Assessment of Immunotoxic Effects in Humans

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The immunotoxic effects of chemicals are varied and markedly different depending on the underlying pathogenesis, namely, direct immunotoxicity (including immunosuppression, immunodepression, and immunostimulation), hypersensitivity, and autoimmunity. A large number of immunological endpoints and functional assays have been proposed for use as biomarkers of immunotoxicity, but they often lack sensitivity or are poorly standardized, so that their relevance in assessing immunotoxic effects in humans is at best ill established. Examining sentinel immunopathological events in individuals with a defined history of chemical exposure is another approach, presumably more cost-effective at the present time. A multicenter collaboration is mandated, however, because these events are rare. We expect that progress in new technologies, e.g., molecular biology, will provide the sensitive and reliable biomarkers of immunotoxicity that are currently lacking.

**Indexing Terms:** immunotoxicity/biomarkers/sentinel events

During the past two decades, evidence has accumulated that the immune system is a possible target organ of toxicity after exposure to a wide array of pharmaceutical products, as well as to industrial and environmental chemicals (1-3). Experimental studies on the immunotoxicity of many substances essentially in rodents have been published (1), but until recently researchers paid only limited attention to immunotoxic effects in humans (2, 3). Nevertheless, as exemplified by two recent panels of experts (4, 5), the assessment of immunotoxic effects in humans is becoming a timely issue.

**Human Consequences of Immunotoxicity**

Because the immune system is involved in many physiological processes as well as pathological conditions, it is not surprising that immunotoxic effects are so varied. Three markedly different subsections of immunotoxicity can be identified (6): direct immunotoxicity, hypersensitivity, and autoimmunity. Each subsection is associated with specific health consequences.

**Direct Immunotoxicity**

Direct immunotoxicity refers to a qualitatively normal immune response that is either abrogated (immunosuppression), impaired (immunodepression), or enhanced (immunostimulation). The main clinical consequences of immunosuppression include more frequent infections and cancers (7). Infections have been shown to be more frequent, more severe, often relapsing, and atypical (e.g., opportunistic infections) in immunocompromised individuals. Because both specific (humoral and cell-mediated) and nonspecific components can be involved in the host defense against microbial invaders, drug and chemical exposures may result in infectious complications through extremely varied mechanisms. Although various cancers, e.g., of skin and kidney, have been described, lymphomas are the commonest malignancies associated with immunosuppression. Lymphomas, which usually develop within months or a few years after the start of immunosuppression, are thought to be mainly related to the activation of dormant viral infections, e.g., Epstein-Barr virus or herpesvirus infections, as a consequence of impaired T-cell control.

Infectious complications and lymphomas have been reported in transplant patients treated with every immunosuppressive drug in current use, especially azathioprine, cyclosporine, OKT3, or tacrolimus (FK506) (8). Interestingly, similar consequences have been described after moderate impairment of the immune responsiveness (i.e., immunodepression) caused by drug treatment, although far less consistently (9). Whether lymphomas associated with pesticide exposure are related to immune impairment is not established (10), but more frequent infections have been reported in human subjects exposed to chemicals (e.g., biphenyls, benzene, cigarette smoke, ethanol, or ozone) known to alter components of immune responsiveness in rodents (11).

Although from a theoretical viewpoint immunodepression is likely to result in the same health consequences as immunosuppression, it is still not known whether a threshold can be identified with regard to the functional reserve capacity of the immune system. Stimulation of immune responses as achieved in patients treated with recombinant cytokines (12) is associated with flu-like reactions (hyperthermia with chills, malaise, and hypotension), with more frequent allergic reactions to unrelated allergens, de novo autoimmune diseases (lupus erythematosus, autoimmune thyroiditis), and impairment of cytochrome P-450-mediated pathways of hepatic biotransformation. Actually, few drugs and chemicals have been shown to induce unexpected enhancement of the immune system (1-3). Interestingly, zinc fume fever has been proposed to be mediated by neutrophil activation [resulting in release of interleukin-1 and (or) tumor necrosis factor] (13), and this is also the mechanism of flu-like reactions associated with cytokine treatments (12).
Hypersensitivity

Hypersensitivity reactions (allergy) are typical immune-mediated consequences of exposure to pharmaceuticals and chemicals, although nonimmune-mediated (or pseudoallergic) reactions are also common (14). Hypersensitivity reactions are either mild to moderate but frequent (e.g., skin eruptions), or severe but rare (e.g., anaphylactic shock). Industrial and environmental chemicals differ from pharmaceutical products in that they are more often direct immunogens (because of larger molecular mass) or haptenize more readily (because of greater chemical reactivity).

