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Effect of Chemical Sensitivity on the Immune System

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Abstract

Immunotoxicology is a science which deals with the effects of physical and chemical agents and other toxic substances on the immune system. The discipline includes detection, occurrence, adverse effects, and mechanisms of chemical-induced immune dysfunction. Many chemicals are known to compromise the immune response of a host. The immune system is extremely vulnerable and sensitive to perturbation by drugs and chemicals. Immunocytes are exposed to chemicals and their metabolites in the circulation, tissues, and organs, as well as in extracellular fluids. Therefore, exposure can be continuous rather than brief, regardless of distribution. The consequences of immune dysfunction may result in increased susceptibility to infectious and neoplastic diseases, or provoke hypersensitivity and autoimmunity. The immunomodulating characteristics of a chemical may be diverse, affecting several components of the immune system, or selectively compromise one particular compartment of the immune response. Many chemicals result in immune dysfunction in animals, but very little information is available to indicate the effects a chemical may have on systemic immunity in humans. A major concern is to identify chemicals that may compromise immune responses at dosages lower than those that produce other signs or symptoms of toxicosis. Several chemicals, following prolonged exposure, alter immune function in animals at low dosages, but the response is transient and recovery is rapid upon removal of the toxicant. In contrast, xenobiotics (excluding drugs) generally will not markedly compromise immune function following a single dose that does not at least incite some other sign or symptom of toxicosis. Therefore, permanent immune dysfunction resulting from chemical exposure would be extremely rare and not expected to occur following a single exposure. A definitive diagnosis for chemical-induced immunomodulation must include several etiologies and body organ systems, since a triad of reciprocal interactions exists between the immune, endocrine, and central nervous systems. Numerous abnormalities of these systems could express immune dysfunction which is completely unrelated to the putative incitive chemical. Finally, although it is dogma that chemicals often modulate immunity in animals, the paucity of information substantiating similar effects in man precludes diagnosing chemical-induced immune dysregulation unless epidemiologic and/or experimental data compiled for humans unequivocally supports and confirms the diagnosis.

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Immune System

The immune system is composed of a delicate network of organs, tissues, cells and cell products that act in unison to protect the living organism against invasion by foreign agents. This complex system, which is dispersed throughout the body of living organisms, regulates body defense mechanisms by monitoring and responding to altered antigenic composition of the body. The regulatory events of the immune system are to: 1)

distinguish, but not react with, normal antigens of the body; 2) identify foreign antigens such as microorganisms and neoplastic cells and successfully destroy and eliminate them from the body; and 3) maintain a delicate balance for optimum performance.

The immune system is regulated by multiple chemical signals operating at different levels. Antigens trigger local chemical signals, which in turn trigger a network of responding cells operating throughout the body. The overall responsiveness of the system is controlled by modification of cellular components and their secretory products, and signals from endocrine glands and the central nervous system. Thymic hormones and corticosteroids in particular have immunoregulatory activities. Disruption of any one of these events may result in immune dysregulation, either suppression or enhancement.

Four major classes of immunocytes are T and B lymphocytes, macrophages, and natural killer cells (NKC). The B cells are primarily the effector cells of humoral immunity (i.e. antibody production), but require the "help" of T cells and macrophages for optimal response to most antigens. The T cells are composed of both effector and regulator subpopulations. The effector T cells can be divided into at least two subsets based on function and phenotype. These are the cytotoxic T cells and T cells that mediate delayed hypersensitivity reactions. Effector T cells act mainly via cell-cell contact, as opposed to production of humoral factors like antibodies, and therefore are responsible for what is termed cell-mediated immunity. The regulatory T cells can be roughly divided into helper (HT) and suppressor (ST) subsets, based on phenotype and regulatory activity. The phenotypic surface molecules for the lymphocytes responsible for HT, mixed lymphocyte reactions, and cutaneous hypersensitivity in mice are Lyt 1^+ (T_4 in humans), while Lyt 23^+ (T_8 in humans) are the surface molecules expressed on ST and cytotoxic T lymphocytes.

Macrophages are also composed of effector and regulator subsets. Macrophages are phagocytic cells which can act directly to ingest, kill, or inactivate foreign agents, or "process" the antigen so it is recognizable to other types of immunocytes as foreign.

Natural killer cells (NKC) are a population of large granular immunocytes which have some characteristics of both lymphocytes and macrophages. The NKC are considered to be an important "first line defense mechanism" (i.e. innate immunity) since, unlike T cells, they react immediately with foreign agents without prior sensitization. The NKC are believed to be especially important in immune surveillance to viruses and neoplastic cells.

In addition to direct effector actions of different types of immunocytes, these cells also produce a variety of immunoregulatory cytokines. Prominent among these cytokines are the lymphokines, interleukin 2 (IL2) and interferons (IFN), and the monokines, interleukin 1 (IL1) and prostaglandins (PGE). These cytokines act on activated effector immunocytes in an interrelated manner to regulate the magnitude of ongoing immune responses. The interleukins are required for clonal proliferation of antigen-activated effector T cells, but also act on B cells and NKC to augment their effector functions. Interferons can act synergistically or antagonistically with interleukins to regulate B cell, T cell, macrophage or NKC response. Interferons are probably best known for their antiviral activity. Prostaglandins are arachidonic acid metabolites produced by activated macrophages (and other cells), and are generally considered to be immunosuppressive. Production of prostaglandins may be one mechanism by which suppressor macrophages operate to down-regulate responsiveness of other immunocytes.

