Immunotoxicology

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Introductory article

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Immunotoxicology can be defined as the study of adverse effects on the immune system resulting from exposure to physical factors (e.g. ionizing an ultraviolet radiation), chemicals (including drugs), biological materials, medical devices 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 chronic inflammation.

Introduction

Although adverse effects by chemicals on the immune system have been long suggested, 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 by 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, most often for sensitizers. 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 better identify individuals that may be more susceptible than the general population, such as the very young or elderly, those with preexisting disease or those genetically predisposed. To now consider immunotoxicology solely as an adjunct to risk assessment would be incorrect. Both mechanistic and applied research is actively pursued and the results often have had significant health implications for humans 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 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 diseases, postulated to stem from PCB-induced immunotoxicity.

In humans, immunotoxicity can be manifested as one of several distinct pathologies, which include allergic disease, immunodeficiency, autoimmunity or chronic inflammation. In the former case, the immune system responds to a low- (hapten) or high-molecular-weight agent (i.e. allergen), 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 or neoplastic diseases. Autoimmunity can be triggered in susceptible individuals by certain environmental chemicals or therapeutics. In the case of drug-induced autoimmune disease, the symptoms usually dissipate following drug removal. Environmental agents thought to exacerbate preexisting autoimmune disease, usually systemic forms, include silica, asbestos and organic solvents. Regarding chronic inflammation, the clearest examples are active agents, such as particulates which persist in the host. The responses to these agents may have an adaptive immune component, such as occurs in chronic beryllium disease (CBD), or may involve predominantly proinflammatory cytokines, such as silicosis. Examples of immunotoxic agents, classified by the type of immunopathology produced, are presented in **Table 1**. Although most studies

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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, dimethylbenzathracine)
Pharmaceuticals (antineoplastics, antibiotics, nucleoside analogues)
Aromatic amines (benzidine, acetylaminofluorene)
Metals (lead, cadmium, mercury, arsenic)
Radiation (ionizing, ultraviolet B)
Abused substances (alcohol, opiates, cannabinoids)
Pesticides (chlordane, hepatochor, parathion)
Organotins (DOTC, di-*n*-octyltin dichloride; DBTC, di-*n*-butyltin dichloride)
Mycotoxins (aflatoxin, ochratoxin A, trichothecenes)
Implicated in autoimmunity
Organic solvents (polyvinyl chloride, trichloroethylene)
Antibiotics (β-lactams, penicillin, sulfonamides, rifampicin)
Particulates (silica, asbestos)
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
Pharmaceuticals (sulfone, pancreatic extracts, antibiotic dusts)
Animal products from (laboratory animals, mites, mealworms, pigeons)
Wood dusts (western red cedar, California redwood, African maple)
Metal salts (chromium, platinum, nickel)
Examples of photoallergens
Antimicrobial (chlorosalicylanilide, hexachlorophene, fenticlor)
Fragrances (musk ambrette, methylcoumarin)
Sunscreens (*p*-aminobenzoic acid, oxybenzones)
Pharmaceuticals (sulfanilamide, chlorpromazine, promethazine)
Examples of contact allergens
Mercaptobenzathiazole
p-Phenylenediamine
Epoxy resin
Black rubber (PPD mix)
Metal salts (chromium, nickel, gold)

in immunotoxicology focus on direct effects on the immune system, some 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. For example, drugs of abuse, including the opiates and the cannabinoids impute their immunotoxicity through their ability to stimulate release of adrenocorticotrophic hormone and corticosteroids. **See also**: Allergy; Autoimmune Disease; Immune System; Immunodeficiency

Hypersensitivity

Allergic 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). Many compounds, such as latex proteins, are capable of producing both dermal (i.e. type 4 or Th1) and respiratory hypersensitivity (i.e. type 1, IgE or Th2) and may depend in part upon the route of exposure to the sensitizer. **See also**: Antibody Classes; Atopy and Asthma; Hypersensitivity: Immunological

Methods to assess low-molecular-weight chemicals for the potential to induce allergic contact sensitivity (ACD) are well established. Two tests that have been used for decades are the guinea-pig maximization test and the Buehler occluded patch test. These protocols assess the actual disease endpoint, erythema and oedema, following sensitization and challenge with the test agent, but can be somewhat subjective. Recently, a more economical, less subjective, test for contact hypersensitivity has been developed using mice. This test, referred to as the local lymph node assay (LLNA), assesses the proliferative response of lymphocytes in the draining lymph node following application of the agent to the ear and is based on our understanding of the immunologic mechanisms responsible for sensitization. The LLNA has been approved as a standalone alternative to the guinea-pig tests by most regulatory agencies. Predictive testing for contact hypersensitivity may be conducted in humans, and is similar to diagnostic testing using multiple occluded patches. The use of human volunteers, however, is limited because of the risk of sensitization from test articles or concern over unknown toxicity, although it is frequently and safely used for consumer products to confirm negative results in animal studies.

