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Chemical Agents and the Immune Response

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Our desire to understand the potential adverse human health effects of environmental chemical exposure has coincided with an increased understanding of the immune system and an appreciation of its complex regulatory network. This has spawned a broad interest in the area of immunotoxicology within the scientific community as well as certain concerns in the public sector regarding chemical-induced hypersensitivity and immunosuppression. The incidence of alleged human sensitization to chemicals has increased, in part, due to the fact that chemical companies are moving to larger and/or different markets. It has been estimated that 35 million Americans suffer from allergic disease, of which 2–5% are from occupational exposure. Although there is not yet a clear understanding of dose-response relationships or disease predisposition, there are many well-defined examples (isocyanates, anhydrides) of chemical sensitizers in humans and experimental animals. Evidence that chemicals suppress immune responses in humans is considerably less well established, although there is a public perception that chemicals generally cause immunosuppression. This perception has been fueled by highly publicized legal cases and scientific controversies within the academic and industrial communities. As a consequence of these public and scientific concerns, many of the regulatory agencies are developing immunotoxicity testing guidelines. At the present, however, there are limitations on adequate human methodology and data that allow the extrapolation of animal data to assess human risk. The potential for human immunosuppression remains of concern, however, because of a large database generated from animal studies that demonstrates immunosuppression as well as reports of immunosuppression in humans inadvertently (e.g., halogenated aromatic hydrocarbons) or occupationally (asbestos, benzene) exposed to xenobiotics. This concern is exacerbated by current knowledge regarding the long-term consequences of immunosuppression that may be associated with pathologic conditions (e.g., cancer, increased infections). Likewise, exposure to immunotoxic xenobiotics may represent additional risk to individuals with already fragile immune systems (e.g., malnutrition, infancy, old age). In another light, there has been considerable public concern regarding "chemical hypersensitivity syndrome" (also referred to as "multiple chemical sensitivities syndrome") and its relationship to hypersensitivity as well as immunosuppression. Although there exists a substantial population who claim to have this disorder, the syndrome is not well understood. Several mediators have been proposed including disorders of immune regulation as well as conditioned responses to odors involving pharmacologic and/or psychologic mechanisms. At present, there is no definitive evidence that these syndromes are immunologically mediated.

Introduction

Under David Rall's leadership and inspiration, the National Institute of Environmental Health Sciences (NIEHS) was one of the first institutions to initiate major research in the area of immunotoxicology. Since its inception, NIEHS has maintained international prominence in this area through extramural funding, sponsorship in numerous programs and conferences, and through active support of intramural research. It is a privilege to contribute to this special *EHP* volume in honor of David Rall's retirement as Director of NIEHS. In this paper, we briefly review some of the present issues confronting immunotoxicology and describe recent research results from our laboratory.

As evidenced by recent documents prepared by the Office of Technology Assessment (*1*) and the National Research Council (*2*) focusing on immunotoxicology, there has been growing interest and concern within the scientific and public communities on the capacity of certain chemical agents to perturb normal immune processes. The types of effects shown to occur are often chemical-specific as well as species-specific and include immunosuppression, targeting either systemic or local immunity (e.g., lung or skin), hypersensitivity disease, manifested as respiratory tract allergies or contact dermatitis, and in certain instances autoimmunity. In addition to environmental pollutants, agents of concern have included certain therapeutics, consumer products, and biologicals (e.g., the therapeutic use of recombinant materials). More recently, interest has also focused on such diverse materials as silicone implants and pollutants common to the indoor environment. The latter include both chemical agents and bioaerosols such as viruses, bacteria, fungi, algae, and protozoa that have the potential to act as either sensitizing agents or mediators of infectious disease.

