Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Toxicology

Immunotoxicology: A brief history, current status and strategies for future immunotoxicity assessment

Dori Germolec[a], Robert Luebke[b], Andrew Rooney[c], Kelly Shipkowski[a], Rob Vandebriel[d] and Henk van Loveren[e]

Abstract

The discipline of immunotoxicology had its origins in the early 1970s, following the recognition of altered immune function and increased sensitivity to infections and cancers after exposure to environmental chemicals and therapeutic drugs. Reduced resistance to infectious disease was a well-documented consequence of primary and acquired immunodeficiencies, but a novel finding following xenobiotic exposure. The awareness of the consequences of altered immune function was likely heightened by the HIV epidemic, leading some to inappropriately characterize xenobiotic-induced immunosuppression as "chemical AIDS", although it is now clear that mild to moderate suppression is the most likely outcome of inadvertent exposure. The human health implications of studies in which chemical exposure reduced resistance to infection, drove an early focus on immunosuppression within the toxicology community. Allergic hypersensitivity was well known to clinicians and symptoms were readily apparent, and therefore was not the initial focus of the developing toxicology subspecialty of immunotoxicology. The first review in the field of immunotoxicology was published by Vos in 1977, and, as research expanded during the years that followed, many of the assays, methodologies and approaches that are currently used to identify potential immunotoxicants were developed. Over the years, advances in our understanding of basic immunology have made it clear that allergy, immunosuppression and, in some cases, autoimmunity, are a matter of polarization of the immune response by immunotoxicants, rather than independent outcomes of chemical exposure.

Addresses

[a] Toxicology Branch, Division of the National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, USA
[b] Cardiopulmonary and Immunotoxicology Branch, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA
[c] Office of Health Assessment and Translation, Division of the National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, USA
[d] Centre for Health Protection, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands
[e] Department of Toxicogenomics, Maastricht University, Maastricht, The Netherlands

Corresponding author: Germolec, Dori (germolec@niehs.nih.gov)

Current Opinion in Toxicology 2017, **5**:55–59

This review comes from a themed issue on **Systems Toxicology**

DOI of original article: http://dx.doi.org/10.1016/j.cotox.2017.06.009

For a complete overview see the Issue and the Editorial

http://dx.doi.org/10.1016/j.cotox.2017.08.002

2468-2020/Published by Elsevier B.V.

Keywords

Immunotoxicology, Immunosuppression, Autoimmunity, In vitro, Hypersensitivity, Tiered testing, Systematic review.

1. Introduction

The discipline of immunotoxicology had its origins in the early 1970s, following the recognition of altered immune function and increased sensitivity to infections and cancers after exposure to environmental chemicals and therapeutic drugs. Reduced resistance to infectious disease was a well-documented consequence of primary and acquired immunodeficiencies, but a novel finding following xenobiotic exposure. The awareness of the consequences of altered immune function was likely heightened by the HIV epidemic, leading some to inappropriately characterize xenobiotic-induced immunosuppression as "chemical AIDS", although it is now clear that mild to moderate suppression is the most likely outcome of inadvertent exposure [1]. The human health implications of studies in which chemical exposure reduced resistance to infection drove an early focus on immunosuppression within the toxicology community. Allergic hypersensitivity was well known to clinicians and symptoms were readily apparent, and therefore was not the initial focus of the developing toxicology subspecialty of immunotoxicology. The first review in the field of immunotoxicology was published by Vos in 1977 [2], and, as research expanded during the years that followed, many of the assays, methodologies and approaches that are currently used to identify potential immunotoxicants were developed. Over the years, advances in our understanding of basic immunology have made it clear that allergy, immunosuppression and, in some cases, autoimmunity, are matters of polarization of the immune response by immunotoxicants, rather than independent outcomes of chemical exposure.

