

# Autoimmunity and Risk Assessment

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Among the issues dealing with identifying potential adverse immunologic effects (i.e., suppression, hypersensitivity, or autoimmunity) associated with xenobiotic exposure, general agreement exists among the regulatory and pharmaceutical communities that predictive tests for autoimmunity are in most need of development in order to improve risk assessment. The estimation of risk (i.e., the probability of a deleterious effect resulting from exposure) involves both the qualitative evaluation of whether a hazard exists and the quantitative evaluation for determining an acceptable level of exposure in humans. Unless adequate human data are available, which is uncommon, this is based on animal studies. Although animal models exist to study autoimmune processes, these models do not readily lend themselves to interpretation in the risk assessment process due, for the most part, to the complexity of autoimmune disease(s), as they are multifactorial and exhibit genetic heterogeneity in humans. To improve the risk assessment process, researchers must develop and validate animal models that not only incorporate mechanistic information into the assessment process but also allow for consideration of potent genetic, physiologic, and environmental influences. *Key words:* autoimmunity tests, immunotoxicology evaluation, immunotoxicology methods, risk assessment, xenobiotic-induced autoimmunity. — *Environ Health Perspect* 107(suppl 5):679–680 (1999).

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## Overview

A study recently conducted by the Environmental Defense Fund (1) indicates that although the number of chemicals tested for immunotoxicity is somewhat less than that for other organ systems, such as the reproductive, developmental, or nervous systems, almost 20% of chemicals present at significant levels in the environment have been examined at some level for immunologic effects. A review of the literature, however, indicated the majority of those chemicals studied were examined for their ability to cause either hypersensitivity or immunosuppression, and only a relatively few were tested for their potential to produce autoimmunity (2). These observations appear at odds with results from a recent survey of the pharmaceutical industry (3). This survey, in which 12 companies responded, suggested that hematologic or dermatologic problems consistent with autoimmunity (or systemic allergy) were the most common preclinical or postlaunch immunologic observations. Furthermore, the survey respondents indicated the most immediate need in the area of immunotoxicology evaluation was the development of predictive tests for autoimmunity. Epidemiologic data support the survey results; approximately 5% of the U.S. population suffers from some form of autoimmune disease (4), and 5–20% of patients receiving certain drugs such as procainamide or hydralazine develop drug-induced autoimmune diseases (5).

The question then arises, Why is there a lack of validated autoimmune models suitable for drug or chemical screening? Certainly a

large number of experimental models are used successfully to study organ-specific and systemic autoimmunity (Table 1). Furthermore, although many questions still remain on the etiology and biology of autoimmune diseases, scientists have provided a good road map of disease development, allowing for successful application of novel biotech therapies such as cytokine antagonists. The primary reason for the lack of validated screening assays probably stems from the complexity of the disease. First, autoimmune disease is not one disease but a group of over 30 diseases affecting different organs, often through different mechanisms. Unless a common early process is identified, a single test would be unlikely to provide an adequate degree of concordance to be useful for predictive risk assessment. Second, although most diseases are governed by genetics, the degree of genetic and epigenetic influences in autoimmune diseases is such that it could drastically alter the outcome of a test. For example, the relative risk for developing autoimmunity from gold salt increases 32-fold in individuals who possess the HL-A DR3 allele (7). Experimental studies of mercury-induced autoimmunity in the Brown-Norway rat and B.10 mouse suggest the same genetic influences apply in animal models (7). Third, development of autoimmune disease may occur through one of several mechanisms, thus increasing the need to conduct multiple tests in risk assessment. For example, at the cellular level, autoimmune disease can occur from either aberrant B-cell or T-cell responses. Thus, if a specific autoimmune disease results from a

defect in T cells, assays designed to detect a defect in B cells would provide a false negative. At the molecular level, depending upon the xenobiotic, an autoimmune disease can develop from the expression (unmasking) of cryptic determinants, altered immunoregulation such as defects in the expression of interleukin 4 or interferon- $\gamma$ , or defects in the establishment of tolerance, which often involve missed deletion or activation of autoreactive T cells. Finally, when using animal models, there is some uncertainty regarding what actually constitutes autoimmunity. This is reflected in humans by a lack of well-defined diagnostic tests for identifying autoimmune disease and is discussed in detail in other articles in this monograph.

Despite these challenges, attempts to develop predictive screening assays for detecting xenobiotic-induced autoimmunity have proceeded in several institutions including Virginia Commonwealth University (Richmond, Virginia) under the National Toxicology Program, the University of Utrecht (Utrecht, The Netherlands) funded by the Dutch Organization for Scientific Research, and the University of Dusseldorf (Dusseldorf, Germany). Models proposed to evaluate the potential of xenobiotics to induce autoimmunity are

- Popliteal lymph node assay with reporter antigens
- Increased titers of autoantibodies
- Examination of immunoglobulin complexes/deposits (immunohistology)
- Spontaneous animal models.

