Published in final edited form as:

Curr Opin Toxicol. 2017 August ; 5: 55–59.

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

Dori Germolec,

Toxicology Branch, Division of the National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC

Robert Luebke,

Cardiopulmonary and Immunotoxicology Branch, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC

Andrew Rooney,

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

Kelly Shipkowski,

Toxicology Branch, Division of the National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC

Rob Vandebriel, and

Centre for Health Protection, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

Henk van Loveren

Department of Toxicogenomics, Maastricht University, Maastricht, the Netherlands

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

Disclaimer: This chapter has been reviewed by the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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 a matter of polarization of the immune response by immunotoxicants, rather than independent outcomes of chemical exposure.

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 [8,9,10]. 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 peri-natal 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.

***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)-1 β 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 have 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 that could be evaluated using *in vitro* techniques [21]. For skin sensitization, these key events include: 1) covalent interaction with skin proteins, 2) activation of inflammatory cytokines and induction of cytoprotective genes, 3) induction of inflammatory cytokine and surface molecules and mobilization of dendritic cells, and 4) activation of T cells [20,22,23]. Many

new *in vitro* techniques have subsequently been developed to assess these key events, including direct peptide reactivity assays (DPRA), the KeratinoSens assay, and human cell line activation tests (h-CLAT) (See Chapters 4-8 for additional information). An *in vitro* profiling strategy, BioMAP, has also been developed. Data on the effects of inflammatory stimuli on human primary cells are being compiled in the BioMAP database for analysis using informatics and data mining strategies [24]. The goal of developing these advanced techniques is to further enhance the predictability of immunotoxicity assays.

With the advent of genomics technology, microarray analysis has been used to examine the effects of immunosuppressive compounds on changes in gene expression [25,26]. Genomic analyses have demonstrated value as a means to identify mechanisms of action of immunotoxic chemicals [27,28], and the hope was that transcriptional profiles could be used to suggest gene signatures associated with immune system toxicity in specific cells and tissues; however, this has proven not to always be the case. On the other hand, the mechanisms of action for contact allergens are well-understood [20,29] and microarray analysis has proven to be a useful component in a testing strategy to identify sensitizers [30].

Machine Learning Approaches

Machine learning approaches are also being used to develop models for evaluating skin sensitization, with the goal of classifying substances as sensitizers or non-sensitizers without requiring animal data. Evaluations of machine learning techniques indicated that an integrated approach of training models using DPRA, KeratinoSens, h-CLAT, read-across, and logP data was more accurate in identifying potential skin sensitizers than *in vitro*, *in chemico*, or *in silico* methods by themselves [31]. Machine learning approaches have also been utilized to develop statistical models that predict skin sensitization potency for the mouse local lymph node assay (LLNA) as well as potential human outcomes. A two-tiered LLNA model, which utilized a support vector machine algorithm in combination with *in vitro* assay and physicochemical data, was shown to be highly accurate in predicting LLNA and human test outcomes, respectively, for up to 120 different substances [32,33].

The Use of Immunotoxicity Data in Risk Assessment

In 2012, a Guidance for Immunotoxicity Risk Assessment of Chemicals was published by the International Programme on Chemical Safety [34]. In that guidance, a table of entry points for the risk assessment process was presented to help the risk assessor identify the type(s) of immunotoxicity suggested by the data. The guidance suggests a weight of evidence approach for the spectrum of available immunotoxicology data. Human studies, such as the epidemiological studies showing an association between prenatal exposure to polychlorinated biphenyls and dioxins and increased risk of wheeze and infections in infants [35], or studies on prenatal exposure to perfluoralkyl substances that demonstrate an association with altered vaccine antibody levels and immune-related health outcomes [36], provide the strongest type of evidence that chemical exposures present a potential hazard to the immune system. However, epidemiological studies often lack precise information on exposure and may not control for important confounding variables. Laboratory animal data typically do not suffer the same shortcomings, but extrapolation to likely human effects may

be problematic because of differences in metabolism, bioavailability, etc. For both approaches, dose–response relationships, biological plausibility and mode of action are critical aspects that need to be considered and from which uncertainty factors may be determined.