The complexity of the immune system negates the use of any single, currently avail-

able assay to adequately assess overall immune competence. Substantial documentation amply demonstrates that one major arm of the immune system may be impaired or enhanced while other major components remain relatively normal in function. Therefore, a battery of immunoassays is required which are designed to monitor different aspects of the immune system in order to comprehensively assess the immune status of an individual.

Immunotoxicology

Immunotoxicology as a discipline was spawned in the early 1970s and is currently recognized by toxicologists, the medical profession, and governmental agencies who are concerned that chemicals which pollute the environment may dramatically alter immune mechanisms, thereby increasing susceptibility of an individual to infectious and neoplastic agents. Such effects could occur at low levels of exposure in absence of overt toxicity (subclinical) and, thus, remain unrecognized as a contributor in the process of infectious disease and neoplasia. Therefore, it is of considerable public health concern not only to be able to identify accurately those substances that alter immunity, but also to determine exposure levels and mechanisms by which those reactions occur.

Immunotoxicology, as a science, deals with the effects of physical and chemical agents and other toxic substances on the immune system. The discipline includes detection of occurrence, adverse effects, and mechanisms of chemical-induced immune dysfunction. Immunopharmacology parallels and admixes with this discipline, but deals predominantly with the effects of drugs and biological response modifiers on the immune system of living organisms. Both fields are rapidly expanding because of the ubiquitous spread of chemicals in the environment and the widespread use of drugs that can potentially impact the immune responsiveness of human beings.

Chemicals can have a marked effect on the immune system. These effects may be advantageous when chemicals are used therapeutically, or may be detrimental if exposure results from toxic substances that contaminate the environment. Occasionally, a therapeutic agent that may be effective for treatment of selected nonimmune-related disorders or diseases may inadvertently compromise immune function. When this occurs, the side effects may preclude use of the chemical for the intended purpose because an immunosuppressed host may be highly susceptible to infectious agents, carcinogens, or spontaneous neoplasia.

Many chemicals alter the immune response of a host.[1-3] For some chemicals, these effects are insidious, whereas for others they are more tangible and must be considered in the overall action of the compound. Because certain chemicals alter immune function and these effects are frequently subtle or occur at dosages well below desired (drugs) or toxic amounts (toxicants), development of sensitive assays is vital for detection of those alterations.

The immune system is extremely vulnerable and sensitive to perturbation by drugs and chemicals. It is a rapidly proliferating and differentiating target organ system, composed of many cell types regulated by a network of cellular components and soluble factors representing several organ systems. Thus, there is an inordinate amount of interaction with other body systems. In addition, immune cells are exposed to chemicals and their metabolites in the circulation, tissues, and organs, as well as extracellular fluids. Therefore, exposure can be continuous rather than brief, regardless of distribution. Chemicals can react with cell membranes, receptors, or internal structures and interfere at numerous points in the ensuing cascade of immune events. For example,

these reactions could occur at any level, including the macrophage, which process the antigens and secretes IL1, which in turn stimulates activated T cells to produce IL2 necessary for proliferation of HT cells, which augment B cells to differentiate into antibody-secreting plasma cells.

What are the consequences of immune dysfunction? Immunosuppression results in increased susceptibility to infectious and neoplastic diseases, while immunoenhancement can provoke hypersensitivity and autoimmunity. The extent and degree of immunomodulation must be ascertained to predict the impact on the host. Generally, immunomodulation occurs during exposure to the chemical, and once the source is removed, recovery is relatively rapid. However, once sensitized to an immunotoxic dose of a chemical, permanent damage may be expressed by autoimmunity, contact hypersensitivity, or neoplasia. Nevertheless, a single or acute exposure, unless at toxic dosages, is less apt to alter immune function, while prolonged exposure (chronic) is most apt to produce immunotoxicity. However, the responses frequently do not follow a typical dose response pattern, but may be selective for a specific component(s) of the immune network. Thus, immune responsiveness to a given chemical may affect different components of immune function, precluding a typical linear dose response effect. These effects generally revert to normal within days after removal of the test agent.

Interpretation and extrapolation of chemical-induced immune modulation in laboratory animals is frequently questioned. The immune procedures are generally reproducible, quantitative, and highly sensitive to detect immune mediated events. In fact, many chemicals impair immune function at dosages lower than other toxic responses normally delineated by a battery of standard toxicological indices utilized in drug and chemical efficacy testing programs. However, many of these concerns can be dispelled on the premise that the immune systems of animals and man are comparable, that animal models are available for conditions of immune dysfunction, that positive immunosuppressants such as cyclophosphamide and corticosteroids can be used to validate assays (as well as for extrapolation purposes), and finally, that animal data has been verified in man. These criteria emulate the similarities of immunocompetence for both animals and man. Granted, variations of immune responsiveness do occur between various species, but the principles and phenomena are basically similar and comparable.

