Immunotoxicology

Introductory article

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Immunotoxicology can be defined as the study of adverse effects on the immune system resulting from exposure to chemicals (including drugs), biological materials and, in certain instances, physiological factors, collectively referred to as agents. It encompasses studies of various immune pathologies associated with exposure of humans and wildlife species, including allergy, immune dysregulation (suppression or enhancement), autoimmunity and, in some instances, chronic inflammatory diseases.

Introduction

Although adverse effects by chemicals on the ability of the host to respond to infectious disease have been suggested for many centuries, the merger of immunology and toxicology into a fledgling discipline occurred only in the mid-1970s following the publication of several reviews and international symposia on the subject. Like other subdisciplines in toxicology, the impetus for this type of work originated primarily from public and regulatory concerns that certain agents may adversely affect the immune system in humans. Not surprisingly, much of the earlier efforts in this area were conducted either by regulatory agencies or private industries involved in the manufacture of chemicals or drugs, and were devoted to developing and applying sensitive and predictive tests to identify immunotoxic compounds. Data generated from these studies were ultimately used in the safety or risk assessment process. This remains a major focus for the discipline, although our understanding of the molecular events involved in toxicokinetics and in mounting appropriate immune responses provides opportunities to develop more streamlined and informative tests which can be used to identify genetically susceptible populations and sensitive individuals. To consider immunotoxicology solely as an adjunct to risk assessment, however, would be incorrect. Both basic and applied research in this area are now conducted, which have significant health implications for human populations, as well as domestic animal and wildlife populations. Regarding the latter, for example, both the Atlantic bottled-nose dolphin and Baltic harbour seal populations have been found to be highly susceptible to immunosuppression by polychlorinated biphenyls (PCBs). It is well known that these species concentrate PCBs through the food chain and their populations have been significantly reduced, presumably from infectious disease, postulated to stem from PCB-induced immunotoxicity. PCBs are members of the halogenated aromatic hydrocarbon (HAH) class of chemicals which includes the prototype, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin), the most toxic synthetic chemical known.

In humans, immunotoxicity can be manifested as one of several distinct immunopathologies which include allergic disease, immunodeficiency and autoimmunity. In the former case, the immune system responds to the agent as an allergen of low (hapten) or high molecular weight, resulting in allergic contact dermatitis, respiratory hypersensitivity or food hypersensitivity. In immunodeficiency, the immune system acts as a passive target for the agent and the results may be increased incidence or severity of infectious disease or neoplasia. Autoimmunity, a breakdown in self-tolerance, occurs when an agent directly or indirectly induces an immune response to autologous constituents that result in pathological consequences. Examples of immunotoxic agents, classified by the type of pathology produced, are presented in **Table 1**. Recently, studies in immunotoxicology have also included investigations into the role of inflammatory mediators, particularly cytokines, as these mediators are often produced by or act upon immune cells, and many xenobiotic agents induce chronic inflammatory responses in the target organ. While most studies in immunotoxicology deal with direct effects on the immune system, increasing attention has been devoted to indirect effects by which an agent acts on another organ or system, which then impacts adversely on the immune system. In this respect, there are a number of agents that can affect the immune system indirectly by altering the central nervous system and endocrine function. The most notable of these are drugs of abuse, including the opiates and the cannabinoids, which impute their immunotoxicity from the ability to stimulate release of adrenocorticotrophic hormone and corticosteroids.

Table 1 Examples of agents reported to cause immunotoxicity in humans or experimental animals