Currently, *in vitro* and genomics data are not used for the purpose of risk assessment, other than providing supportive evidence for the mechanisms of toxicity. For example, Hochstenbach et al. [37] established toxicogenomic profiles in relation to maternal immunotoxic exposure and immune functionality in newborns, and Pennings et al. [38] found cord blood gene expression profiles supporting that prenatal exposure to perfluoralkyl substances caused depressed immune functionality in early childhood. *In vitro* assays which evaluate immunosuppression are generally not used to form a basis for risk assessment. Rather, as is the case for toxicogenomics, *in vitro* assays may aid in understanding the mechanisms, hence delivering supportive evidence. With the increased use of *in vitro* data to perform hazard identification for contact sensitizers it is likely that these types of data will soon be the basis for risk evaluation. Implementation of gene expression profiling in *in vitro* systems has proven to be promising in hazard identification of immunotoxicants and unraveling mechanisms [28,30,39], but so far has not been implemented in immunotoxicity risk assessment of chemicals. Dose-response assessment as a basis for risk characterization from *in vitro* studies may eventually be a way forward for risk assessment, but such approaches need further development.

Systematic Review and Hazard Assessment

Systematic review approaches are increasingly being used to address questions in public health because they provide greater transparency and objectivity to the process of developing literature-based assessments and the communication of the results. The National Toxicology Program (NTP) developed a systematic review framework that can be used to identify data gaps, research needs, or reach hazard conclusions based on the integration of human, animal, and mechanistic evidence [40]. These methods were used to develop the NTP Monograph on Immunotoxicity Associated with Exposure to PFOA or PFOS [41]. The framework supports the assessment of individual study quality for human, animal, and *in vitro* studies and transparent consideration of applicability of *in vitro* studies to the research question at hand.

Future Trends in Immunotoxicology

While the primary focus of immunotoxicology has been on suppression and the potential effects of environmental chemicals on the immune system, a major focus in the therapeutic area has been the development of immunostimulatory compounds for the treatment of neoplastic disease. Similarly, there are now many herbal supplements, probiotics and other over-the-counter products that promise to boost the immune response, and most are considered to be safe for use by the general public. In both cases, there have been reports of development or exacerbation of autoimmune disease, and some dietary supplements now carry warning labels with a cause for concern [42,43,44,45]. Developing effective testing strategies, particularly with a forward focus of reducing the use of animals and increasing

the relevance of *in vitro* models should be an area of increased emphasis to assist in our understanding of the potential adverse effects associated with immune stimulation.

Conclusion

In this brief overview, we have tried to convey a sense of the dynamic nature of immunotoxicology, how it has evolved since its inception and how it continues to progress to accommodate advances in our understanding of basic immunology and incorporate new concepts and techniques. The discipline of immunotoxicology has refined a number of powerful tools to assess the safety of new drugs and other products. Novel approaches for assessment of hypersensitivity and cytokine-based assays to examine chemical-specific effects are moving the field away from the use of animals and providing a path forward for hazard identification and risk assessment.

Acknowledgments

We thank Drs. Christal Bowman and Dave Lehmann for their thoughtful review and comments on the manuscript.