Priority candidates for drug and chemical immune assessment should be those that present the greatest exposure to humans for prolonged periods of time. These include products used for long-term therapy; those that persist in the environment, particularly those which accumulate in the food chain; and those used during pregnancy, since the developing fetus is extremely vulnerable to exogenous stimuli. An awareness of immunotoxic properties of chemicals could be assessed both during the development of a drug and during experimental trials in man.

Immunopharmacotoxicology has made considerable advances in the past decade. Interest has been generated not only from hazards to human health, but also from the aspect of development of satisfactory chemo-preventive and -therapeutic drugs to treat infectious diseases and cancer. The advent of biotechnology and discovery of biological response modifiers has provided the momentum necessary to perpetuate and propel this discipline into a formidable science.

Immune Sensitivity to Chemicals

Local Hypersensitivity

Allergic responses are a rather common reaction in man following sequential exposures to specific drugs and chemicals. Typical clinical manifestations may involve any organ in the body, but the most prevalent re-

sponses are either pulmonary or topical. It has been estimated that 15% of all adverse drug reactions are due to allergic responses.[4]

Hypersensitivity responses can be classified into four major types. Type I reactions are initiated by interactions of an allergen or antigen with performed antibodies (IgE) and are immediate hypersensitivities (anaphylaxis and atopy) that generally occur within 30 minutes after exposure to the antigen. This reaction is mediated by the immune complex (Ag/Ab) binding to mast cells or basophils which release pharmacologically active substances (histamine, serotonin, etc.) that are responsible for the inflammatory process.

Type II reactions occur when antibodies (IgG) combine directly with an antigenic component of a tissue cell or with an antigen which has become fixed to tissue cells. These complexes in turn fix complement, which is followed by a cascade of activation, which may lead to increased cell permeability and even cell lysis. Certain complement proteins evoke activation of anaphylatoxins, which results in the release of histamine from mast cells and chemotactic attraction of polymorphonuclear leukocytes (PMNs). This reaction occurs within 2 to 6 hours after exposure to the sensitizing agent. The cytotoxic reactions are frequently directed against elements of the blood vascular system and are emulated by autoimmunity.

Type III hypersensitivity reactions result from deposition of antigen-antibody (IgM/IgG) complexes combined with complement in tissue spaces and walls of small blood vessels. These complexes attract PMNs to the reaction site, which release lysosomal enzymes that are responsible for the cell injury and tissue damage. This reaction (Arthus) generally occurs 2 to 6 hours following exposure to the sensitizing antigen and is typified by serum sickness and chronic membranous glomerulonephritis.

Type IV allergic reactions, or delayed-type hypersensitivity (DTH), are differentially separated from the other three types of hypersensitivities by a delayed onset (12 to 48 hours) of the reaction. This reaction is characteristic of cell-mediated immunity and is elicited by lymphocytes in the absence of antibody. Antibody-mediated reactions are a manifestation of Types I, II, and III. The DTH reaction is characterized by erythema, edema, and histologically by a predominance of a mononuclear cell infiltrate (lymphocytes and monocytes). Delayed-type hypersensitivity is typical of graft vs. host reaction and contact hypersensitivity. This portion of the review will be concerned primarily with Type IV hypersensitivity as a local response to drug and chemical insult.

All contact sensitivity-inducing substances have at least two common features. They have a low molecular weight which permits diffusion through intact skin and, upon percutaneous absorption, they combine with amino acid side chains of proteins to form conjugates that are generally non-antigenic themselves. Contact hypersensitivity results when the sensitizing hapten-carrier conjugates form locally in the skin.

Contact hypersensitivity is regarded as a localized skin reactivity which is not life-threatening. Numerous chemicals are known to be highly allergenic for contact sensitivity and result in various degrees of discomfort. Nevertheless, susceptibility to contact irritants is not indicative of systemic immune responsiveness, and thus the main emphasis of this report will be directed at chemical sensitivity of the systemic immune response, which includes DTH responses.

Recent data, derived from rodent studies, implies that DTH reactions are biphasic, involving both classical immediate and delayed hypersensitivity, and that both are mast cell-dependent.[5-7] A cascade of immune events occurs upon introduction of antigen. Sensitized T cells (Ly-1$^+$) secrete a soluble factor that activates mast cells to release serotonin, which augments local vascular permeability. Consequent to this early event, a second T

cell (Ly-1$^+$) population enters the reaction site and interacts with the antigen, which elicits a late response responsible for the immunocytes and PMN infiltrate. In contrast, others[8] dispute the hypothesis that mast cell products are required for leukocyte emigration or swelling associated with DTH. Their contradictory results reveal that mast cell–deficient mice expressed DTH reactions of equal intensity or in excess of that of heterozygous litter-mate controls. Additional investigations will be necessary to definitively ascertain the mechanistic events which precipitate and perpetuate DTH reactions.

DTH responses are controlled by accessory (macrophages) cells and a complex ST cell circuit.[9,10] Macrophages secrete numerous monokines such as IL1, IFN and PGEs, which can affect proliferation and differentiation of all T cell subsets. Therefore, classical DTH reactions encompass an intricate network of cells and their secretory products which regulate the intensity of the DTH effector T lymphocytes. Drugs and chemicals may alter the magnitude of response at several points in this pathway.