Inhibitors of immune function
Polyhalogenated aromatic hydrocarbons (dioxins, polychlorinated biphenyls)
Polycyclic aromatics (benzo[*a*]pyrene)
Pharmaceuticals (cytoreductives, antibiotics, nucleoside analogues)
Aromatic amines (benzidine, acetylaminofluorene)
Metals (lead, cadmium, mercury, arsenic)
Radiation (ionizing, ultraviolet B)
Abused substances (alcohol, opiates, cannabinoids)
Pesticides (chlordane, malathion, hexachlorocyclohexane)
Implicated in autoimmunity
Organic solvents (polyvinyl chloride, trichloroethylene)
Industrial chemicals (silica, poly(brominated/chlorinated) biphenyls)
Antibiotics (β-lactams, penicillin, sulfonamides, rifampicin)
Antidiabetics (chlorpropamide, tolbutamide)
Analgesics (acetaminophen, ibuprofen, phenacetin)
Anticonvulsants (phenytoin, carbamazepine)
Miscellaneous (gold salts, diphenylhydantoin, digitoxin)
Immediate respiratory sensitizers
Anhydrides (maleic, phthalic, trimellitic)
Proteins (latex, alcalase letic)
Dyes (reactive black, rifafix yellow, red BBN)
Isocyanates (toluene diisocyanate, diphenylmethane diisocyanate, hexamethylene)
Pharmaceuticals (sulfone, pancreatic extracts, antibiotic dusts)
Animal products from (laboratory animals, mites, mealworms, pigeons)
Wood dusts (western red cedar, California redwood, African maple)
Metals (platinum salts, nickel)
Examples of photoallergic contact dermatitis
Antimicrobial (chlorosalicylanilide, hexachlorophene, fenticlor)
Fragrances (musk ambrette, methylcoumarin)
Sunscreens (*p*-aminobenzoic acid, oxybenzones)
Pharmaceuticals (sulfanilamide, chlorpromazine, promethazine)
Plant derivatives (balsam of Peru, lichen mixture)
Examples of contact allergens
Mercaptobenzathiazole
p-Phenylenediamine
Formaldehyde
Epoxy resin
Black rubber (PPD mix)
Nickel sulfate

Hypersensitivity

Hypersensitivity, a common manifestation of immunotoxicity, falls into two broad categories that are distinguished from each other both mechanistically and temporally. These include immediate hypersensitivity, which is mediated usually by immunoglobulin (Ig) E, and appears within minutes following challenge to an allergen, and delayed-type hypersensitivity, a cell-mediated response, which occurs within 24–48 h following challenge. Hypersensitivity responses are induced by a variety of compounds, including naturally occurring proteins found in foods, animal dander, pollens, metals, pharmacological agents, such as β-lactam antibiotics, as well as industrial materials (**Table 1**). The response can be manifested in the gastrointestinal tract (food allergy), skin (allergic contact dermatitis) or respiratory tract (rhinitis, asthma). A number of compounds, such as latex proteins, are capable of producing both dermal and respiratory hypersensitivity. The most studied class of occupational respiratory allergens is isocyanates, represented by toluene diisocyanate (TDI), a low molecular weight chemical used in the production of polyurethanes. TDI is the leading cause of occupational asthma in the Western world. However, it is difficult to diagnose and control, in part because IgE antibodies cannot always be found and the determinants of exposure have not been well defined.

Tests to identify potential respiratory sensitizers are difficult to undertake and, as such, efforts to validate screening models are limited. Although the guinea-pig has some significant immunological differences compared with humans (e.g. IgG1 versus IgE reaginic antibodies), it has proved to be a predictable animal model for humans given the limited comparative data available, and can be used to test for both low and high molecular weight sensitizers. As with guinea-pig skin tests, the method includes both a sensitization and a challenge phase, the latter usually by aerosol. There is also a need to measure both immediate and delayed-onset responses, although this does not distinguish between nonspecific pulmonary hyperreactivity and specific immune responses. The latter is commonly established by examining sera for the presence of reaginic antibodies. Rat and mouse models using raised serum IgE levels as a biomarker for respiratory sensitization hold early promise, but have yet to be validated for screening purposes.

The guinea-pig is also used to test for potential skin sensitizers, and guinea-pig assays are included in many regulatory test guidelines. Although variations in guinea-pig hypersensitivity assays exist, animals are usually exposed topically or intradermally to the test agent and challenged with the same agent 10–14 days later. Because guinea-pigs are large, several graded doses of antigen may be tested simultaneously and an entire dose–response curve can be generated by comparing skin reactions in individual animals. However, guinea-pigs are expensive to

purchase as well as to maintain, there are few inbred strains, and immunological reagents are not widely available. As such, efforts are presently underway to replace guinea-pig assays with the mouse local lymph node assay (LLNA). This procedure determines proliferation in cells from the lymph nodes that drain the site of chemical application. Compared with guinea-pig assays, the mouse LLNA is considerably less complex and time consuming. This procedure can also be modified to allow examination of potential photoallergens (**Table 1**). However, the LLNA may fail to detect weak sensitizers and may provide false-positive results for strong irritants.