References

1. Luebke RW, Parks C, Luster MI. Suppression of immune function and susceptibility to infections in humans: association of immune function with clinical disease. *J Immunotoxicol.* 2004; 1:15–24. [PubMed: 18958637]
2. Vos JG. Immune suppression as related to toxicology. *Crit Rev Toxicol.* 1977; 5:67–101.
3. Dean JH, Padarathsingh ML, Jerrells TR. Assessment of immunobiological effects induced by chemicals, drugs or food additives. I. Tier testing and screening approach. *Drug Chem Toxicol.* 1979; 2:5–17. [PubMed: 548256]
4. Dean JH, Luster MI, Boorman GA, Lauer LD. Procedures available to examine the immunotoxicity of chemicals and drugs. *Pharmacol Rev.* 1982; 34:137–148. [PubMed: 6280211]
5. Luster MI, Munson AE, Thomas PT, Holsapple MP, Fenters JD, White KL Jr, Lauer LD, Germolec DR, Rosenthal GJ, Dean JH. Development of a testing battery to assess chemical-induced immunotoxicity: National Toxicology Program's guidelines for immunotoxicity evaluations in mice. *Fundam Appl Toxicol.* 1988; 10:2–19. [PubMed: 3280374]
6. Exon JH, Bussiere JL, Mather GG. Immunotoxicity testing in the rat: an improved multiple assay model. *Int J Immunopharmacol.* 1990; 12:699–701. [PubMed: 2272731]
7. Vos, JG., van Loveren, H. Immunotoxicity testing. In: Burger, EJ, Tardiff, RG., Bellanti, JA., editors. *The Rat, Advances in Modern Environmental Toxicology.* Vol. 13. Princeton Scientific Publishing; Princeton, N.J: 1987. p. 167-180.
8. Luster MI, Portier C, Pait DG, White KL Jr, Gennings C, Munson AE, Rosenthal GJ. Risk assessment in immunotoxicology. I. Sensitivity and predictability of immune tests. *Fundam Appl Toxicol.* 1992; 18:200–210. [PubMed: 1534777]
9. Luster MI, Portier C, Pait DG, Rosenthal GJ, Germolec DR, Corsini E, Blaylock BL, Pollock P, Kouchi Y, Craig W, et al. Risk assessment in immunotoxicology. II. Relationships between immune and host resistance tests. *Fundam Appl Toxicol.* 1993; 21:71–82. [PubMed: 8365588]
10. Keil D, Luebke RW, Pruett SB. Quantifying the relationship between multiple immunological parameters and host resistance: probing the limits of reductionism. *J Immunol.* 2001; 167:4543–4552. [PubMed: 11591782]
11. Office of Prevention, Pesticides and Toxic Substances (OPPTS). Health Effects Test Guidelines: 870.7800, Immunotoxicity. United States Environmental Protection Agency; Aug. 1998
12. Food and Drug Administration (FDA). HHS, International Conference on Harmonisation; Guidance on S8 Immunotoxicity Studies for Human Pharmaceuticals. Notice, Fed Regist. 2006; 71:19193. Availability. [PubMed: 16612859]

