REVIEW

Biomarkers of immunotoxicity in man

Jacques Descotes, Brigitte Nicolas, Thierry Vial and Jean-François Nicolas

The immunotoxic consequences of chemical exposures include direct immunotoxicity (namely immunosuppression and immunostimulation), hypersensitivity and autoimmunity, and because the mechanisms involved are markedly different, no single immune parameter is likely to ever predict or assess all three types of immunotoxicity. A fairly large number of immunological endpoints have been proposed for use as biomarkers of immunotoxicity in man. Unfortunately, they are often not sensitive enough and/or poorly standardized, so that their relevance for assessing immunotoxic effects in humans is debatable, and actually debated. Immune-mediated sentinel events detected in individuals with a defined history of chemical exposure, may prove helpful until methodological advances, notably with the introduction of technologies derived from molecular biology, provide reliable parameters to be used as biomarkers of immunotoxicity.

Keywords: *immunotoxicity, immunosuppression, hypersensitivity, autoimmunity.*

Introduction

It is now well established that the immune system is a target organ of toxicity following exposure to pharmaceutical, industrial and environmental chemicals (Descotes 1988, Burrell *et al.* 1992, Newcombe *et al.* 1992). Studies in laboratory animals showed the potential immunotoxic hazards of many substances, but surprisingly only limited attention has been paid to identifying immunotoxic effects in man until recently (Selgrade *et al.* 1995). Therefore, confusion inevitably arises when addressing this issue. As little, if anything, is known of the immunotoxic effects of most chemicals in humans, no clear distinction in the use of immune parameters as biomarkers of immunotoxicity is generally made between those parameters recommended as predictors of immunotoxicity for an unrecognized human immunotoxicant, and those parameters recommended as indicators of immunotoxicity in a specific group of individuals exposed to a recognized human immunotoxicant.

Jacques Descotes, Brigitte Nicolas, Thierry Vial *and* ***Jean-François Nicolas*** *are in the Laboratory of Immunopharmacology and Medical Toxicology, and at INSERM U80, Alexis Carrel Faculty of Medicine, Lyon, France.*

Immunotoxic effects in humans

Because the immune system is involved in many physiological and pathological processes, immunotoxic effects are varied and can be classified into direct immunotoxicity, hypersensitivity, and autoimmunity (Luster 1994). Importantly, each group is associated with specific health consequences, and should be investigated using different assays.

Direct immunotoxicity

Direct immunotoxicity refers to a qualitatively normal immune response which is either decreased (immunosuppression) or increased (immunostimulation). Immunosuppression is associated with more frequent infections and cancers (Revillard 1990). Infections are more frequent, and generally more severe, often relapsing and atypical (e.g. opportunistic infections) in immunocomprised individuals. As both specific (namely humoral and cell-mediated) and non-specific components of the host defences against microorganisms can be involved, infectious complications develop through extremely varied mechanisms. Lymphomas are the commonest malignancies associated with immunosuppression.
B lymphomas develop within months or a few years after the start of immunosuppression, and are thought to be mainly related to the activation of dormant viral infections, e.g. Epstein–Barr or Herpes virus infections.

Interestingly, infectious complications and lymphomas have been reported in patients treated with every immunosuppressive drug (Vial and Descotes 1996). More frequent infections and a few isolated cases of lymphomas in patients treated with low-dose immunosuppressive drugs, e.g. methotrexate, have also been reported. Although from a theoretical viewpoint, a moderate impairment of immune responses is likely to result in the same health consequences as immunosuppression, it is not known whether a threshold is to be identified due to the functional reserve capacity of the immune system.

Stimulation of the immune responses as in patients treated with recombinant cytokines (Vial and Descotes 1995), is associated with flu-like reactions (hyperthermia with chills, malaise, and hypotension), more frequent allergic reactions to unrelated allergens, *de novo* autoimmune diseases (lupus erythematosus, autoimmune thyroiditis), and impairment of cytochrome P450-mediated pathways of hepatic biotransformation.