Systemic Immunity

An issue that remains controversial in immunotoxicology, and is likely to remain so in the near future, is the question of whether chemical agents can suppress the immune response within the

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general population and, if so, if this can result in clinical disease. Such diseases would most likely be manifested as an increase in the frequency or severity of infections and increased incidences of certain cancers such as Kaposi's sarcoma or non-Hodgkin's lymphoma, malignancies often observed in immunosuppressed individuals. This concern is fueled by a number of clinical and experimental observations. First, although the association between primary immunodeficiency diseases and the incidence of recurrent infections and neoplastic diseases is well recognized, a similar association to these diseases has been recognized with the chronic low-level use of immunosuppressive agents (*3*), which presumably induce moderate levels of immunosuppression. For example, in one study, 30% of cardiac transplant patients treated with cyclosporin developed pulmonary infections within the first year after surgery (*4*), whereas in another study, 50% of renal transplant patients on immunosuppressive therapy were found to develop cancer within 10 years after surgery (*5*).

Second, there is an increasing number of reports describing various immune changes in individuals who have been inadvertently or occupationally exposed to chemical agents. These range from unconfirmed reports with putative compounds such as trichloroethylene and methyl isocyanate to more substantiated studies with polychlorinated biphenyls (PCBs), asbestos, and silica (*2*). As may be expected, it is considerably more difficult to identify subtle changes in immune function after inadvertent or occupational exposure compared to the severe immune dysfunction that occurs in HIV infection or primary immunodeficiency diseases. Several difficulties encountered include a lack of sensitive assays that usually accompany routine clinical assessment, problems in identifying recently exposed, well-defined cohorts, and considerable immunologic variability that exists in the general population.

The third observation, which has fueled interest in chemical-induced immunosuppression, stems from *in vitro* and *in vivo* experimental studies suggesting that many environmental chemicals can inhibit the immune system and alter host resistance to infectious agents or tumor cells [see reviews by Luster et al. (*6*) and Dean and Murray (*7*)]. Additional credence is ascribed to such studies because the immune system of laboratory animals, including rodents, is remarkably similar to that of humans with respect to organization, function, and responsiveness. However, the relevance of the dose levels employed in some of these studies, compared to likely human exposure, is of considerable concern. Immunotoxic compounds identified from such studies, some of which have been reported to produce effects in humans. include certain halogenated aromatic hydrocarbons, organic solvents, heavy metals, mycotoxins, oxidant gases, and abused drugs (i.e., alcohol, cocaine) (Table 1).

A study of particular interest, recently conducted through Health and Welfare, Canada, indicated that monkeys chronically exposed to low levels of a PCB mixture, representative of that found in the environment, develop immunosuppression primarily characterized by decreased CD4:CD8 ratios and antibody responses (*8*). Furthermore, these effects were selective for the immune system in that other toxicities (e.g., liver, reproductive, etc.) were not observed. A major concern was that immunosuppression was prevalent in offspring exposed perinatally. That the developing immune system is highly susceptible to chemical injury has been suggested in a number of experimental studies with halogenated aromatic hydrocarbons, as reviewed by Vos and Luster (*9*). Interestingly, the types of immunological effects seen in PCB-exposed primates were similar to those reported to occur in individuals from Taiwan and Japan who were exposed to rice oil inadvertently contaminated with PCBs and dibenzofurans (*10*). As a possible consequence of this immunosuppression, some of these individuals also developed increased incidences of sinopulmonary infections (*1*).

Table 1. Xenobiotics reported to inhibit immune function and decrease host resistance.[a]

Class	Examples
Polyhalogenated aromatic hydrocarbons	TCDD, PCB, PBB
Metals	Lead, cadmium, arsenic
Aromatic hydrocarbons	Benzene, toluene
Polycyclic aromatic hydrocarbons	DMBA, BaP, MCA
Pesticides	Trimethyl phosphorothioate carbofuran, chlordane
Organotins	Dibutyltin chloride
Aromatic amines	Benzidine, acetylaminofluorene
Oxidant gases	NO_2, O_3, SO_2
Particulates	Asbestos, silica, beryllium
Mycotoxins	T-2, ochratoxin
Drugs	Cyclosporin, methotrexate, diphenylhydantoin
Abused drugs	Cocaine, alcohol, marijuana

Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PCB, polychlorinated bipenyls; PBB, polybrominated biphenyls; DMBA, dimethylbenzanthracene; BaP, benzo[*a*]pyrene; MCA, methylcholanthrene; T-2, trichothecene mycotoxin.