A major problem with hypersensitivity reactions is that many mechanisms can be involved, sometimes simultaneously, e.g., IgE-mediated anaphylaxis, IgM-mediated cytopenia, or T cell-mediated contact dermatitis, quite apart from non-immune-mediated reactions including direct complement activation or histamine release. Despite evidence recently obtained on the role of (TH1/TH2) T-helper subsets, the fundamental mechanisms of chemical allergenicity, in particular the involvement of cytokines, have not yet been fully elucidated (15).

Autoimmunity

Autoimmune reactions of chemical origin are uncommon. Many pharmaceutical drugs are reportedly involved, particularly in drug-induced lupus; however, this usually occurs in only a few patients (16). By contrast, few chemicals are suspected, e.g., adulterated oil in the Spanish toxic oil syndrome, 1-tryptophan in the eosinophilia–fasciitis syndrome, silicone breast prosthesis in scleroderma, or mercury in autoimmune kidney damage. Their role, if any, and the mechanisms involved are still matters of debate.

One major difficulty is our current lack of a clear understanding of what autoimmunity actually is (17). The diagnosis of autoimmune diseases is usually based on the presence of both clinical and biological criteria in a given patient. In particular, the relevance of identifying autoantibodies in asymptomatic patients is unknown.

Biomarkers of Immunotoxicity

Clinical immunologists have devoted extensive efforts to designing assays for the diagnosis of primary or secondary immunodeficiencies, allergic reactions, and autoimmune diseases. Thus, several immune indicators can in principle be used as biomarkers of immunotoxicity, the detailed description of which falls far beyond the scope of this overview. It should be kept in mind that the applicability of these indicators in epidemiological or field studies remains to be fully established (4, 5).

Direct Immunotoxicity

The humoral and the cellular limbs of the immune response have been the subjects of major studies. Humoral immunity can be assessed by measuring total IgG, IgM, and IgA serum concentrations; specific serum antibodies to defined antigens (e.g., tetanus toxoid or influenza vaccine); and lymphocyte proliferation induced by B-cell mitogens (e.g., lipopolysaccharide) in vitro or ex vivo. Cellular immunity can be assessed by measuring skin reactivity to recall antigens (e.g., tuberculin, Candida, mumps, trichophytton, tetanus, or diphtheria toxoid), and lymphocyte proliferation induced by T-cell mitogens (mainly phytohemagglutinin and concanavalin A) or mixed lymphocyte culture, in vitro or ex vivo.

The finding that AIDS patients have a decreased number of CD4+ cells has led to the growing use of this assay. However, the immunotoxicological relevance of a decreased CD4+ count or CD4:CD8 ratio induced by chemicals is unknown (5), and this endpoint was not recommended for use by the US National Research Council panel of experts (4). Surprisingly, natural killer (NK) cell activity, which is best assayed by the 51Cr release method, has seldom been used, although it has been shown to be a very good correlate of increased susceptibility to cancer in rodents (18, 19). Finally, neutrophil or macrophage functions, e.g., phagocytosis and chemotaxis, have been extensively assessed.

Functional indicators of the humoral and cellular immune responses, lymphocyte subset analysis, and NK cell activity have been shown to be reliable predictors of direct immunotoxicity in rodents (18, 19), but the immunotoxicological relevance of such chemically induced defects in humans is unclear, as exemplified by lead-exposed workers. Workers with increased blood lead concentrations compared with controls show decreased (20) or normal (21) serum Ig and complement concentrations, impaired phagocytosis and chemotaxis (22, 23), and decreased CD4+ cell counts (24). However, no evidence is available that lead exposure is associated with more frequent infections.

One major problem in using functional immune endpoints is the lack of both standardized reagents and methods, so it is uncertain that results obtained in one laboratory can be readily reproduced in another. Many discrepancies and conflicting results can be found in the literature (1), and we feel that they cannot all be explained only by uncertainties or variations in the actual level of exposure. Another major concern is the unknown relevance of these endpoints from a toxicological viewpoint. As indicated above, the immune system is characterized by a large functional reserve, so that it has generally not been established to what extent, if any, a decrease in one given endpoint can be considered as evidence of immunotoxicity (25). We know, e.g., that many pharmaceutical products such as most psychotropic drugs and antimicrobials can induce slight to moderate immune changes without clearly established health consequences (1), although we should keep in mind that only limited attention has so far been paid to seeking such consequences in treated patients.