Hypersensitivity

Hypersensitivity reactions include immune-mediated (allergic) and non immune-mediated (or pseudo-allergic) reactions (Dukor *et al.* 1980). They are either mild to moderate, but frequent (e.g. skin eruptions), or severe but rare (e.g. anaphylactic shock). Industrial and environmental chemicals often act as direct immunogens (due to larger molecular weight) or haptenize more readily (due to greater chemical reactivity) than pharmaceutical agents.

A major problem is that many mechanisms can be involved, sometimes simultaneously: for instance IgE-mediated anaphylaxis, IgM-mediated cytopenia, or T-cell mediated

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contact dermatitis, as well as non-immune-mediated reactions including direct complement activation or histamine release. Overall, the fundamental mechanisms of chemical allergenicity, in particular the involvement of cytokines, have not yet been established.

Autoimmunity

Autoimmune reactions of chemical origin are uncommon. Many drugs have been suggested to be involved, in particular as regards the drug-induced lupus, but in a few patients only (Kosuda and Bigazzi 1995). By contrast, only a few chemicals have been suspected, for instance adulterated oil in the Spanish toxic oil syndrome, L-tryptophan in the eosinophilia-fasciitis syndrome, or silicone breast prosthesis in scleroderma. One major difficulty is the lack of understanding of what autoimmunity actually is. Therefore, the diagnosis of many autoimmune diseases is uncertain.

Immunological biomarkers

In the recent past, efforts have been paid to the diagnosis of primary or secondary immune deficiencies, allergic reactions and autoimmune diseases. A large number of immune parameters can in principle be used as biomarkers of immunotoxicity even though their applicability in epidemiological or field studies is unknown (US National Research Council 1992, Straight *et al.* 1994). As a matter of fact, no studies specifically addressed the relative value of various immune parameters as predictors or indicators of immunotoxicity in man, so that recommendations on which immune parameters can be used are generally based on theoretical considerations or even mere speculations.

Direct immunotoxicity

Humoral immunity can be assessed by measuring total IgG, IgM and IgA serum levels, specific serum antibodies to defined antigens (e.g. tetanus toxoid or influenza vaccine), and lymphocyte proliferation induced by B-cell mitogens (e.g. LPS). Cellular immunity can be assessed by measuring skin reactivity to recall antigens (e.g. tuberculin, *Candida*, mumps, trichophyton, tetanus or diphtheria toxoid), and lymphocyte proliferation induced by T-cell mitogens (mainly phytohaemagglutinin and concanavalin-A) or mixed lymphocyte culture, *in vitro* or *ex vivo*. The finding that AIDS patients have a decreased number of $CD4^+$ cells led to the growing use of this assay, but the immunotoxicological relevance of decreased $CD4^+$ count or CD4:CD8 ratio induced by chemicals is unknown (Straight *et al.* 1994) and this endpoint was not recommended for use by the US NRC panel of experts (US National Research Council 1992). Surprisingly, natural killer (NK) cell activity, although shown to be a very good correlate of increased susceptibility to cancer in rodents (Luster *et al.* 1992, 1993), has seldom been used. Finally, neutrophil or macrophage functions, e.g. phagocytosis and chemotaxis, have been extensively assessed.

Functional parameters 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 (Luster *et al.* 1992; 1993), but the immunotoxicological relevance of such chemically-induced immune changes in man is unclear or unknown (US National Research Council 1992, Straight *et al.* 1994) so that their value as biomarkers of immunotoxicity in man is again unknown.

One major problem is the lack of standardization in both reagents and methods so that results obtained in one laboratory are unlikely to be readily reproduced in another laboratory. Discrepancies and conflicting results are widespread in the literature (Descotes 1988), and they cannot all be explained only by uncertainties or variations in the actual level of exposure. Another concern is the unknown relevance of these endpoints from a toxicological perspective. Due to the large functional reserve of the immune system, it is absolutely essential to establish to what extent, if any, a decrease in one given endpoint can be considered as evidence of immunotoxicity (Dayan 1990). For instance, the majority of psychotropic drugs and antimicrobials can induce slight to moderate immune changes without clearly established health consequences (Descotes 1988), so that it must be stressed that immune changes are not synonyms of immunotoxicity.