13. Organisation for Economic Co-operation and Development (OECD). Test No. 443: Extended One-Generation Reproductive Toxicity Study. OECD Publishing; Paris: 2011. DOI: <http://dx.doi.org/10.1787/9789264122550-en>
14. Lankveld, DPK., van Loveren, H., Baken, KA., Vandebriel, RJ. *In vitro* testing for direct immunotoxicity: state of the art. In: Dieter, RR., editor. Immunotoxicity Testing: Methods and Protocols, Methods in Molecular Biology. Vol. 598. Springer International Publishing; New York, N.Y: 2009. p. 401-423.
15. National Academies Press (NAP). Toxicity testing in the 21st century: a vision and a strategy. 2007. <https://www.nap.edu/download/11970#>
16. Rich IN. High-throughput *in vitro* hemotoxicity testing and *in vitro* cross-platform comparative toxicity. Expert Opin Drug Metabol & Toxicol. 2007; 3:295–307.
17. Langezaal I, Hoffmann S, Hartung T, Coecke S. Evaluation and prevalidation of an immunotoxicity test based on human whole-blood cytokine release. Altern Lab Anim. 2002; 30:581–595. [PubMed: 12513684]
18. Kimura Y, Fujimura C, Ito Y, Takahashi T, Aiba S. Evaluation of the Multi-ImmunoTox Assay composed of 3 human cytokine reporter cells by examining immunological effects of drugs. Toxicol In Vitro. 2014; 28:759–768. [PubMed: 24603311]
19. Mehling A, Eriksson T, Kolle S, Ramirez T, Teubner W, van Ravenzwaay B, Landsiedel R. Non-animal test methods for predicting skin sensitization potentials. Arch Toxicol. 2012; 86:1273–1295. [PubMed: 22707154]
20. Organisation for Economic Cooperation and Development (OECD). Guidance Document No. 168: The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins: Part 1, Part 2. 2012. <http://www.oecd.org/chemicalsafety/testing/seriesontestingandassessmentpublicationsbynumber.htm>
21. Kleinstreuer N, Sullivan K, Allen D, Edwards S, Mendrick DL, Embry M, Matheson J, Rowlands JC, Munn S, Maull E, Casey W. Adverse outcome pathways: from research to regulation; scientific workshop report. Reg Toxicol Pharmacol. 2016; 76:39–50.
22. Reisinger K, Hoffmann S, Alépée N, Ashikaga T, Barroso J, Elcombe C, Gellatly N, Galbiati V, Gibbs S, Groux H, et al. Systematic evaluation of non-animal test methods for skin sensitization safety assessment. Toxicol In Vitro. 2014; 29:259–270.
23. Rovida C, Alépée N, Api AM, Basketter DA, Bois FY, Caloni F, Corsini E, Daneshian M, Eskes C, Ezendam J, et al. Integrated testing strategies (ITS) for safety assessment, Workshop Report. ALTEX. 2015; 32:25–40. [PubMed: 25413849]
24. Kleinstreuer N, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, et al. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014; 32:583–591. [PubMed: 24837663]
25. Baken KA, Arjusz J, Pennings JL, Vandebriel RJ, van Loveren H. *In vitro* immunotoxicity of bis(tri-n-butyltin)oxide (TBTO) studied by toxicogenomics. Toxicology. 2007; 237:35–48. [PubMed: 17553608]
26. Frawley R, White K Jr, Brown R, Musgrove D, Walker N, Germolec D. Gene expression alterations in immune system pathways in the thymus after exposure to immunosuppressive chemicals. Environ Health Perspect. 2011; 119:371–376. [PubMed: 21041162]
27. Katika MR, Hendriksen PJ, van Loveren H, Peijnenburg A. Exposure of Jurkat cells to bis (tri-n-butyltin) oxide (TBTO) induces transcriptomics changes indicative for ER- and oxidative stress, T cell activation and apoptosis. Toxicol Appl Pharmacol. 2011; 254:311–322. [PubMed: 21601586]
28. Shao J, Katika MR, Schmelts PC, Hendriksen PJ, van Loveren H, Peijnenburg AA, Volger OL. Toxicogenomics-based identification of mechanisms for direct immunotoxicity. Toxicol Sci. 2013; 135:328–346. [PubMed: 23824090]
29. Vandebriel RJ, Pennings JL, Baken KA, Pronk TE, Boorsma A, Gottschalk R, van Loveren H. Keratinocyte gene expression profiles discriminate sensitizing and irritating compounds. Toxicol Sci. 2010; 117:81–89. [PubMed: 20566472]
30. van der Veen JW, Rorije E, Emter R, Natsch A, van Loveren H, Ezendam J. Evaluating the performance of integrated approaches for hazard identification of skin sensitizing chemicals. Regul Toxicol Pharmacol. 2014; 69:371–379. [PubMed: 24813372]

