

Chapter 2

Developmental Immunotoxicity (DIT): The Why, When, and How of DIT Testing

Rodney R. Dietert and Jamie DeWitt

Abstract

Developmental immunotoxicity (DIT) has emerged as a serious health consideration given the increases in the prevalence of many immune-based childhood diseases and conditions, including allergic diseases and asthma, recurrent otitis media, pediatric celiac disease, and type 1 diabetes. As a result, the use of DIT testing to identify potential environmental risk factors contributing to these and other diseases has become a higher priority. This introductory chapter considers: (1) the basis for an increased and earlier use of DIT testing in safety evaluations and (2) the general features of DIT testing strategies designed to reduce health risks.

Key words: Developmental immunotoxicity, DIT, Developmental immunotoxicology, Pediatric health risks, Safety testing, Autoimmunity, Allergic hypersensitivity, Inflammation, Immunosuppression, Host resistance

1. Introduction

Developmental immunotoxicity (DIT) testing is a significant consideration under the larger umbrella of immunotoxicity testing covered in this book. DIT received only occasional research consideration before the mid-1990s (1–3). However, it has grown sufficiently in scope and impact to be the subject of a stand-alone book by Holladay (4) and has been an integral part of virtually every book on immunotoxicology to appear since that time (5–10).

Luster et al. (10) recently defined developmental immunotoxicology as “the effects on the immune system resulting from pre- and/or postnatal exposure to physical factors (e.g., ionizing and ultraviolet radiation), chemicals (including drugs), biological materials, medical devices, and in certain instances, physiological factors, collectively referred to as agents.” DIT increases the risk

of autoimmunity, allergic hypersensitivity, susceptibility to infectious diseases and cancer, and inflammatory diseases in humans as well as in wildlife. The increased risk exists because the immune system is central not only to host defense but also to physiological homeostasis. Since environmentally induced immune dysfunction encompasses both suppression and inappropriate enhancement of immune responses, DIT testing should be capable of detecting both types of changes.

This introductory chapter on DIT considers three key topics that significantly impact DIT testing. These are: (1) the “why” of DIT testing or the scientific basis for early-life vulnerability that has led DIT testing to become a central issue within safety testing, (2) the “when” of DIT testing or the circumstances that would be expected to result in DIT testing, and (3) the “how” of DIT testing or the key considerations that can guide an effective testing strategy for health-risk reduction.

2. The “Why” of DIT Testing

Epidemiological studies and animal studies have consistently demonstrated the adverse effects of exogenous agents on the developing immune system that last longer or that occur at lower doses than effects of the same agents on adults. Therefore, assessing immunotoxicity in adult animals may not adequately reflect the severity or the persistence of the adverse effects following developmental exposure. From a risk assessment perspective, if early-life exposure to toxicants poses the greatest environmental risk for the immune system, then it poses the greatest effect on human health (11).

Epidemiological studies of humans environmentally exposed to exogenous agents provide concrete examples of how developmental exposure to toxicants alters immunocompetence and subsequent susceptibility to infections (12–17). Populations in Canada, China, the Netherlands, and Japan accidentally exposed to polychlorinated biphenyls (PCBs) and their associated breakdown products are well-documented examples of DIT. In each of these populations, rates of recurrent otitis media (inflammation of the inner ear), recurrent respiratory infections, and other types of immune dysfunction were higher in developmentally exposed children than in matched controls. Myriad animal studies with PCBs and other chlorinated compounds corroborate these epidemiological data that developmental exposure increases the risk of infections later in life.

Numerous other exogenous agents have been implicated as developmental immunotoxicants in animal models, exposed human populations, or both. These include therapeutic agents

(diazepam, diethylstilbestrol, and dexamethazone), additional environmental chemicals such as pesticides (chlordane, heptachlor, and hexa-chlorobenzene), and metals (lead, mercury, and organotin compounds). Luebke et al. (18) compared the immunotoxicity of five different compounds (diethylstilbestrol, diazepam, lead, TCDD, and tributyltin oxide) following adult or developmental exposure and concluded that until information to the contrary is available, the developing immune system is more sensitive to toxicant exposure than the adult immune system. One important consideration is that the timing of exposure determines the immuno-toxicological outcome, which means that DIT produces a myriad of effects. For example, if lead exposure occurs throughout gestation, the juvenile and adult delayed-type hypersensitivity (DTH) response (a functional measure of cell-mediated immunity) is decreased; if exposure is restricted to the first half of gestation, macrophage function is impaired but later-life DTH response is unaffected (19). Therefore, the evaluation of DIT requires: (1) the understanding of immune system development, (2) the utilization of relevant age-based exposure regimes, and (3) the selection of appropriate immunological outcomes for assessment. These considerations are discussed in the subsequent section.