These models are clearly different from those described in Table 1, as the latter are designed to investigate the mechanisms of a specific autoimmune disease. Of the four assays listed, immunohistology, in which immunoglobulin deposits or complexes are evaluated either on suspect organs or in the periphery, has been evaluated the least, whereas the popliteal lymph node assay with reporter antigens has been studied the most (8). Details

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**Table 1.** Examples of experimental models used to study autoimmune diseases.<sup>a</sup>

Organ-specific autoimmunity
Induced by immunization (EAE, AA)
Spontaneous mice (NOD, <sup>b</sup> transgenics)
Toxicant-induced (streptozotocin, Cd)
Systemic autoimmunity
Allogeneic reactions
Neonatal thymectomy
Spontaneous mice (New Zealand mixed) <sup>c</sup>

Abbreviations: AA, autoimmune arthritis; EAE, experimental autoimmune encephalitis; NOD, nonobese diabetic. <sup>a</sup>Adapted from Pelletier et al. (6). <sup>b</sup>Develop immune diabetes. <sup>c</sup>Prone to develop systemic lupus erythematosus.

of these methods and the results so far obtained have been discussed by Pieters and Albers (9). However, none of these models have undergone a vigorous validation process to provide sufficient confidence for use in risk assessment. Such a validation process would involve, at the minimum, establishing an acceptable level of concordance and an inter/intralaboratory validation exercise. The former would involve determining the assay's sensitivity and specificity using agents known to induce autoimmune disease in humans. The inter/intralaboratory validation component is somewhat less defined but would examine assay reproducibility, feasibility, accuracy, and in certain instances, cost effectiveness.

Data used in risk assessment are derived primarily from animal toxicology studies. When available, findings from epidemiologic and controlled clinical exposure studies take precedent. Results obtained from *in vitro* studies, structure-activity relationships, (SARs), or mechanistic investigations are normally used only in a supporting role. Mechanistic studies are particularly helpful, however, as without them all defaults in the risk assessment process are assumed valid (e.g., threshold, animal vs human sensitivity, interindividual variability). Epidemiologic data represent the "gold standard" in risk assessment. Epidemiologic studies involving autoimmune diseases would be complicated by the likelihood that the disease incidence in the exposed population would be relatively low because of the multifactorial and poly-genetic nature of the disease. This would be further confounded by difficulties in clinical diagnosis because of a lack of defined end points available. Some of these problems would be circumvented in controlled clinical studies. However, such studies may be limited with

**Table 2.** Structure-activity relationships of potential interest.

Activity	Chemical example
Estrogenic	Diethylstilbestrol
Thymolytic	Cyclosporin A, cyclophosphamide
Formation of protein adducts	Haloethane
Altered immune regulation	Mercury, interferon- $\gamma$
Myeloperoxidase substrates	Procainamide
Altered methylation	Hydralazine

respect to the size of the population and the length of treatment and could only be conducted with pharmaceuticals.

Despite the challenges in developing appropriate tests for autoimmunity that can be used in risk assessment, the considerable amount of data generated by immunologists and pharmacologists pertaining to basic mechanisms of chemical-induced autoimmune diseases have provided a conceptual framework that allows the establishment of potential SARs (Table 2) (6, 10). These SARs are by no means definitive. As the database increases, no doubt some will not be supported and others will be added. In all cases these relationships are supported by basic understanding of immunologic and pharmacologic processes. For example, estrogens are known to be a major factor in classic autoimmune diseases, presumably because of their ability to stimulate certain components of the immune system (11). Laboratory studies have shown that thymolytic chemicals can induce autoimmunity when given neonatally by altering normal patterns of autoreactive T-cell deletion, a process that occurs in the thymus early in life (12). Chemicals that form protein adducts or damage tissue in such a way to allow expression of cryptic determinants would provide novel host antigens that could be recognized by T cells. Agents that have adjuvant activity or biologicals that stimulate certain cytokines may shift the balance of T-helper 1 and T-helper 2 cells and allow exacerbation of preexisting autoimmune disease (13). Common features associated with many drugs that induce autoimmune diseases are that they serve as myeloperoxidase substrates and/or cause changes in methylation. The underlying biology for the latter associations are less clear but may involve formation of the specific antigenic epitopes responsible for

the autoimmune response. With regard to the association with myeloperoxidase substrates, it has been suggested that many of the chemicals require metabolism in proximity to immune cells in order to be antigenic; immune cells such as monocytes contain high levels of myeloperoxidase (14).

In any case, based upon the need to develop predictive screening models to identify the potential of xenobiotics to induce or exacerbate autoimmunity, combined with our increased understanding of immune and pharmacologic mechanisms and the rapidly increasing array of available animal disease models, suitable tests should be forthcoming.

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