Tests to identify potential respiratory or food sensitizers are difficult to undertake and, as such, efforts to validate screening models are limited. Although expensive and not amenable for most labs the potential to induce respiratory sensitization by different proteins or chemical agents has been assessed following exposure by the respiratory route in guinea pigs or mice and monitoring for the development of cytophilic antibody (immunoglobulin G1 (IgG1) in guinea pigs; immunoglobulin E (IgE) in mice and humans) as well as increased respiratory rate following pulmonary challenge following sensitization. In humans, similar endpoints are used to assess individuals for respiratory

sensitivity or allergic asthma. The skin prick test, in which different proteins are injected under the skin, tests for the presence of cytophilic antibodies and helps to identify which proteins provoke the immune response in an individual. In certain settings patients may be exposed via the respiratory route to potential allergens (antigen-specific broncho-provocation test) and respiratory function monitored. As respiratory challenge may provoke systemic anaphylactic reactions in sensitized individuals, predictive testing for respiratory sensitization is usually not recommended in human subjects. **See also**: Immunology: Comparative Immunology of Mammals

Databases from agents that have been tested for skin sensitization potential in experimental animals and humans have been used to develop computational models to aid in identifying untested agents. These employ chemical structure and physicochemical properties, such as absorption through the skin and potential to react with skin-associated proteins, to derive qualitative structure–activity relationships (QSARs) or structural alerts for skin sensitization although so far with mixed success.

In laboratory rodent studies air pollutants, such as diesel exhaust and residual oil fly ash, have been shown to behave as adjuvants (i.e. they enhance allergic pulmonary sensitization to common allergens). These studies have been undertaken in a research setting using a variety of strategies and, subsequently there is no standard approach to assessing chemicals for such effects. Likewise, epidemiology has shown an association between episodes of high air pollution and exacerbation of asthmatic symptoms requiring hospital emergency room visits. Again, however, there is no standard approach to testing chemicals for the capacity to exacerbate allergic symptoms in sensitized individuals. **See also**: Hypersensitivity: Anaphylactic (Type I)

Immune Regulation

Although allergic hypersensitivity is the most commonly reported immunotoxic effect following exposure to drugs or environmental chemicals, altered immune regulation (i.e. suppression or stimulation of the local or systemic immune responses) may represent a potentially more significant human health hazard and, as such, has been afforded considerable attention. Initially, studies were focused on employing data from routine experimental animal toxicology studies (e.g. haematological results) to identify agents that could potentially modulate the immune system. Results collected from these studies were most often used for risk assessment. Subsequent studies were conducted to develop more predictive test methods to assess the immune system, to determine mechanisms of action and to identify human populations where the immune system may have been adversely affected. There is now extensive literature to indicate that a number of environmental and occupational chemicals, therapeutics, microbial products and ionizing and ultraviolet radiation (UVR) can suppress various components of the immune system and enhance susceptibility to both infectious and neoplastic disease in laboratory animals (**Table 1**). In addition, clinical and epidemiologic studies, although limited to a few agents, have reported suppression of immune function and/or increased frequency of infectious disease in humans. One of the most studied classes of environmental chemicals, from the standpoint of human health effects as well as basic laboratory research, is the halogenated aromatic hydrocarbon (HAH), which includes PCBs and dioxins, particularly 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the prototype of this chemical class. TCDD inhibits antibody responses in laboratory mice at doses of less than 1 $\mu g\,kg^{-1}$ bodyweight; a level that many believe is close to the exposure level that occurs in humans living in industrialized countries. Epidemiological studies have helped support these observations demonstrating that children exposed *in utero* and/or at birth via feeding breast milk to increased levels of HAHs develop subtle immune changes, such as altered CD4+/CD8+ ratios and may be more susceptible to infectious diseases. Although the exact mechanisms by which HAHs exert immunotoxicity are unknown, these compounds mediate their effects through binding to 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) to regulate gene expression. **See also**: Protein Kinases: Physiological Roles in Cell Signalling

Incorporating experimental animal tests to evaluate the immune system as part of toxicological evaluations 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 often a general screen for immunotoxicity and, usually incorporates a functional test, such as the measurement of antibody responses after *in vivo* antigenic challenge, as well as nonfunctional parameters (e.g. lymphoid organ histology). The second tier usually consists of tests that identify specific target cells and alterations in resistance to infectious agents or neoplastic disease. An alternative to this approach has been utilized in a recent guideline prepared by the International Congress of Harmonization (ICH), a group composed of representatives from regulatory agencies dealing with therapeutics, such as the Food and Drug Administration (FDA), and the major pharmaceutical industries. This guidance proposes to conduct immune studies only when routine preclinical tests identify changes that suggest an immune system effect, such as altered spleen weight, and only if the immune system can be shown to be the primary target. This weight of evidence approach has been shown to misclassify therapeutics that may have subtle effects on the immune system but will likely identify the more potent representatives. Laboratories conducting antibody-response tests currently use one of several platforms including enzyme-linked immunosorbent assay (ELISA) and plaque formation as well as one of several antigens including sheep red blood cells (SRBCs) and keyhole lymphet

haemocyanin (KLH), although the tests are collectively referred to as TDAR, meaning T-dependent antibody response.