3. The “When” of DIT Testing

As discussed in the prior section, the developing immune system is generally accepted as a more sensitive toxicological target compared with the immune system of an adult. In fact, even the nature of adverse immune outcomes resulting from early-life exposures is not reliably predictable, based on adult-exposure immunotoxicity results (11). This disconnection between adult-exposure immunotoxicity data and early-life immunotoxic risk raises two key questions in immunotoxicity testing: (1) Are age-relevant immunotoxicity data needed to ensure adequate protection of the nonadult from exposure to a chemical or drug? and (2) When should DIT studies be conducted in safety testing?

In recent years, there has been an increased concern over the protection of children’s health that has been reflected in new government and international agency-sponsored activities (20–25). Not surprisingly, this has extended to an increased interest in DIT. Since many of the significant chronic diseases of childhood feature immune dysfunction (26), this increased interest appears warranted.

A comparison of DIT publications from the years 1982–1994 vs. 1996–2008 makes it clear that the number of DIT studies, workshops, conference symposia, and reviews has

increased significantly in recent years (10, 11, 18, 26–36). As a result, more information is now available on age-based immune safety for selected chemicals and drugs. However, this increase appears to be largely a result of increased government- and industry-funded research into DIT as well as recent National Toxicology Program (NTP) contracts for early-life exposure studies. In contrast, regulatory expectations for DIT testing have remained relatively unchanged across the years with limited exceptions (e.g., pesticide safety).

As illustrated in Fig. 2.1, current immunotoxicity testing of drugs and chemicals expected by regulatory agencies is focused on adult-exposure assessment. Additionally, the collection of specific immune data from adult exposures is often predicated on a “cause for concern” triggered by initial, more general data. DIT exposure assessment is not routinely expected and would usually be triggered only by evidence of clear immunotoxicity from the adult-exposure data. The problem with this priority of testing is that adverse immune outcomes from an insensitive toxicological response system (the fully matured adult immune system) are used as a prerequisite for pursuing DIT testing.

The strategy of evaluating risk to the developing immune system only if and when the fully matured immune system (i.e., the adult trigger) is altered should produce two types of errors. The first is essentially a quantitative error. In this case, the adult-exposure immunotoxicity data would not provide an indication of the dose–response sensitivity, persistence of adverse effects, or range of adverse effects that the same chemical or drug might produce with early-life exposure (11, 18). If direct DIT testing is pursued, this information is then available. But in the absence of additional DIT testing, addition of safety factors may be applied to reduce age-based risk for some immunotoxicants. Because age-based sensitivities vary widely (18), these may or may not be sufficient.

The second type of error is more qualitative and of greater concern. If a chemical or drug is not identified as an immunotoxicant (based on adult-exposure results), then it may never be tested for DIT. Yet, the chemical or drug may be capable of producing an adverse immunotoxic outcome in early life at relevant exposures. While it is not clear if examples of this second type of error do exist, it is also likely that adult-defined “non-immunotoxicants” will never be tested for early-life risk of facilitating allergy, autoimmunity, or targeted immunosuppression. Therefore, the data may not exist to address the likelihood of overlooking developmental immunotoxicants.

One advantage of using DIT testing earlier in the safety testing regime (shown in Fig. 2.1) is the benefit of having evaluated the most sensitive age-group for potential immunotoxicity. A negative finding in a comprehensive DIT assessment should

provide safety information that extends to the adult immune system as well. However, the reverse is not true in that negative adult immune exposure results provide little assurance for early-life immune safety.