Immunotoxicity of AIDS Therapeutics

Preclinical assessment of imune function is important for many therapeutics, and in particular AIDS therapeutics, because HIV-infected patients represent an already immunologically compromised population. Recognizing this, the National Institute of Health AIDS program has taken an active interest in assessing the immunotoxicological effects of a variety of anti-AIDS drugs. Certain nucleoside analogs are potent inhibitors of HIV and have been used with some benefits in AIDS patients. The dose-limiting hematological toxicities of nucleoside analogs include macrocytic anemia and granulocytopenia for 3′-azido-3′-deoxythymidine (AZT) (*12*) and neutropenia and thrombocytopenia for 2′,3′-dideoxycytidine (ddC) (*13*). In rodent studies it was reported that hematotoxicity could be detected within 5 days after initiation of treatment and was reversible after cessation of treatment (*14*). These observations correlate with experimental studies showing drug-induced inhibition of bone marrow progenitor cells after *in vitro* or *in vivo* exposure (*15,16*). A number of nucleoside analogs also produce immunotoxicity after *in vivo* treatment in mice. For example. 2′,3′-dideoxyadenosine (ddA) and 2′,3′-dideoxyinosine (ddI) have been shown to suppress the antibody plaque-forming cell response to sheep red blood cell immunization (*17*). In recent studies, the nucleoside analog, 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T) produced a pattern of myelotoxicity that was similar to AZT in that erythroid progenitors were more sensitive to drug treatment than myeloid progenitors (*18*). Similar hematotoxicities have been observed in clinical trials with humans manifested by anemia after administration of d4T.

In addition to therapeutics that inhibit HIV replication, a number of treatments are being used that target one or more of the opportunistic infections that occur in AIDS patients. Penta-

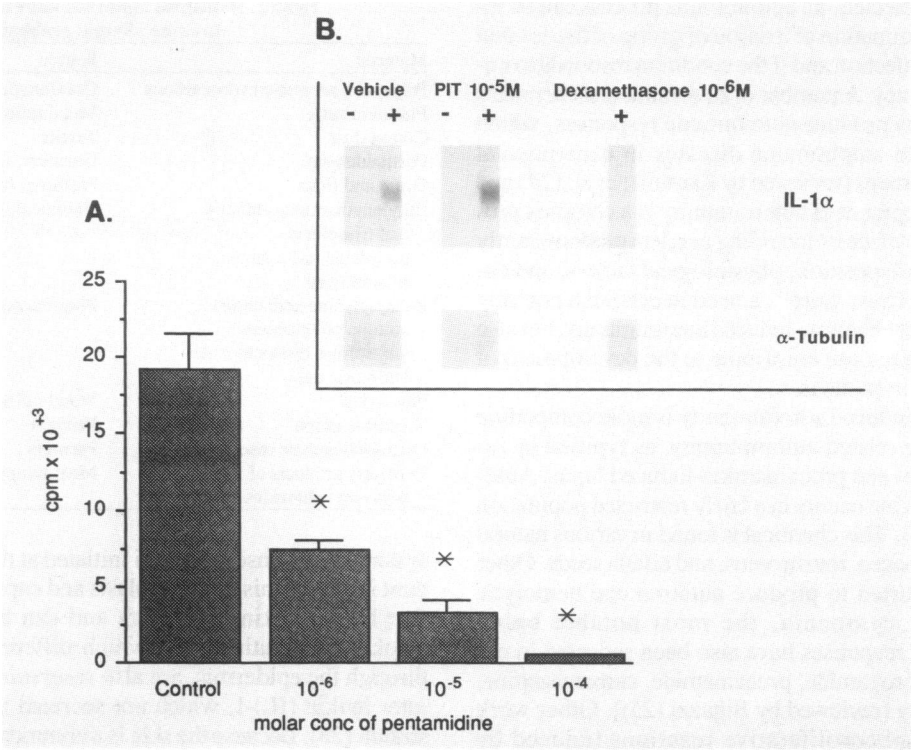

FIGURE 1. (*A*) Interleukin-1 (IL-1) release from pentamidine-exposed macrophages as measured by the thymocyte co-culture assay. Data are represented as mean counts per minute ± SE of supernatants derived from quadruplicate cultures of alveolar macrophages exposed to vehicle or pentamidine. (*B*) Northern blot analysis of IL-1a mRNA. For determination of IL-1a mRNA levels, alveolar macrophages were preincubated in media with or without 10 μM pentamidine or 1 μM dexamethasone and subsequently stimulated for 3 hr with lipopolysaccharide (1 μg/mL).