Systemic Sensitivity

The intact immune system provides protection of the host against invasion by foreign organisms and neoplastic agents. Dysregulation of the system may suppress or enhance immune responses which may be beneficial (destroy microorganisms, neoplastic cells, etc.) or detrimental (autoimmunity, hypersensitivity) to the host. In the past decade, it has been well documented that certain environmental chemicals, at relatively low dosages, do adversely affect normal function of the mammalian immune system[1-3] Until recently, the impact and implications of these reactions in man have not been fully realized or appreciated. However, attention has been directed at attaining information concerning the health hazards of many xenobiotics that are ubiquitous in the environment. In addition, a particular concern is that a small percentage of the human population may be innately hypersensitive to chemicals. This article will review chemical-induced immunomodulation and discuss some of the ramifications associated with this phenomenon.

A myriad of assays are available by which to assess the integrity of the immune system both *in vivo* and *in vitro*. A consensus of most immunotoxicologists is that a panel of immunoassays should include, as a minimum, assessment of humoral immunity, cell-mediated immunity (CMI), macrophage function, pathotoxicologic examination of lymphoid tissues, and host resistance to an infectious or oncogenic agent. These assays have more recently been expanded to include natural killer cell (NKC) and production/activity of numerous regulatory cytokines. Most of these procedures are quantitative and inclusive, in order to formulate a complete immunotoxic profile for a specified drug or chemical. Therefore, a battery of immunoassays is available in order to comprehensively assess the immune status of an animal.

Humoral immunity is characterized by B lymphocytes, which differentiate into plasma cells that synthesize and secrete antibody into the circulation. Antibody can be easily enumerated by several acceptable techniques, particularly by two commonly used procedures, radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA), that are quantitative and possess an extremely high degree of sensitivity. Table I lists several chemicals that are known to significantly ($p \leq 0.05$) alter antibody synthesis in animals.[1-3,11-17] These agents represent several classes of chemicals and compromise immunity in several species of animals. Many of the effects occurred at dosages lower than those that notably alter other standard

Table I
Chemicals Known to Alter Antibody Synthesis in Animals

Chemical	Species	Effect[1]
Lead	Mouse, rat, rabbit	D
Cadmium	Mouse, rat, rabbit	D
Mercury	Mouse, rabbit	D
Selenium	Mouse, rat	I,D
Nickel	Rat	D
Tetrachlorodibenzodioxin	Mouse, guinea pig	D
Tetrachlorodibenzofuran	Mouse	D
Pentachlorophenol	Mouse, rat	D
Polychlorinated biphenyl	Mouse, rat, monkey, rabbit, guinea pig	D
Polybrominated biphenyl	Mouse	D
Benzo-a-pyrene	Mouse	D
Dimethylbenz(a)anthracene	Mouse	D
Methylcholanthrene	Mouse	D
Dimethyl vinyl chloride	Mouse	D
Urethane	Mouse	D
T phorbol acetate	Mouse	D
Lindane	Rat	D
Toxaphene	Mouse, rat	D
Monochloramine	Rat	D
Chlorine dioxide	Rat	D
Ethyl nitrosourea	Rat	D
2,4 dichlorophenol	Rat	I
Tetrachlorobiphenyl	Mouse	D
Diethylnitrosamine	Mouse	D
Zinc	Mouse	D

[1] I = increase, D = decrease.

Table II
Chemicals Known to Alter Delayed-type Hypersensitivity in Animals

Chemical	Species	Effect[1]
Lead	Mouse, rat	D
Selenium	Rat	D
Tetrachlorodibenzodioxin	Mouse, guinea pig, rat	D
Tetrachlorodibenzofuran	Guinea pig	D
Polychlorinated biphenyl	Rabbit	D
Methylcholanthrene	Mouse	D
Carbofuran	Rabbit	D
2,4 Dichlorophenol	Rat	D
Sodium hypochloride	Rat	D
Diethylnitrosamine	Mouse	I
Triphenyltin	Rat	D
Dialkyltin	Mouse	D

[1] I = increase, D = decrease.

toxicological indices such as weight loss, altered serum chemistries, or impaired mixed function oxidase activity. The ease of assessing humoral immunity has contributed to the numerous chemicals tested (positive) for altered antibody synthesis, more so than for other immune parameters. It is noteworthy that most chemicals suppress humoral immune responses. The humoral immune response could be particularly sensitive to perturbation by chemicals, since this system is regulated by other components (T lymphocytes and macrophages) of the immune network.

Drugs and chemicals tend to inhibit the primary immune response more so than the secondary response. The anamnestic response is more resilient to respond to repeated chemical insult. The stages of the immune response also differ markedly in their susceptibility to immunomodulators. Suppressive agents are most effective when used before antigenic challenge or during the induction phase of the immune response.[18] Further, many drugs and chemicals express a differential toxicity for B and T lymphocytes, i.e., cyclophosphamide produces a proportionately greater reduction in B than T cells. Subsequent to discontinuance of exposure, the modulating properties of these agents are generally of brief duration and transient. These factors must be considered to fully appreciate the immune dysregulatory activity of drugs and chemicals.