A large database exists for chemicals that have been tested for skin sensitization in experimental animals and humans. Results from these studies have been incorporated into computer models which use chemical structure and physicochemical properties, such as absorption through the skin and potential to react with skin-associated proteins, to derive structure–activity relationships or structural alerts for skin sensitization. Predictive testing for contact hypersensitivity may be conducted in humans, and is similar to diagnostic testing using multiple occluded patches. Because of the risk of sensitization from test articles, however, use of human volunteers is generally limited to confirmation of negative results. As respiratory challenge may provoke systemic anaphylactic reactions in sensitized individuals, predictive testing for respiratory sensitization is not recommended in human subjects.

Immune Regulation

Altered immune regulation (i.e. suppression or stimulation of the local or systemic immune responses) may represent a potentially more significant human health hazard than hypersensitivity and, as such, has been afforded considerable attention. Initially, studies were focused on employing *in vitro* or rodent models to identify agents that could potentially suppress the immune system. Results collected from these studies were often used in hazard identification. Subsequently, studies were conducted to develop more predictive test methods, to determine mechanisms of action, and to identify human populations that may have been adversely affected. One of the most studied classes of environmental chemicals, from the standpoint of human health effects as well as basic laboratory research, are the HAHs, which include PCBs and TCDD, the latter representing the prototype of this chemical class. TCDD inhibits antibody responses in laboratory mice at doses of less than 1 microgram per kilogram bodyweight (μg per kg bodyweight), a level that many believe is close to the exposure level that occurs in humans living in industrialized countries. Epidemiological studies support, but do not confirm, that subtle immunological effects can occur in humans, particularly neonates, exposed to low levels of these chemicals. Although the exact mechanisms by which HAHs exert immunotoxicity are unknown, these compounds mediate their effects through the aryl hydrocarbon receptor, a basic helix–loop–helix, ligand-dependent transcription factor, which translocates to the nucleus and interacts with 'dioxin-responsive elements' on deoxyribonucleic acid (DNA) which regulate gene expression. Some of the genes that are affected regulate tyrosine kinases which are responsible for B-cell differentiation. Recently, TCDD has been demonstrated to downregulate the expression of the class II molecule on the major histocompatibility complex gene and to modulate fas–fas ligand systems on lymphocytes which is thought to regulate apoptosis of autoreactive immune cells.

Although immunological changes have been reported in human and wildlife populations exposed to dioxins and PCBs, the clinical significance of these changes is not clear. One view states that the reported immune changes are not of sufficient magnitude to be considered relevant biologically and is supported by lack of evidence that exposed populations show increased frequencies of immune-associated diseases, such as infections. The opposing view states that even small changes in the immune system can translate to increased frequency or severity of disease, but the ability to discern this is dependent on the size of the population exposed and how closely individuals are monitored. This would imply that, although the immune system may possess redundancy, it does not possess significant reserve. The latter hypothesis is supported by mathematical models derived from experimental databases. Databases derived from human studies are not available to confirm these quantitative relationships. A recent epidemiological study exemplifies the debate: a dose–response relationship was observed between internal exposure to TCDD or PCBs and changes in the number of lymphocyte phenotypes, particularly cluster of differentiation (CD) 3+ CD8+ T cells, in infants exposed via contaminants in breast milk. Supporting these clinical observations, similar findings were reported previously in mice and nonhuman primates receiving chronic, low-level dioxin exposure. The infants, however, showed no evidence of increased disease, and their phenotype values, which show coefficient of variation as high as 30% in normal populations, fell within background levels. Presumably, improved disease monitoring and use of standardized methods will help establish the clinical significance of such observations.