midine (4,4′-diaminodiphenoxy-pentane) isethionate, widely used since the 1930s as an antiprotozoal agent, is often used against AIDS-related *Pneumocystis carinii* pneumonia (PCP). PCP is a frequent cause of morbidity and mortality in immunocompromised patients and afflicts more than 60% of patients with AIDS. Although a high risk of toxicity occurs with parenteral administration of pentamidine, administration via inhalation has been successfully used for the treatment and prophylaxis of PCP in AIDS patients (*19*). The exact mechanism of action for pentamidine on *P. carinii* pneumonia is unclear, although impairment of oxidative phosphorylation, nucleic acid synthesis, glucose metabolism, and dihydrofolate reductase have all been suggested to contribute to its efficacy.

Alveolar macrophages are the first immune cell to contact aerosolized pentamidine. Considering the critical role of macrophages in antimicrobial activity, we have studied the immunomodulatory effects of pentamidine on alveolar macrophages and have found that the secretion but not the transcription of the inflammatory cytokines, interleukin 1 (IL-1) and tumor necrosis factor (TNF) (*20*), are inhibited by pentamidine at pharmacological doses (Fig. 1). Although the precise physiologic role for these cytokines in the lower lung has not been established, it has been suggested that reduced production of these mediators contribute to defective cellular responses, while overproduction enhances local tissue damage. For instance, TNF displays antiviral activity and a protective role in protozoan infections, whereas it is implicated in inflammatory tissue destruction and granuloma formation (*21*). Interestingly, alveolar macrophages recovered from AIDS patients with PCP, but not in AIDS patients without the infection, exhibit markedly increased spontaneous production of TNF. Thus, the efficacy of pentamidine against PCP may in part be mediated by its ability to inhibit TNF release from bystander cells in the lung.

Interferon-α (IFN-α), an endogenous protein with antiviral activity, has also been used successfully in HIV-infected individuals. The effectiveness of IFN-α in AIDS appears to be due to its pleiotropic effects on target cells and processes integral to the disease. For example, although antiviral activity by IFN-α has been observed when used alone or in conjunction with AZT (*22*), it is also effective against the growth of Kaposi's sarcoma, a common neoplastic disease in AIDS patients. Interferon-α acts directly against tumor cells as well as indirectly through stimulating NK cells. However, IFN-α also possesses immunotoxic properties in that it inhibits T- and B-lymphocyte proliferative ability as well as stem cell differentiation in the bone marrow (*23*).

Autoimmunity

Autoimmune diseases are those in which an individual's own immune system attacks one or more tissues or organs, resulting in functional impairment, inflammation, and sometimes permanent tissue damage. Usually these diseases are associated with the loss of immune tolerance to self-antigens and demonstrable immune responses to one or more tissue antigens. When no spe-

cific antigen can be detected, an autoimmune process can be inferred if there is inflammation of a tissue or group of tissues that show no evidence of infection and if the condition responds to immunosuppressive therapy. A number of environmental chemicals and therapeutic agents produce autoimmune responses, which in some cases lead to autoimmune diseases in experimental animal models and humans [reviewed by Kamuller et al. (*24*) and Bigarzi (*25*)]. Development of autoimmunity is a complex process that involves many factors including gender (predominantly females), genetic predisposition, physiological factors, and environmental factors. Thus, there is a need to establish not only the prevalence of direct chemical-induced autoimmunity, but also whether chemical agents can contribute to the development of autoimmune disease in humans.