Cell-mediated immunity (CMI) is expressed by T cells composed both of effector and regulator subpopulations. The effector T cells are derived from two distinct lineages and are divided into subsets based on function and phenotype. These include cytotoxic T lymphocytes (CTL), cells that mediate delayed-type hypersensitivity (DTH), and

Table III
Chemicals Known to Alter Macrophage Phagocytic Activity in Animals

Chemical	Species	Effect[1]
Lead	Mouse, rat	I,D
Cadmium	Mouse	I,D
Mercury	Mouse	N
Nickel	Mouse, rat, rabbit	D
Pentachlorophenol	Mouse, rat	I
T phorbol acetate	Mouse	I
Toxaphene	Mouse	D

[1] I = increase, D = decrease.

Table IV
Chemicals Known to Alter Natural Killer Cell Activity in Animals

Chemical	Species	Effect[1]
Polychlorinated biphenyl	Rat	D
Chlorine dioxide	Rat	I
Selenium	Rat	I
Ethylnitrosourea	Rat	D
Diethylnitrosamine	Mouse	I
Methylcholanthrene	Mouse	D
Manganese	Mouse	I
Nickel	Mouse	D

[1] I = increase, D = decrease.

those active in mixed lymphocyte reactions (MLR). The regulatory T cells consist of helper (HT) and suppressor (ST) subsets. These cells synthesize and secrete soluble products (lymphokines) which mediate their activity.

The test accepted by immunotoxicologists as most representative of CMI is various modifications of DTH. These techniques have been modified and adapted to a particular species and have proven to be quite sensitive as well as quantitative to assess chemical-mediated dysfunction of CMI.

Table II lists several chemicals known to significantly ($p \leq 0.05$) alter DTH in animals.[1-3,11-17] The majority of the agents depress CMI, which would suggest that the host would be more receptive to invasion by mycobacteria, fungi, viruses and other organisms that replicate intracellularity, as well as carcinogens that are immunoresponsive.

Macrophages are phagocytic cells which process antigens. The phagocytic index has not proven to be a satisfactory method to assess immunotoxicity of xenobiotics. This procedure appears to be relatively insensitive, since macrophages can compensate for considerable insult before marked effects in phagocytosis can be detected. It is noteworthy that some chemicals can either stimulate or depress phagocytic activity, depending on the amount and time or dose of the chemical (Table III).[1-3,11-17] Monokines appear to be a much more sensitive indicator of macrophage function.

Natural killer cells (NKC) do not require prior antigen sensitization to be activated and thus are considered as an important first line defense mechanism (innate immunity). These cells are of particular importance in initial immune surveillance to virus-infected and neoplastic cells.

The NKC procedure has recently been incorporated in immunotoxicity assessment and has proven to be an extremely sensitive procedure to detect chemical-induced immunomodulation. Table IV lists some chemicals known to significantly ($p \leq 0.05$) alter NKC activity in animals.[17] Carcinogens or promoters of neoplasia tend to decrease NKC, while other chemicals, such as selenium, an inhibitor of promotion, amplify the NKC response. This quantitative procedure appears to have great promise for immunopharmacology/toxicology efficacy testing programs.

T lymphocytes and macrophages produce a variety of immunoregulatory cytokines such as interleukin 1 (IL1), interleukin 2 (IL2), interferons (IFN), and prostaglandins (PGE). These cytokines act in concert to regulate the magnitude of ongoing immune responses. The interleukins and interferons stimulate immune response, while prostaglandins, which are produced primarily by activated macrophages, generally "down-

Table V
Chemicals Known to Alter Cytokine Production/Activity in Animals

Chemical	Species	Cytokine	Effect[1]
Lead	Rat	Interleukin 2	D
Selenium	Rat	Prostaglandin E_2	D
Manganese	Mouse	Interferon	I
Polychlorinated biphenyl	Rat	Interleukin 2	D
Diethylnitrosamine	Rat	Prostaglandin E_2	D
Chlorine dioxide	Rat	Interleukin 2	I
Chlorine dioxide	Rat	Prostaglandin E_2	I
Sodium hypochlorite	Rat	Prostaglandin E_2	I
Monochloramine	Rat	Prostaglandin E_2	I

[1] I = increase, D = decrease.

Table VI
Immune Components of Animals Affected by Selected Chemicals

Chemical	Lymphocyte		Macrophage	Natural Killer Cell
	B	T		
Polychlorinated biphenyls	D[1]	D	D	D
Pentachlorophenol	D	D	I	—
Toxaphene	D	NE	D	—
Selenium	I/D	D	I/D	I
2,4 Dichlorophenol	I	D	NE	—
Dialykyltin	NE	D	NE	—

[1] I = increase, D = decrease, NE = no effect, — = not done.

regulate" immune response. An extremely small quantity (picogram) of cytokines will provoke a reaction which makes these "immunohormones" particularly sensitive and desirable for assessment of immunotoxicants. These indices have been utilized in our laboratory and have provided a better understanding of the mechanisms by which chemicals compromise immunity.