Incorporating experimental animal data on toxicant-induced immune alterations as part of the evaluation for drugs and chemicals in human risk assessment has become increasingly common. Because of the complexity of the immune system, the initial strategies devised to detect immunotoxic agents were to select and apply a tiered panel of assays. The first tier is usually a general screen for immunotoxicity and, depending on the testing agency, may or may not include functional tests such as the measure-

ment of antibody responses after *in vivo* antigenic challenge or the natural killer cell assay, as well as nonfunctional parameters (e.g. lymphoid organ histology and serum immunoglobulin levels). The second tier usually consists of tests that identify specific target cells and examines alterations in resistance to infectious agents or neoplastic disease. The qualitative and quantitative relationships that exist used between immune and host resistance tests have been evaluated using a database of over 50 chemicals from environmental, industrial and pharmacological sources. These studies have shown that assessment of only a minimal number of appropriate tests may be needed to predict immunotoxicity successfully in rodents. In particular, assessment of humoral immunity, via quantitation of antigen-specific T-dependent antibody responses, has been shown to be the best single indicator to determine the potential for a compound to induce alterations in immune function. These studies also found that linear relationships often existed between immune function and host resistance models, suggesting that even small immunological changes may have clinical significance.

Autoimmunity

Environmental influences, as well as viral, genetic and hormonal factors, play a role in the development of autoimmune diseases. In humans, the most well known class of agents involved in autoimmune diseases are pharmaceuticals, such as penicillamine gold salts (**Table 1**). Drug-induced autoimmune diseases, however, differ from their idiopathic counterparts in that the disease remits in the absence of the drug and does not present the same clinical or immunological spectrum. Physical factors in the environment, such as ionizing radiation, have been associated with the induction of autoimmune diseases. In addition, clinical reports and experimental studies have suggested that occupational or environmental exposure to certain chemical agents can produce autoimmune responses or autoimmune-like diseases in humans and laboratory animals (**Table 1**). For example, a strong association exists between exposure to halogenated hydrocarbons and the development of glomerulonephritis, including the presence of antibodies to glomerular basement membrane. Oral ingestion of certain food or food contaminants has also been associated with autoimmune phenomena. The most notable example occurred in Spain when, in 1981, adulterated rapeseed oil was sold as cooking oil. The disease, referred to as 'toxic oil syndrome', was manifested as vasculitis, sicca syndrome and indurated thickened skin, and was attributed to an aniline derivative present in the oil. Autoimmunity occurs when the mechanisms of tolerance break down. Chemicals and drugs associated with autoimmunity may influence the process by various mechanisms. For example, halothane, cadmium and methimazole can interact with host molecules to form self determinants for which there is no tolerance. Other chemicals, such as cyclosporin, may affect the ability of autoreactive T and B cells to escape deletion, whereas metals, such as gold and mercury, activate subsets of autoreactive T cells. As sex steroid hormones influence autoimmune disease, it has been suggested that chemicals with oestrogenic activity can exacerbate autoimmunity.

As multiple factors participate in the development of autoimmune diseases, and each disease presents both diverse clinical symptomology and organ specificity, appropriate experimental screening models have been difficult to develop. A method that has received considerable attention is the popliteal lymph node assay with the use of reporter antigens. This method employs a modification of the graft-versus-host response combined with immunization to model antigens. Animal models for autoimmune diseases, such as immunization with myelin basic protein to induce experimental autoimmune encephalomyelitis, or autoimmune predisposed rodents have also been employed. However, none of these models has been sufficiently validated for use in risk assessment. The need to develop and validate sensitive and predictive screening assays for autoimmune disease was recently brought to the attention of the toxicology community by the silicone gel-filled breast implant controversy. A number of case reports have associated the implant to various connective tissue diseases, although controlled epidemiological studies have consistently failed to demonstrate any link.

Inflammation and Immunotoxicology

The role of inflammation in toxicological responses is viewed by many immunologists and toxicologists with growing interest as increasing evidence suggests that many diseases, ranging from Alzheimer disease to idiopathic pulmonary fibrosis and chronic hepatitis, present an inflammatory component in their pathophysiology. The underlying hypothesis that links inflammation to these diseases, as well as to immunotoxicology, is that injury, whether induced by xenobiotics or other (e.g. infectious) agents, produces focal areas of tissue damage. In the case of toxic chemicals, this may occur from any one of a number of mechanisms, such as lipid peroxidation or mitochondrial damage. In response to this localized damage, tissue-fixed macrophages and circulating monocytes migrate to the damaged site, undergo activation and secrete products that cause additional cell damage. Some of these products are short-lived, such as reactive oxygen species and the nitrogen-centred radical, nitric oxide. Other products, such as proinflammatory cytokines, regulate the production of additional inflammatory mediators and, thus, amplify as well as propagate these responses. The overall effect of this