Evidence for drug-induced autoimmunity is more compelling than environmentally related autoimmunity, as typified by reports of penicillamine- and procainamide-induced lupus. Autoimmunity from hydrazine occurs in a fairly restricted population (i.e., slow acetylators). This chemical is found in various natural products including tobacco, mushrooms, and alfalfa seeds. Other drugs have been reported to produce autoimmune hemolytic anemia or thrombocytopenia, the most notable being methyldopa. Similar responses have also been reported in patients receiving chlorpropamide, procainamide, carbamazepine, and interferon therapy [reviewed by Bigazzi (*25*)]. Other work has focused on lymphoproliferative reactions induced by diphenylhydantoin, the development of thyroid antibodies in patients receiving lithium, and drug-induced hepatitis in association with halothane, nitrofurantoin, or isoniazid treatment. Evidence of autoimmunity induced by environmental chemicals, particularly in humans, is limited. Occupational or inadvertent exposure to vinyl chloride and/or quartz has been linked to a disorder resembling scleroderma. This disorder, like idiopathic scleroderma, shows a prevalence for individuals with the HLA-DR5 haplotype. Certain metals, such as mercury, cause immune-complex glomerulonephritis in humans (*26*), although the extent of mercury-induced autoimmune disease in humans is unknown. It is well established that low levels of mercury administered to susceptible strains of mice and rats result in immune-complex glomerulonephritis and nuclear autoantibodies. An autoimmunlike disorder, referred to as toxic oil syndrome (TOS), has also been reported in a large population of Spanish residents who inadvertently ingested aniline-adulterated rapeseed oil (*24*). The symptoms associated with TOS reveal similarities to those observed in some humans receiving hydantoin-related compounds.

Local Immunity in the Skin

The skin is not merely an inert barrier that physically prevents entry of foreign materials, but possesses biologically active systems and products, including immunological systems, that function as an effective defense system. The immune system of the skin, called "skin-associated lymphoid tissue" (SALT) by Streilein (*27*), contains multiple cell types that participate in immune-mediated processes. These include Langerhans cells, which possess antigen-presenting capabilities, and dendritic T-cells, which retain helper and suppressor function. Additionally, immune cells and active components (e.g., antibody–antigen complexes) are readily recruited to the skin from the circulatory system in response to stimuli initiated at the skin surface. Abundant in the dermis are fibroblasts and capillary endothelial cells that bear cytokine receptors and can be induced to secrete cytokines. Keratinocytes, which differentiate as they ascend through the epidermis, are also reservoirs of cytokines such as inter leukin (IL)-1, which are secreted in response to various stimuli (*28*). Because the skin is a common site of exposure to environmental agents, it is not surprising that SALT is a common target. Depending on the chemical agent and the dose, toxicity can be manifested as either contact hypersensitivity, inflammation, or immunosuppression.

Table 2. Industrial materials known or presumed to cause allergic problems.

Material	Source
Polycyclic aromatic hydrocarbons	Combination of fossil fuels
Platinum salts	Metal refining
Cotton dust	Textile
Formaldehyde	Garment, laboratory
Grain and flour	Farming, baking, mill operating
Ethylenediamine, phthalic and trimellitic anhydride, toluene diisocyanate	Chemical, plastic, rubber
Phenylglycine acid chloride, sulfone chloramides, amprolium hydrochloride antibiotic dust	Pharmaceutical
Wood dust	Wood mills, carpenters
Vegetable gums	Printers
Organophosphate insecticides	Farmers
Pyrolysis products of polyvinyl chlorides	Meat wrappers

Contact hypersensitivity reactions in the skin are common, affecting literally millions of Americans. The incidence associated with environmental or occupational exposure is unknown, but has been estimated to be approximately 5–10% of all cases. The events associated with induction and elicitation of chemical-induced hypersensitivity reaction have been intensely investigated. It is thought that Langerhans cells initially interact with antigen in the skin and transport it to the draining lymph nodes where the antigen is presented to immunocompetent T-lymphocytes in context with class II antigens. This initiates an immune response whereby subsequent exposure to the antigen can evoke elicitation in sensitized individuals. This involves the interaction of processed antigen with sensitized lymphocytes. The sensitized lymphocytes are transformed into lymphoblasts, which proliferate and secrete various biologically active products including antibodies (e.g., IgE) and lymphokines. These products, either directly or indirectly, are responsible for the generation of inflammatory mediators such as chemoattractants, adhesion molecules, and pharmacological mediators.