Other matters of concern in the use of biomarkers for immunotoxicity include (26, 27): (a) the careful selec-
tion of appropriate control subjects, as many factors can interfere with immune competence (e.g., age, sex, smoking, nutritional status, or illness); (b) the requirement that chemical exposure be sufficiently high and well documented; (c) the overcoming of difficulties caused by sample acquisition at sites geographically distant from the investigator's laboratory; and (d) the very high cost of such field studies.

Unfortunately, studies meeting all these requirements adequately are scarce; we should also bear this in mind when interpreting the results obtained as well as the relevance or usefulness of selected endpoints (28).

Hypersensitivity

Detection of antibodies specific to a chemical substance is considered a diagnostic tool for hypersensitivity reactions. This is particularly true for IgE, but a limited number of conjugates are available commercially (4), and IgE-mediated reactions are unlikely to be the commonest. Numerous reactions involve cellular mechanisms (i.e., effector lymphocytes) that, upon a new contact with the offending chemical, are activated and release cytokines together with other humoral factors. Classical assays such as the lymphoproliferative assay (29) are routinely used in research laboratories only, and because samples must be processed within hours, these assays are not well suited to field studies. Available cytokine assays, even those involving ELISA techniques, are not sensitive enough to be useful in this context (30).

Autoimmunity

Since we largely ignore the pathogenesis of chemically induced autoimmunity, it is doubtful that autoantibodies detected in individuals without any clinical manifestations can be used reliably as early biomarkers (4, 5).

Sentinel Immune Diseases

Because no currently available biomarkers of immunotoxicity have attained the required degree of sensitivity, reproducibility, and reliability, we propose another approach for detecting immunotoxic effects in humans associated with chemical exposure. The concept of sentinel events has already been used in various fields of toxicology, e.g., mutagenicity (31) and occupational diseases (32), and several experts (4, 16, 33) proposed this concept to be used for assessing immunotoxic effects in humans.

A network for the detection of immune-mediated sentinel events was recently started in France (34). Nine autoimmune diseases (lupus erythematosus, rheumatoid arthritis, scleroderma, dermatomyositis, Sjögren syndrome, mixed connective tissue disease, Hashimoto thyroiditis, pemphigus, and myasthenia gravis) and non-Hodgkin lymphoma were selected; these diseases have all been described after drug or chemical exposure in some patients (8, 9, 16), and their diagnosis is based on widely accepted criteria. Physicians from several poison centers, post-marketing drug surveillance units, and hospital departments of internal medicine, occupational medicine, rheumatology, dermatology, and oncology agreed to collaborate. When a patient with a newly diagnosed disease of relevance is identified, a standard questionnaire is used to document his or her recent history of chemical and drug exposure. A sentinel event is defined as the combination of a selected disease in a patient with a reasonably well-documented exposure. It should be stressed that this network does not set out to establish causal relationships between exposure and disease, but to document coincidences only. When such coincidences are found to occur more frequently than expected as compared with the disease incidence in the general population, a warning signal is generated (35). Specific studies, in particular epidemiological work, will then be conducted to confirm the hypothesis thus generated.

Summary

Even though more and more toxicologists are concerned with the immunotoxic consequences of drug and chemical exposure, little is actually known of the immunotoxic effects of chemicals in humans. Available immunological assays have been designed to address specific problems in certain patients, notably children with primary immunodeficiencies and patients with severe allergies or autoimmune diseases, and thus the assays have not been shown to be readily adaptable to immunotoxicity assessment.

Few biomarkers can now be recommended for use on the basis of rigorous previous experience, and there is no general agreement on what biomarkers to use. The US Agency for Toxic Substances and Disease Registry adopted a basic immune test battery (5), including antinuclear antibodies; serum C-reactive protein; IgA, IgG, and IgM concentrations; total white blood cells; lymphocytes; eosinophils; and CD4+ counts. This basic test battery can be supplemented with focused tests when special concerns arise regarding immunodeficiency, hypersensitivity, or autoimmunity. The US National Research Council panel of experts (4) recommended a three-tiered approach of immune tests, from the simplest to the most invasive. However, recommendations from both panels of experts heavily emphasized the lack of sensitivity of available tests and the need to identify and design better biomarkers of immunotoxicity. A similar opinion was recently expressed by Vos and Van Loveren (36), who could not identify reliable biomarkers of immunotoxicity in humans and thus advocated the use of comparisons between animal and human results. Importantly, we should remember that no epidemiological study including immune endpoints has thus far been published (5).

Thanks to the introduction of new techniques, expected developments such as those derived from molecular biology (30) are likely to change this situation in the future. However, these techniques are available only in research laboratories and will
not be adaptable to field studies for a long time. In the meantime, sentinel events may prove useful for detecting the immunotoxic effects of chemical exposure in humans.

References