Table 2 Examples where inflammatory cytokines have been implicated in organ-specific toxicity

Organ	Agent
Lung	Ozone, silica, asbestos, bleomycin, beryllium, sulfur dioxide, diesel particles, chromium
Liver	Carbon tetrachloride, dimethylnitrosamine, ethanol, acetaminophen, cadmium
Skin	Ultraviolet light, contact irritants (e.g. phenol)
Kidney	Cadmium
Brain	Organotin, lead

response can range from almost complete recovery, as often occurs in hepatotoxicity induced by carbon tetrachloride, to progressive organ failure characterized by extensive fibrosis as seen with lung particulates, such as silica or asbestos. This effect is dependent on variables such as the physicochemical properties of the inducing agent, the concentration and distribution in the target organ, the nature of the damage to the organ and the duration of exposure.

Although many factors contribute to the inflammatory response, tumour necrosis factor (TNF) α arguably plays a major role in regulating this process. That TNFα mediates many organ-specific toxic responses is suggested by the following: (1) raised TNFα levels are often found in target organs following exposure to certain occupational and environmental agents; (2) administration of TNFα in experimental animals mimics many of the pathophysiological responses associated with the toxic response; (3) inhibition of TNFα, either through administering neutralizing agents or use of TNF-deficient transgenic mice, prevents many of the pathophysiological responses from occurring; and (4) organs in which these types of responses occur contain tissue-fixed macrophages and/or other cell types capable of producing TNFα. Some examples of toxic agents in which chronic inflammatory mediators, particularly cytokines, have been implicated to play a significant role in the pathological sequelae are listed in (**Table 2**). Of particular significance have been experimental studies demonstrating that administration of TNFα-soluble receptors or neutralizing TNFα antibodies prevents silica- and bleomycin-induced pulmonary fibrosis as well as liver damage and repair caused by carbon tetrachloride.

Risk Assessment

To date, regulatory efforts to examine the immunomodulatory potential of chemicals and drugs has focused primarily on the ability of these agents to induce hypersensitivity. Recent efforts in this area have focused on harmonization of testing requirements in the European Community and multiple US regulatory agencies. With respect to hypersensitivity, the Organization for Economic Cooperation and Development (OECD) has accepted the LLNA as a first stage in assessment of skin sensitization, but has required that guinea-pig testing be conducted if the LLNA is negative. The US Food and Drug Administration (FDA) is currently considering incorporation of the LLNA as an alternative test method for the assessment of the skin sensitization.The LLNA is the first assay to be evaluated as an alternative method by the Interagency Coordinating Committee on Alternative Methods (full scientific evaluation and peer review were completed in 1999). With regard to immunosuppression, the US Environmental Protection Agency is currently the only agency with specific regulatory requirements for functional assessment of the potential immunosuppressive effects of chemicals. However, test guidelines that incorporate various levels of immune function tests are being developed by the OECD and several centres within the FDA.Testing batteries have also been proposed to assess immune function in occupationally or environmentally exposed human populations by the Agency for Toxic Substances and Disease Registry/Centers for Disease Control and Prevention, and the National Research Council. These clinical evaluation protocols incorporate a comprehensive evaluation of the immune system, as well as traditional tests such as lymphoproliferative responses. Many of these tests are similar to those used for assessment of immunotoxicity in laboratory animals and should help to predict the probability of developing suppressed host resistance or clinical disease in exposed human populations. As regulatory guidelines and requirements are subject to update and modification, the reader should refer to the appropriate agency for the most current version specific to a biological or chemical agent, or device.

Conclusions

In summary, immunotoxicology encompasses aspects of traditional basic and clinical immunology, and toxicology. The discipline evolved from studies showing that a variety of immunopathologies may result from exposure to physical, chemical and biological agents, including suppression, hypersensitivity and autoimmunity as well as chronic inflammatory disease. These pathologies may be manifested systemically or in specific target organs. As a

subdiscipline of toxicology, immunotoxicological studies are providing valuable information for risk assessment in humans and, to a lesser extent, in wildlife. To improve risk assessment, however, it will be necessary to improve human and wildlife immunoepidemiological studies, to increase our understanding of mechanisms for immunotoxic effects and to understand better the quantitative relationships between immunological tests and disease.

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