A number of chemical agents have the capacity to produce contact hypersensitivity, a widely recognized environmental and occupational problem [reviewed by Menne (*29*)]. A partial list of compounds known or presumed to produce allergic responses is shown in Table 2. The characteristic that sets allergic responses apart from immune mechanisms involved in host defense is that the reaction is excessive and often leads to tissue damage. Chemical-induced hypersensitivities fall into two categories distinguished not only mechanistically but temporally: delayed-type hypersensitivity, a cell-mediated response that occurs within 24–48 hr after challenge, and immediate hypersensitivity, which

is mediated by immunoglobin, most commonly IgE, and manifests within minutes after exposure to an allergen. The type of immediate hypersensitivity response elicited (i.e., anaphylatic, cytotoxic, Arthus or immune complex) depends on the interaction of the sensitizing antigen or structurally related compound with antibody. In contrast, delayed-type hypersensitivity responses are characterized by T-lymphocytes, bearing antigen-specific receptors, which, on contact with cell-associated antigen, respond by secreting cytokines. Metals such as beryllium, mercury, cobalt, nickel, platinum, chromium, and gold can induce a spectrum of hypersensitivity responses, from delayed-onset to immediate. Nickel is considered a medium-to- strong contact sensitizer in humans and has been used as a "gold standard" in development of new assays for assessing hypersensitivity.

In addition to contact hypersensitivity responses, some chemicals and environmental agents can alter the normal processes associated with SALT and in certain instances suppress systemic immunity in laboratory animals. In the former, for example, the disappearance or functional loss of Langerhans cells is associated with dermal exposure to ultraviolet light, particularly UV-B radiation (*30*), or certain chemical agents such as dimethylbenzanthracene (*31*), lanthanides (*32*), pentamidine (*33*), or phorbol esters (*34*). Subsequently, the ability to elicit contact hypersensitivity with known sensitizers is lost when the skin is pretreated with these agents. On the other hand, some compounds, such as those with antioxidant activity, exacerbate contact hypersensitivity in mice after dermal exposure. This is associated with an increase in Langerhans cell function as evidenced by increases in Ia antigen density on the cell surface (*35*). Systemic immunosuppression may occur in experimental animals at doses of UV–B higher than that which suppresses local immunity (*36*). This is characterized by an inability to respond to sensitizers when applied to unirradiated as well as irradiated sites and a decrease in delayed hypersensitivity responses. The mechanism(s) responsible for these effects are unclear, but may depend on the induction of antigen-specific suppressor T-lymphocytes and bioactive products released from UV-damaged keratinocytes. An association between skin cancers and immunosuppression by UV-B has been established in laboratory animals where UV-induced immunosuppression affects the rejection of UV-induced skin tumors (*37*). UV-B radiation may also affect SALT in humans, as suggested by the observation that UV exposure inhibited the subsequent ability to induce contact hypersensitivity at the irradiated site in 40% of normal subjects and in 90% of patients with sunlight-induced skin cancer (*38*).

Chemical agents may also produce local inflammatory responses in the skin through nonspecific mechanisms. The keratinocyte, which represents the vast majority of cells that compose the epidermis (> 95%), is the primary source of immuno-active cytokines. It has been postulated that the release of these mediators in response to various stimuli orchestrate many of the immunological and inflammatory responses that occur in the skin after exposure to dermatotoxins (*39*). In this respect, the pathogenesis of psoriasis, a chronic skin disease characterized by excessive keratinocyte proliferation and inflammatory cell infiltrates in psoriatic plaques, is closely associated with altered regulation of keratinocyte-produced cytokines (*40*) and may serve to characterize chemical-induced dermatotoxicity. The mechanisms and events by which these processes occur are currently major areas of research. Cytokines known or presumed to be products of keratinocytes include IL-1α and β, 1L-3, IL-6, IL-8, granulocytic macrophage colony-stimulating factor, tumor growth factor-α and β, and TNF-α. Based upon several lines of *in vitro* and *in vivo* evidence, a cytokine network theory has been proposed (*39*). In this theory, environmental stimuli, which includes contact allergens, ultraviolet light, or certain dermatotoxic chemicals, can act directly on keratinocytes, resulting in the release of IL-1 and TNF-α, as well as the expression of ICAM-1, an adhesion ligand for lymphocytes. The secretion of IL-1 and TNF-α leads to the expression of surface leukocyte adhesion molecules (e.g., VCAM-1) as well as the release of keratinocyte-derived IL-8, a potent attractant for T-lymphocytes and polymorphonuclear leukocytes. Tumor necrosis factor-α and/or IL-1 may also stimulate keratinocytes in an autocrine fashion. When the initial environmental stimulant is antigenic, such as in the case with $NISO_4$, the response involves increased apposition of mononuclear cells with subsequent involvement of sensitized T-cells, which increases the intensity and perpetuation of the response.