31. Strickland J, Zang Q, Paris M, Lehmann DM, Allen D, Choksi N, Matheson J, Jacobs A, Casey W, Kleinstreuer N. Multivariate models for prediction of human skin sensitization hazard. *J Appl Toxicol*. 2016; 37:347–360. [PubMed: 27480324]
32. Strickland J, Zang Q, Kleinstreuer N, Paris M, Lehmann DM, Choksi N, Matheson J, Jacobs A, Lowit A, Allen D, Casey W. Integrated decision making strategies for skin sensitization hazard. *J Appl Toxicol*. 2016; 36:1150–1162. [PubMed: 26851134]
33. Zang Q, Paris M, Lehmann DM, Bell S, Kleinstreuer N, Allen D, Matheson J, Jacobs A, Casey W, Strickland J. Prediction of skin sensitization potency using machine learning approaches. *J Appl Toxicol*. 2017
34. International Programme on Chemical Safety (IPCS). Guidance for Immunotoxicity Risk Assessment for Chemicals. World Health Organization; Geneva: 2012. <http://www.inchem.org/documents/harmproj/harmproj/harmproj10.pdf>
35. Stølevik SB, Nygaard UC, Namork E, Haugen M, Engelstad KH, Meltzer HM, Alexander J, van Delft JHM, van Loveren H, Løvik M, Granum B. Prenatal exposure to polychlorinated biphenyls and dioxins is associated with increased risk of wheeze and infections in infants. *Food Chem Tox*. 2011; 49:1843–1848.
36. Granum B, Haug LS, Namork E, Stølevik SB, Thomsen C, Aaberge IS, Van Loveren H, Lovik M, Nygaard UC. Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. *J Immunotoxicol*. 2013; 10:373–379. [PubMed: 23350954]
37. Hochstenbach K, van Leeuwen DM, Gmuender H, Gottschalk RW, Stølevik SB, Nygaard UC, Løvik M, Granum B, Namork E, Meltzer HM, et al. Toxicogenomic profiles in relation to maternal immunotoxic exposure and immune functionality in newborns. *Toxicol Sci*. 2012; 129:315–324. [PubMed: 22738990]
38. Pennings JLA, Jennen DGJ, Nygaard UC, Namork E, Haug LS, van Loveren H, Granum B. Cord blood gene expression supports that prenatal exposure to perfluoroalkyl substances causes depressed immune functionality in early childhood. *J Immunotoxicol*. 2016; 13:173–180. [PubMed: 25812627]
39. Shao J, Berger LF, Hendriksen PJM, Peijnenburg ACM, van Loveren H, Volger OL. Transcriptome-based functional classifiers for direct immunotoxicity. *Arch Toxicol*. 2014; 88:673–689. [PubMed: 24356939]
40. Rooney AA, Boyles AL, Wolfe MS, Bucher JR, Thayer KA. Systematic review and evidence integration for literature-based environmental health assessments. *Environ Health Perspec*. 2014; 122:711–718.
41. National Toxicology Program (NTP). Monograph on immunotoxicity associated with exposure to perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). Research Triangle Park; NC: 2016. http://ntp.niehs.nih.gov/ntp/ohat/pfoa_pfos/pfoa_pfosmonograph_508.pdf
42. Bergner, P. Cautions with echinacea in auto immune disease?. *Medical Herbalism*. Available from: <http://medherb.com/92ECHAUT.HTM>
43. Day D, Hansen AR. Immune-related adverse events associated with immune checkpoint inhibitors. *BioDrugs*. 2016; 30:571–584. [PubMed: 27848165]
44. Lee AN, Werth VP. Activation of autoimmunity following use of immunostimulatory herbal supplements. *Arch Dermatol*. 2004; 140:723–727. [PubMed: 15210464]
45. Tan TG, Sefik E, Geva-Zatorsky N, Jua L, Naskar D, Teng F, Pasman L, Ortiz-Lopez A, Jupp R, Wu HJJ, et al. Identifying species of symbiont bacteria from the human gut that, alone, can induce intestinal Th17 cells in mice. *Proc Natl Acad Sci USA*. 2016; 113:E8141–E8150. [PubMed: 27911839]

Highlights

- 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.
- As methods to evaluate immunotoxicity became standardized, tiered approaches became a potentially useful tool to evaluate specialized toxicity to the immune system from a regulatory standpoint.
- Numerous *in vitro* techniques have become routine in assessments of immunotoxicity.
- In 2012, a Guidance for Immunotoxicity Risk Assessment of Chemicals was published by the International Programme on Chemical Safety. In that guidance, a table of entry points for the risk assessment process was presented and a weight of evidence approach is used to evaluate the spectrum of available immunotoxicology data.