A few chemicals known to significantly ($p \geq 0.05$) alter cytokine production/activity in animals are listed in Table V.[17,19,22,23] Generally, the cytokine data directly correlates with effector cell activity. Further investigations will delineate and confirm the application of cytokines for immunopharmacological/toxicological testing purposes.

These data support the philosophy that non-toxic dosages of chemicals result in immune dysfunction. Frequently, these effects are selective for specific components of the immune network. Table VI depicts the selectivity certain chemicals express. For instance, polychlorinated biphenyls (aroclor 1254) suppress virtually all aspects of the immune system, but are relatively weak inhibitors of CMI.[1,3,17] Pentachlorophenol will depress humoral immunity (HI) and CMI, while actually augmenting macrophage activity.[15] Toxaphene spares CMI, but impairs both HI and the macrophage.[13] Selenium may actually stimulate HI and the macrophage with moderate excess dosages,[2] but larger dosages will suppress HI, CMI, and the macrophage but enhance NKC activity.[23] 2,4 dichlorophenol will potentiate HI but reduce CMI responses while sparing the macrophage.[24] Dialkyltin is selective in depressing CMI in the absence of affecting the other major arms of immunity.[25] These examples illustrate that the immunotoxic profiles of chemicals differ even within a given class, thereby prohibiting the prediction of the immunotoxic characteristics for similar agents.

Table VII
Chemicals Known to Modulate Immunity in Humans

Chemical	Parameter	Effect[1]
Polychlorinated biphenyl	IgA, IgM	D
	Membrane receptors	D
	DTH	D
	Active T and HT cells	D
Polybrominated biphenyl	Mitogens	D
	T lymphocytes	D
Lead	Resistance to infectious agents	D
	IgA	D
Nickel	IgA, IgM, IgG	I
Cobalt	IgA	I
Zinc (deficiency)	Response T-dependent antigens	D
Selenium (deficiency)	Cytocidal capacity PMN	D

[1] I = increase, D = decrease.

Chemical Sensitivity in Humans (Systemic)

A paucity of information, scattered in the literature, indicates that chemicals elicit immune dysfunction in humans. The accidental exposure of humans to PCBs in Yusho, Japan, and western Taiwan have permitted scientists to assess the immunotoxic properties of this compound over an extended period. Patients exposed to PCB had significant decreases in serum IgA and IgM, a deficiency of membrane receptors on monocytes and PMNs, depressed DTH responsiveness, and reduced percentages of total T, active T, and HT cells[26] (Table VII). Suppressor T cells were unaffected.

The effects of lead on bacterial and viral infections in humans have never been adequately studied, but there is evidence to suggest that human host resistance may be lowered by lead. Children with persistently high blood lead levels who were infected with *Shigella enteris* had prolonged diarrhea.[27] In addition, lead workers with blood lead levels of 22–89 μg/dl have been reported to manifest more colds and influenza than non-lead exposed individuals.[28] This study also indicated that secretory IgA levels were suppressed significantly in lead workers with a medium blood lead level of 55 μg/dl. Secretory IgA is a major factor in immune defense against respiratory as well as gastrointestinal infections. Epidemiological investigations could ascertain if lead alters the immune system of man and consequently increases susceptibility to infectious agents and neoplasia.

In 1973, a commercial preparation of polybrominated biphenyl (PBB) was inadvertently substituted as a supplement of dairy cattle feed and was distributed throughout Michigan. Contaminated dairy products were consumed for the ensuing 5 years, which resulted in exposure of a substantial percent of the Michigan population, particularly to the dairy producers' families. Immune dysfunction in humans has been associated with PBB exposure.[29,30] The immune dysregulation certainly is not definitive but rather suggestive, since a small percentage of the dairy farm residents exhibited a decrease in the percent of absolute number of T lymphocytes, had a concomitant increase of lymphocytes without detectable membrane surface markers, and showed reduced response to antigen stimulation.

Serum immunoglobulin concentrations have been assessed in individuals exposed to nickel or cobalt. A significant increase in IgM, IgG and IgA levels was present in workers exposed to nickel, while those exposed to cobalt had elevated serum IgA.[31] In

addition, both nickel sulfate and mercuric chloride possess mitogenic properties and stimulate blastogenesis of lymphocytes.[32] A deficiency of some essential elements will contribute to immune dysfunction. For instance, zinc deficiency results in atrophy of the thymus and decreased capacity to respond to many T-dependent antigens.[33] Granulocytes from selenium-deficient individuals had a lower cytocidal capacity than those from selenium-supplemented recipients.[34] A considerable amount of epidemiological and experimental data must be obtained for humans to actually confirm or refute the data compiled from animals as to the relevance of chemical-induced immune dysregulation in man.