Local Immunity in the Lung

The lung represents one of the principal portals of entry for many chemical agents. To deal with the influx of such foreign material, the lung has evolved a complex immunological framework of cells and mediators. Inhaled microbes or antigens may be phagocytized in the conducting airways by macrophages or may be processed for subsequent presentation by dendritic cells. The lung also contains significant lymphoid tissue in the form of lymph nodes positioned in the mediastinum and hilar areas of the lungs as well as interstitially in parenchymal tissue. Additionally, the luminal surface secretions contain significant amounts of immunoglobulin, which is predominantly of the IgA class.

Lung immunity can be a target of airborne chemicals as well as function as a pulmonary defense mechanism against chemical-induced cell injury. Because of their strategic location within the lung, alveolar macrophages provide the initial defense system for the body against inhaled toxicants, and numerous compounds have been shown to modulate its activity (*41*). Additional immunologic defenses operating in the lung include humoral immunity and cell-mediated immunity derived from bronchial-associated lymphoid tissue as well as an interstitial lymphocyte compartment within lung tissue. In particular, lung-associated NK activity has been shown to be either up- or downregulated by a number of agents including IFN-α (*42*) and phosgene (*43*). Overall suppression of such systems can predispose the host to infectious agents or tumor development, as has been shown to occur for pollutant gases such as ozone, a photochemical oxidant resulting from atmospheric reactions of hydrocarbons and nitrogen oxides catalyzed by sunlight. In experimental animals, ozone decreases pulmonary resistance to bacterial challenge including Streptococcus sp., *Pasteurella haemolytica,* and *Mycobacterium tuberculosis* (*44*). This increased susceptibility may relate to impairment of alveolar macrophage functions, although recent studies have suggested that T- and B-cell functions may also be impaired after ozone inhalation. Inhalation of asbestos modulates pulmonary and systemic immunity. Asbestos exposure in humans is also associated with respiratory diseases including asbestosis, fibrosis, malignant mesothelioma, and bronchogenic carcinoma. Alterations in cellular and humoral

immune responses often accompany these conditions. Impairments in cell-mediated immunity in humans by asbestos is characterized by decreases in delayed hypersensitivity responses, the numbers of circulating T cells, and T-cell proliferation. In contrast, patients with asbestosis present hyperactive T-cell responses, often manifested by increased levels of serum immunoglobulins and secretory IgA.

Acute hypersensitivity disease or pulmonary allergic responses represent one of the major sources of immunotoxicological complaints. Pulmonary hypersensitivity, allergic alveolitis, and asthma are syndromes with very complex etiologic backgrounds. It is estimated that more than 10 million persons in the United States suffer from asthma. In the general population, asthma prevalence rates increased 29% from 1980 to 1987, and, during the same time period, asthma-associated mortality increased 31% (2891 mortalities in 1980 versus 4360 in 1987) (*45*). Asthma is characterized by airway hyperresponsiveness, which can be manifested by an exaggerated bronchoconstriction response to many physical changes (e.g., cold air) as well as chemical and pharmacological agents. Although numerous factors may airway contribute to the development of hyperesponsiveness, clinical and experimental evidence suggests, that altered lung immunity is a major factor. Morphological studies have shown that bronchial infiltration of inflammatory cells occurs in severe disease as well as mild episodes. Similarly, there is evidence to suggest that altered cellular responses and increased levels of inflammatory mediators are associated with asthma and airway hypersresponsiveness. While there appears to be a genetic component to the pathogenesis of asthma, numerous environmental factors may also be involved, including allergens, cigarette smoke, and viral agents. Airborne pollutants, including ozone sulfur dioxide, and nitric oxide, have also been cited to contribute to the pathogenesis or exacerbation of asthma. A number of studies have shown an association between ambient concentrations of these pollutants and increased hospital admissions for asthma or increased asthma symptoms (*46*). However, the immunological changes produced by these compounds that predispose the host to pulmonary hypersensitivity remains to be determined.