Autoimmunity

In humans, the most well-known class of agents involved in autoimmune diseases are pharmaceuticals (**Table 1**). Drug-induced autoimmune diseases, however, differ from their idiopathic counterparts in that, although they significantly affect the patient, the disease remits in the absence of the drug and does not present the same clinical or immunological spectrum. Observational studies, particularly in worker populations, have also suggested that occupational or environmental exposure to certain chemical agents can be important triggers for expression of systemic autoimmune diseases and have been suggested to both induce onset and modulate disease severity. Other factors such as diet, smoking, therapeutic and recreational drug use, infections and exposure to UVR have also been suggested to be modifiers of autoimmune disease. Dietary factors such as iodine, nutritional supplements such as omega fatty acids and food contaminants have also been linked with autoimmune disorders. 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, consistent with scleroderma, and was attributed to an aniline derivative present in the oil.

Autoimmunity occurs when the mechanisms of self-tolerance break down. Chemicals and drugs associated with autoimmunity may influence the process by various mechanisms. For example, halothane, cadmium and methimazole may interact with host molecules to form novel self-determinants for which there is no tolerance. Other chemicals, such as cyclosporine, 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 also exacerbate autoimmunity.

As multiple factors participate in the development of autoimmune diseases, and each disease presents both diverse clinical symptoms and organ specificity, appropriate experimental screening models have been difficult to develop. Although there are now a variety of animal models designed to mimic different types of autoimmune disease, much of the information currently available comes from associations based on human observations. Animal models for autoimmune diseases frequently use tools such as immunization with targeted molecules (i.e. myelin basic protein to induce experimental autoimmune encephalomyelitis), knockout of regulatory cell populations or cytokines or genetic predisposition. However, none of these models have been sufficiently validated for use in risk assessment, and the nature of these studies makes it very difficult to make definitive cause/effect statements about aetiology, much less mechanisms underlying the disease process. **See also**: Autoimmune Disease: Animal Models; Autoimmune Disease: Pathogenesis

Inflammation and Immunotoxicology

There are a number of disorders, particularly associated with the workplace, where cell-mediated immunity along with chronic inflammation play major aetiological roles. One example is hypersensitivity pneumonitis (HP), also called extrinsic allergic alveolitis. HP is a complex syndrome caused by sensitization to repeated inhalation of dusts containing organic antigens. The dusts can be derived from various sources, such as dairy and grain products, animal dander and protein, wood bark and water reservoir vapourizers. The most common forms are farmer's lung and bird fancier's lung caused by thermophilic actinomycetes and avian proteins, respectively. HP occurs only in previously sensitized patients and is manifested by diffuse inflammation in the lower airways and poorly formed noncaseating interstitial granulomas.

Similar to HP, beryllium (Be) exposure has been associated with pulmonary syndromes including a granulomatous lung disease referred to as CBD, or berylliosis. CBD is clinically similar to other granulomatous diseases such as sarcoidosis. As the disease progresses, the granulomas become organized and eventually form small, fibrous nodules and progressive impairment of pulmonary function occurs. Diagnosis of CBD is dependent upon evidence of sensitization to beryllium based upon a positive beryllium lymphocyte proliferation test (BeLPT) and the presence of compatible lung pathology, usually manifested by nonnecrotizing granulomas on lung biopsy. Patients with a positive finding on blood BeLPT but no lung pathology are considered sensitized to beryllium, but not having CBD. Patients with CBD show a remarkably strong genetic association with HLA-DP beta alleles that contain glutamic acid in position 69 (Glu69) of their beta chain.

Chronic inflammation, without apparent involvement of adaptive immunity, 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 that either initiates or sustains the disease process. The underlying hypothesis that links inflammation to these diseases, as well as to immunotoxicology, is that the initial injury 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

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

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

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 response can range from almost complete recovery, to progressive organ failure characterized by extensive fibrosis as is sometimes seen with lung particulates, such as silica or asbestos. The severity of the effect is dependent on variables such as the physicochemical properties of the agent responsible, the concentration and distribution in the target organ, the nature of the damage to the organ and the duration of exposure. Examples are given in **Table 2**. **See also**: Alzheimer Disease; Inflammation: Acute; Inflammation: Chronic

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 dysregulation, 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 currently 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 the immunological tests conducted in the laboratory and actual disease in human populations.

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