It is obvious that chemicals can compromise immune function. In retrospect, can altered immunity provoke other syndromes that may be somewhat dissociated with immune function, as has been envisioned in the past half-century? For example, the immune system was considered to be somewhat autonomous in regulation and action until the recent discoveries that there is, indeed, a regulatory interaction between the nervous, endocrine, and immune systems. Convincing evidence has been presented which suggests that a significant reciprocal interaction occurs between these three systems.[35-38] For instance, some classical neuroendocrine hormones and neurotransmitters possess immunomodulating activity, while immunocytokine hormones have been discovered to affect the central nervous system and function as endocrine glands.[37,38] These interregulatory patterns confirm the existence of a nervous-endocrine-immune axis, which further illustrates the complexity and sophistication of immunity.

The regulatory role of the central nervous system on immunity is best exemplified by the ability of stressful situations, including emotional stress, to compromise immune responses. For instance, death of a spouse can impair immune responsiveness for up to 6 weeks.[39] In animals, a loud noise, electric shock, infant-mother separation, and overcrowding can result in marked suppression of the immune response.[40] Individuals who are separated, divorced, or widowed are susceptible to immunological dysfunction which is thought to predispose these subjects to cancer.[41] Since severe emotional and mental dysfunction can be accompanied by immunological abnormalities, these factors must be carefully considered in a definitive diagnosis of chemical-induced immune dysregulation. The putative causes of immune modulation, particularly in humans, are quite diverse and, therefore, must at least include emotional distress as a contributing factor.

In summary, the fact that certain drugs and chemicals alter immunity is dogma. Many drugs are purposely developed and manufactured for their immunomodulatory characteristics and value for chemoprevention and -therapy. A major concern, however, is the immunomodulating properties of drugs used for non-immunological purposes, as well as chemicals that are ubiquitous in the environment and enter the food chain, or may present potential hazards to human health by other routes of exposure. Since the immune system is extremely complex and sophisticated, many drugs and chemicals frequently compromise immune function at dosages much lower than those which produce other signs or symptoms of toxicosis. These features, whether suppressive or stimulatory, may be detrimental to the host and expressed by several types of immunologically related syndromes. Several chemicals, generally during prolonged exposure, alter immune function at low dosages, but the response is transient and the subject rapidly recovers upon discontinuance of exposure to the chemical. Contrary to multiple exposure, a single exposure to an immunotoxicant (non-drug) rarely results in immune dysfunction at dosages that do not incite

other toxicologic signs or symptoms. There is no known chemical where exposure occurs in this capacity that produces permanent immune dysregulation. Finally, the interregulatory network and circuit consisting of the immune, endocrine, and central nervous systems suggests that, particularly in man, abnormalities of these two systems also could contribute to immune dysfunction. Therefore, a definitive diagnosis of chemical-induced immune dysregulation is inclusive of several etiologies and body systems which must be adequately assessed before a diagnosis incriminating a chemical can be confirmed. Additional information must be compiled for man to implicate and equivocate chemical perturbation of immune reactivity in humans.