A number of additional problems have been identified (Biagini *et al.* 1994, Kimber 1995). They include the requirement for a careful selection of control subjects to avoid confounding factors interfering with the immune competence (e.g. age, sex, smoking, nutritional status, or illness). The level of chemical exposure must be sufficiently high and well documented. Difficulties due to sample acquisition at sites geographically distant from the investigator's laboratory must be overcome, and last but not least, the very high cost of such field studies should be taken into account.

Hypersensitivity

The detection of antibodies specific to a chemical substance is a diagnostic tool. This is particularly true for IgE, but IgE-mediated reactions are unlikely to be the commonest. Skin tests can also be used. A number of reactions involve cellular mechanisms, namely effector lymphocytes. Classical assays, such as the lymphoproliferative assay (Mroz *et al.* 1991), are routinely used but because samples must be processed within hours, this assay is not well suited to field studies. Available cytokine assays, even those using ELISA, are not sensitive enough (Sundraram and Wing 1995).

Autoimmunity

As the pathogenesis of chemically-induced autoimmunity is largely unknown, autoantibodies detected in individuals without any clinical manifestations cannot be used reliably (US National Research Council 1992, Straight *et al.* 1994).

Sentinel immune diseases

No proposed biomarkers of immunotoxicity have so far attained the required degree of sensitivity, reproducibility and reliability. Another approach is the detection of immune-mediated sentinel events (US National Research

Council 1992, Sundaram and Wing 1995, Descotes *et al.* 1996). A sentinel event is defined as the combination of a selected disease in an individual with a reasonably well documented exposure. A network for the detection of nine autoimmune diseases (lupus erythematosus, rheumatoid arthritis, scleroderma, dermatomyositis, Sjögren's syndrome, mixed connective tissue disease, Hashimoto's thyroiditis, pemphigus and myasthenia gravis) plus non-Hodgkin's lymphoma was recently started in France (Pham *et al.* 1995). When a patient with a newly diagnosed disease is identified, a standard questionnaire is used to document his or her recent history of chemical and drug exposure. The aim is definitely not to establish causal relationships between exposure and disease, but to document coincidences. When coincidences seem to occur more frequently than expected as compared with the disease incidence in the general population, a warning signal is generated. Specific studies, in particular epidemiological studies, will be conducted to confirm the hypothesis thus generated. A major limitation to the use of sentinel events is their lack of sensitivity, whereas validation may be inferred from many case reports in the literature showing that lymphomas or autoimmune diseases can be induced by drug treatment or chemical exposure.

Conclusion

Despite growing concern on the immunotoxicity of drug and chemical exposure, little is known in humans. Available assays have been designed to address specific problems of diagnosis in given patients, such as children with primary immune deficiencies, and patients with severe allergies or autoimmune diseases. Thus, they have generally not been shown to be readily adaptable to immunotoxicity assessment. At the present time, few immune parameters can be recommended as biomarkers of immunotoxicity due to our limited experience and the lack of general agreement on what immune parameters to use. Clearly, it is not yet possible to clarify those parameters that can be used to predict immunotoxicity in humans exposed to an unrecognized immunotoxicant, from those that can be used as biomarkers in humans exposed to a known immunotoxicant.

A test battery was adopted by the US Agency for Toxic Substances and Disease Registry (1994): it includes antinuclear antibodies, serum C reactive protein, IgA, IgG and IgM levels, total white blood cells, lymphocytes, eosinophils, and $CD4^+$ counts. This basic battery can be supplemented with focused tests when special concern is raised regarding either immunosuppression, hypersensitivity or autoimmunity. The US NRC panel of experts (1992) recommended a three-tier approach from the simplest to the most invasive immune tests. Importantly, 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 Van Loveren *et al.* (1995) who could hardly identify reliable biomarkers of immunotoxicity in humans and thus advocated the use of comparisons between animal and human results. Importantly, it should be remembered that no epidemiological study including immune endpoints has so far been published (US National Research Council 1992).

Thanks to the introduction of new techniques, expected developments, e.g. those derived from molecular biology, are likely to change this situation in the future. However, these techniques are only available in research laboratories and will not be adaptable to field studies for some time. In the meantime, sentinel events may prove useful in the recognition of immunotoxicants in humans.

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