In addition to environmental exposure, occupational factors are thought to contribute to 2% of all asthma cases. Trimellitic anhydride, which reacts as a hapten, as well as chromium, acid anhydrides, ethanolamines, complex amines, and plicatic acid, are all industrial products capable of inducing asthmatic conditions or sequela in humans (Table 2). One of the most studied chemicals regarding its potential to induce lung hypersensitivity is toluene diisocyanate (TDI), a classical representative of the isocyanate compounds used in the production of plastics. In industrial workers, TDI exposure can cause asthma (5–10% of all exposed workers) as well as contact dermatitis. Characteristics of TDI, shared by many chemical agents that induce hypersensitivity, are a low molecular weight (500–1000) and high reactivity to macromolecules, particularly proteins. Studies in guinea pigs demonstrated that a threshold concentration is required for pulmonary sensitization.

Beryllium, a metal used in the manufacture of a variety of industrial components, has long been associated with pulmonary hypersensitivity including acute pneumonitis and chronic pulmonary granulomatous disease (berylliosis) (*47*). The relationship between beryllium exposure and induction of cell-mediated immunity is well established with T lymphocyte blastogenesis in the presence of beryllium, serving as a diagnostic tool for berylliosis.

Conclusions and Future Direction

The immune system is composed of several cell populations whose maturation is subject to orderly control by endogenous hormones and/or exogenous bacterial products. These mediators possess activation, growth promotion, or differentiation properties and are under the influence of potent and well-defined regulators. From studies in rodents and limited observations in humans, it is apparent that a number of environmental and chemical agents can adversely affect the immune systems, resulting in either immunosuppression, hypersensitivity, or autoimmune disease. These examples and our current knowledge about the pathogenesis of disease support the possibility that chemical-induced damage to the immune system may be associated with a wide spectrum of diverse pathological conditions, some of which may become detectable only after a long latency. Likewise, exposure to chemical agents might represent an additional risk to individuals with already fragile immune systems (e.g., malnutrition, infancy, old age).

The value of incorporating immunological experimental data for the toxicological assessment of drugs, chemicals, and biologicals for human risk assessment has been increasingly accepted. For example, in addition to previously established test guidelines proposed by the Environmental Protection Agency (EPA) for hypersensitivity testing, the EPA (*48*) and the Food and Drug Administration (*49*) have recently discussed the benefits of testing the immunosuppressive potential of biochemical pest control agents and antiviral drugs, respectively. Furthermore, EPA has established reference doses (Rf or NOAEL/SF) using immunotoxicity data for several compounds including 1,1,2-trichloroethane, 2,4-dichlorophenol, and dibutyltinoxide, and the Agency for Topic Substances and Disease Registry has derived, "minimum risk levels" for arsenic, dieldrin, nickel, 1,2-dichloroethane, and 2,4-dichlorophenol from immune endpoints (M. Selgrade, personal communication). The preceding decade of research has provided a database of immunotoxic and nonimmunotoxic compounds, a better understanding of the mechanisms responsible for hypersensitivity disease, studies correlating immune dysfunction and altered host resistance, and more predictive methods for detecting immunomodulatory chemicals. Future research is needed to *a*) further refine and validate immune function tests and host resistance assays, particularly in the rat as well as tests for autoimmunity; *b*) establish better test methods to evaluate the effects of chemical exposure on lung and skin immunity; *c*) develop and evaluate *in vitro* methodology for detecting chemical-induced immunotoxicity; *d*) develop and implement a testing battery to examine immune changes in humans occupationally or environmentally exposed to chemicals shown to be immunotoxic in laboratory animals; and *e*) establish appropriate mathematical models to allow for extrapolating experimental studies.

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