References

1. Vos JG: Immune suppression as related to toxicology. CRC Critical Rev Toxicology 5:67-101, 1977.
2. Koller LD: Effects of environmental chemicals on the immune system. Adv Vet Sci Comp Med 23:267-295, 1979.
3. Dean JH, Luster MI, Boormana GA: Immunotoxicology. In: Research monographs in immunology. Immunopharmacology. (Sirois P, Pleszczynski MR, eds). Elsevier Biomedical Press, Amsterdam, Vol 4, pp 349-398, 1982.
4. Parke AC: Clinical manifestations of drug-induced syndromes. In: Immunotoxicology. Gibson GG, Hubbard R, Parke DV, eds, Academic Press, London, pp 27-40, 1983.
5. Askenase PW, Van Loveren H: Delayed-type hypersensitivity: Activation of mast cells by antigen-specific T-cell factors initiates the cascade of cellular interactions. Immunol Today 4:259-264, 1983.
6. Van Loveren H, Askenase PW: Delayed-type hypersensitivity is mediated by a sequence of two different T cell activities. J Immunol 133:2397-2401,1984.
7. Van Loveren H, Kato K, Meade R, et al: Characterization of two different LY-1$^+$ cell populations that mediate delayed-type hypersensitivity. J Immunol 133:2402-2411, 1984.
8. Galli SJ, Hammel I: Unequivocal delayed hypersensitivity in mast cell deficient and beige mice. Science 226:710-713, 1984.
9. Claman HN, Miller SD, Sy MS, et al: Suppressive mechanisms involving sensitization and tolerance in contact allergy. Immunol Rev 50:105-132, 1980.
10. Knop J, Malorny U, Macher E: Selective induction of delayed hypersensitivity T-effector and T-suppressor lymphocytes *in vitro* by haptenized bone marrow-derived macrophages. Cell Immunol 88:411-420, 1984.
11. Kerkvliet NI: Halogenated aromatic hydrocarbons as immunotoxicants. In: Chemical regulation of immunity in veterinary medicine, Alan R. Liss, Inc., New York, NY, pp 369-387, 1984.
12. Exon JH: The immunotoxicity of selected environmental chemicals, pesticides and heavy metals. In: Chemical regulation of immunity in veterinary medicine, Alan R. Liss, Inc., New York, NY, pp 355-368, 1984.
13. Allen AC, Koller LD, Pollock GA: Effect of toxaphene exposure on immune responses in mice. J Toxicol Environ Health 11:61-69, 1982.
14. Koller LD, Exon JH, Moore SA, et al: Evaluation of ELISA for detecting *in vivo* chemical immunomodulation. J Toxicol Environ Health 11:15-22, 1982.
15. Exon JH, Koller LD: Immunotoxicity of pentachlorophenol in rats and mice. J Toxicol Environ Health (Submitted for Publication).
16. Exon JH, Koller LD: Effects of chlorinated phenols on immunity in rats. Inter J Immunopharmacol 5:131-136, 1983.
17. Exon JH, Koller LD, Talcott PA: Effect of lead, polychlorinated biphenyls and cyclophosphamide on rat natural killer cells, interleukin 2, and antibody synthesis. Fund Appl Toxicol 5:158-164, 1985.
18. Fudenberg HH, Stites DP, Caldwell JC, et al: Immunosuppression and immunopotentiation. In: Basic and clinical immunology, 3rd ed., Lange Med Publishers, Los Altos, CA, pp 313-326, 1980.
19. Exon JH, Koller LD, Bercz PJ: Effect of chlorine-based drinking water disinfectants on immune responses of rats. Toxicol Appl Pharmacol (Submitted for Publication).
20. Talcott PA, Exon JH, Koller LD: Alteration of natural killer cell-mediated cytotoxicity in rats treated with selenium, diethylnitrosamine and ethylnitrosomine. Cancer Lett 23:313-322, 1984.
21. Smialowicz RJ, Rogers RR, Riddle MW, et al: Manganese chloride enhances murine cell-mediated cytotoxicity: Effects on natural killer cells. J Immunopharmacol 6:1-23, 1984.
22. Smialowicz RJ, Rogers RR, Riddle MW, et al: Immunologic effects of nickel. Environ Res 36:56-66, 1985.
23. Koller LD, Exon JH, Talcott PA, et al: Immune responses in rats supplemented with selenium. Clin Exp Immunol (Submitted for Publication).
24. Exon JH, Henningsen GM, Osborne CA, Koller LD: Toxicologic, pathologic and immunotoxic effects of 2,4-dichlorophenol in rats. J Toxicol Environ Health 14:723-730, 1984.
25. Seinen W: Immunotoxicity of alkytin compounds. In: Immunologic considerations in toxicology, Vol. I, Shurman RP, ed, CRC Press, Inc., Boca Raton, FL, 1981, pp 103-119.
26. Lui Y-C, Wong P-N: Dermatological, medical and laboratory findings of patients in Taiwan and their treatments. Am J Ind Med 5:81-115, 1984.
27. Sachs HK: Intercurrent infection in lead poisoning. Am J Dis Child 132:315-316, 1978.
28. Ewers U, Stiller-Winkler R, Idel H: Serum immunoglobulin, complement C3, and salivary IgA levels in lead

workers. Environ Res 29:351-357, 1982.
29. Bekesi JG, Holland JF, Anderson HA, et al: Lymphocyte function of Michigan dairy farmers exposed to polybrominated biphenyls. Science 199:1207-1209, 1978.
30. Bekesi JG, Roboz JP, Solomon S, et al: Altered immune function in Michigan residents exposed to polybrominated biphenyls. In: Immunotoxicology, Gibson GG, Hubbard R, Parke DV, eds, Academic Press, Inc., London, pp. 181-191, 1983.
31. Bencko V, Wagner V, Wagnerova M, Reichrtova E: Immunobiochemical finds in groups of individuals occupationally and nonoccupationally exposed to emissions containing nickel and cobalt. J Hyg Epid Micro Immunol 27:387-394, 1983.
32. Nordland K, Henze A: Stimulating effect of mercuric chloride and nickel sulfate on DNA synthesis of thymocytes and peripheral blood lymphocytes in children. Inter Arch Aller Appl Immunol 73:162-165, 1984.
33. Chandra RK, Dayton DH: Trace element regulation of immunity and infection. Nutr Res 2:721-733, 1982.
34. Arvilommi H, Poikonen K, Jokinea I, et al: Selenium and immune functions in humans. Infect Immun 41:185-189, 1983.
35. Besedovsky H, del Rey A, Sarkin E: Immunoregulation by neuroendocrine mechanisms. In: Neuroimmunology, Behan P, Spreafico F, eds, Raven Press, New York, pp 445-450, 1984.
36. Martin JB: Neuroendocrine regulation of the immune response. In: Neuroimmunology, Behan P, Spreafico F, eds, Raven Press, New York, pp 433-443, 1984.
37. Blulock JE: Relationships between neuroendocrine hormones and lymphokines. Lymphokines 9:1-13, 1984.
38. Blalock JE: The immune system as a sensory organ. J Immunol 132:1067-1070, 1984.
39. Bartrop RW, Lazarus L, Luckhurst E, et al: Depressed lymphocyte function after bereavement. Lancet 1:834-836, 1977.
40. Landenslager ML, Ryan SM, Drugan RC, et al: Coping and immunosuppression: Inescapable but no escapable shock suppresses lymphocyte proliferation. Science 221:568-570, 1983.
41. Sklar CS, Anisman H: Stress and cancer. Psyc Bull 89:369-406, 1981.