quinidine	**Penicillamines**
Anticonvulsant drugs	**Chlorpromazine**
Phenytoin	**Propylthiouracil**
Ethosuximide	**Griseofulvin**
Primidone	**Oxyphenisatin**
Antimicrobial drugs	**Vinyl chloride**
Penicillin	**Methylcholanthrene**
Sulfonamides	
Isoniazid	
Nitrofurantoin	

SOURCE: Office of Technology Assessment, 1991; based on P.E. Bigazzi, "Mechanismsof Chemical-induced Autoimmunity," *Immunotoxicologyand Immunopharmacology J.* H. Dean, MI. Luster, A.E. Munson, et al. (eds.) (New York, NY: Raven Press, 1985).

humoral, cell-mediate~ and nonspecific immunity have been validated in one experimental animal, the mouse, and validation efforts are underway for other species. Tests that measure immune cell numbers have advanced more quickly than tests that measure functional immunity, but tests to evaluate specific immune functions are available.

immunotoxicological testing–like all toxicology–presents an investigator with significant challenges. The results of tests on experimental animals often differ depending on the test subject's age, species, or sex. Environmental factors, such as diet or smoking, also affect immune system performance. Choosing the appropriate test dose and the means and duration of exposure can prove difficult when resources are limited and the point of the exercise is to extrapolate from the test to the consequences of human exposure.

Scientists believe that the immune system has a reserve capacity, although the size of that reserve is as yet undetermined. Thus tests that measure impairment of one immune system component may not, in fact, indicate overall immunotoxicity, since other immune components or processes may compensate for the impairment. In several of the studies cited in the preceding text, a chemical produced a discernible decrease in a specific immune function without producing a measurable decrease in host resistance to disease. Many scientists believe that without increased incidence of infection or cancer, there is no evidence of immunotoxicity. There is still much to be

Box3-C-Chemical-InducedAutoimmunity Spanish Toxic Oil Syndrome

Understanding of autoimmune responses to chemical exposure lags far behind hypersensitivity and immunosuppression. Following a poisoning episode in Spain, scientists made one of the few attempts to examine whether an autoimmune mechanism could explain the symptoms experienced after toxic exposure.

In May 1981, an unknown disease affecting approximately 20,000 people was reported in Madrid and northwest of the city. About 3 months after the acute phase of the outbreak, a subpopulation of individuals developed a severe neuromuscular and scleroderma-like syndrome, causing at least 350 deaths. Epidemiologic evidence supported a conclusion that adulterated rapeseed oil, sold as olive oil, was responsible for the disease.

Kammuller, et al., reported that a contaminant, l-phenyl-5-vinyl-2-imidazolidinethione (PVIZT), was isolated in certain case-associated oil samples. Because the chemical structure of PVIZT is closely related to hydantoins and thioureylenes, which can cause autoimmune-like disorders in man, the researchers conclude that PVIZT could account for the syndrome. Patients with toxic oil syndrome presented symptoms similar to those found in known human autoimmune diseases. In addition, the researchers measured immunological changes, including high nonspecific IgE antibody levels, marked eosinophilia, decreased T suppressor cells, and several types of autoantibodies.

SOURCE: Office of Technology Assessment, 1991; based on M.E. Kammuller, N. Bloksma, and W. Seinen, "Chemical-Induced Autoimmune Reactions and Spanish Toxic Oil Syndrome: Focus on Hydantoins and Related Compounds" *Clinical Toxicology 26(3&4):157-174, 1988.*

learned about the long-term consequences of weakening individual components of the immune system.

Immunotoxicologists have identified many substances that have demonstrable immunotoxic effects in laboratory animals, and in a few instances, the effects of these substances have been observed in humans as well. Drugs developed to control graft rejections and cancer definitely suppress the human immune system, and patients receiving these drugs provide good human data on the consequences of prolonged immunosuppression. Occupational experience (see table 3-3) has provided some evidence of substances' inadvertent immunotoxic effects in humans. Accidental exposures to suspected immunotoxicants have, in a few cases, provided the opportunity for gathering human data (see boxes 3-A and 3-B). For the most part, however, data are sparse on the effects of general exposure to immunotoxicants in the environment. The scientific community recognizes that the immune system is an important target organ for toxicity. Most scientists agree that the lack of human test data should not preclude efforts to control human exposures to suspected immunotoxicants, but the absence of data will ensure continued disagreement about suitable means and levels of control.

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