2. The early framework for immunotoxicity testing

Although the experimental methods adopted by immunotoxicologists to evaluate immune function were common to immunology laboratories, experimental

designs were often *ad hoc*. This lack of standardization often made it difficult to compare chemical-specific results obtained in different labs and led Dean et al. [3] to propose a "tiered testing" paradigm for assessments in the mouse. This tiered approach contained both screening assays to detect immunologic effects (Tier I) and a comprehensive suite of assays to provide an in-depth assessment of immune function and host resistance endpoints (Tier II). A group of assays from the screening tier were subsequently tested in mice against the known immunosuppressant, cyclophosphamide, for performance and reproducibility, then further refined and validated in multiple laboratories [4,5]. A similar suite of assays was developed for immunotoxicity screening in the rat, the traditional species used in industrial chemical toxicity studies [6,7]. The next logical step in the evolution of the tiered-testing approach was the use of sophisticated statistical analyses to evaluate the predictive value of data generated by these studies. A number of groups have examined the sensitivity, specificity and predictive value of various immune endpoints as well as analytical strategies to evaluate data [8–10]. As methods to evaluate immunotoxicity became standardized, the tiered approaches became a potentially useful tool to evaluate specialized toxicity to the immune system from a regulatory standpoint. Testing guidelines that include evaluation of immunotoxicity have been developed for industrial and environmental chemicals [11] and a harmonized guideline is in place for the assessment of pharmaceutical agents [12]. In recognition of the potential vulnerability of the developing organism, specific requirements for the assessment of immune effects following pre- or perinatal exposure have also been implemented, such as the inclusion of an immunological cohort in the Organisation for Economic Cooperation and Development (OECD) Extended One-Generation Reproductive Toxicity Study testing guideline [13]. These efforts have shaped the evolution of testing methods by providing additional insight into modes and mechanisms of immunotoxicity, and the functional or observational endpoints that best predict changes in immune function. They have also set the stage for the development of *in vitro* testing strategies to assess immune function in an effort to reduce the use of animals and identify the specific targets of immunotoxicants.

3. *In vitro* and high throughput approaches to assess immunotoxicity

Over the past forty years, significant progress has been made regarding the use of *in vitro* assessments to evaluate immunotoxicity. The advantages of *in vitro* approaches include higher chemical throughput, the ability to explore multiple mechanisms of potential immunosuppression, and the use of mechanism-focused data to extrapolate potential immune effects to multiple species; however, the primary advantage is the significant reduction in cost and use of animals [14]. It has been proposed that advances in toxicogenomics, bioinformatics, systems biology, epigenetics, and computational toxicology could transform immunotoxicity testing from a system based on whole-animal testing to one founded primarily on *in vitro* methods that evaluate changes in immunologic processes using cells, cell lines, or cellular components, preferably of human origin [15].

Numerous *in vitro* techniques have become routine in assessments of immunotoxicity. Similar to *in vivo* hazard assessment frameworks, *in vitro* testing has been performed in a two-tiered approach; the first tier using myelotoxicity, or bone marrow suppression, assays to evaluate general immunotoxicity, and the second tier focused on lymphotoxicity [14]. The assessment of myelotoxicity provides a broad measure of the potential impact of chemicals on growth and development of immune cells in general, as all immune-related cells develop from pluripotent, hematopoietic stem cells in the bone marrow. Both human and murine colony forming units-granulocyte/macrophage (CFU-GM) assays have been validated for assessing xenobiotic-induced myelotoxicity, and *in vitro* bone marrow stem cell assays are now commercially available and routinely used in pharmaceutical screening [16].

Standard assessments of lymphotoxicity utilize both *in vitro* and *ex vivo* assays that evaluate different functional parameters of the immune response; however, the reliability of these techniques for predicting immunotoxicity varies between assays [14]. The human whole-blood cytokine release assay, which is currently the only cytokine-based assay that has been validated for *in vitro* immunotoxicology assessments, measures interleukin (IL)-1β and IL-4 release in human blood samples in response to lipopolysaccharide (LPS) or staphylococcal enterotoxin B (SEB) [17]. The Multi-ImmunoTox assay, which uses reporter cell lines derived from Jurkat and THP-1 cells to examine cytokine changes following chemical treatment, has shown promising results in early validation efforts [18]. Additional *in vitro* tests include the lymphocyte proliferation assay, mixed lymphocyte reaction (MLR) assay, the anti-CD3 T cell proliferation assay, cytotoxic T-lymphocyte (CTL) assay, natural killer (NK) cell activity assay, and the dendritic cell maturation assay [14].

To date, the majority of progress in using *in vitro* models to assess immunotoxicity has focused on chemical sensitization, and, in particular, dermal hypersensitivity and irritancy [14,19]. The OECD recently developed an adverse outcome pathway (AOP) for skin sensitization [20]. The goal of an AOP is to link molecular initiating events and cellular and tissue effects to specific adverse outcomes, which helps to identify individual key events