4. The “How” of DIT Testing

The specific protocols described in the assay-specific chapters within this book are directly relevant not only to adult-exposure assessment but also to DIT testing. Therefore, they should be considered as having direct relevance across age groups. Hence, there is no need to repeat details of these same assays in this background chapter on DIT. Most of the scientific discussions surrounding the use of specific assays and immune parameters in DIT testing concern: (1) the most informative version of each assay to use, (2) the optimum (or minimum) combination of assays needed for an informative DIT assessment, and (3) the timing of applying these assays in DIT assessment.

There is general agreement that a routine screen of DIT would normally include exposure to the environmental agent over the entire developmental period of the nonadult (prenatal, neonatal, juvenile) with immune assessment occurring during the juvenile and/or young adult periods (8). More restricted or specialized exposure regimes could be applied as needed to address specific age-based concerns or the most relevant human exposure (e.g., proposed use of a drug in the pediatric population). DIT assessment should be performed on a challenged immune system (immunized or exposed to an infectious agent) to provide the opportunity to detect potential immune dysfunction (32). Beyond those basic suggestions, the optimum combination of assays and parameters to be employed in the assessment is the subject of considerable discussion. However, given that DIT testing is unlikely to be structured into a multiple tier approach, as are some adult immunotoxicity testing regimes, the probable one-time assessment needs to include a sufficient range of immune measures to address health concerns.

Like adult-exposure immunotoxicity assessment, DIT testing is designed to identify potentially problematic exposures and to reduce immune-associated health risks. To accomplish the latter, it is useful to identify target diseases that could be impacted by a reduction of childhood and adult adverse immune outcomes and would serve as the justification for DIT testing. Dietert (26) started with eight of the most significant diseases or conditions of children and young adults having environmental risk factors and featuring immune dysfunction. Most of these diseases are chronic in nature, have increased in prevalence in recent decades and

include: allergies (including asthma), autism, childhood leukemia, late-onset sepsis, multiple sclerosis, otitis media, pediatric myalgic encephalomyelitis, and type 1 diabetes. A reverse engineering approach was taken toward the goal of optimizing DIT testing for human health-risk reduction. The author asked how one might identify the earliest signs of the immune problems found with the eight childhood diseases and then progressed backwards to the immune parameters that were most useful for this identification. The results from this exercise led to a series of generalized priorities that may prove helpful in guiding effective DIT testing.

These are presented here as a series of nine questions that should be useful in a consideration of specific DIT testing regimes and the desired combination of immune parameters to be included in DIT assessments.

1. Was the immune system adequately challenged to permit detection of immune dysfunction, including those parameters prominent during the secondary immune responses?
2. Did the measures permit a sensitive detection of changes in T helper (Th) balance (Th1, Th2, Th17)?
3. Was there an adequate assessment of cell-mediated immunity?
4. Was the potential risk of autoimmunity determined (involving changes in T regulatory cells and/or T cell receptor diversity)?
5. Was innate immune maturation adequately evaluated?
6. Was the status of marginal zone B lymphocytes and the potential responsiveness to T-independent antigens determined?
7. Was the status of resident macrophage populations such as microglia evaluated?
8. Did the assessment parameters evaluate immune cell recruitment and trafficking?
9. Was mucosal immune status determined [including the status of the bronchus-associated lymphoid tissue (BALT) and the gastrointestinal-associated lymphoid tissue (GALT)]?

5. Summary

Numerous immunotoxicity testing protocols are detailed in the subsequent chapters of this book and virtually all of these are directly applicable to use in DIT testing. A broader issue concerns the conditions under which these protocols would be applied to DIT testing within a safety testing regime. In the past, DIT testing has been a relatively rare regulatory requirement. However,

the increasing prevalence of immune-based childhood diseases coupled with the uncertainties inherent in applying adult-exposure immunotoxicity data to predict early-life health risks should increase the role of DIT testing in safety evaluation. With this in mind, it is important that DIT testing designs optimize detection of those adverse immune outcomes that contribute to the most significant health risks of the at-risk population (prenatal, neonatal, juvenile). This introductory chapter has provided disease-pertinent immune information that should prove useful in designing DIT testing strategies.

Acknowledgments

The authors thank Janice Dietert for her editorial assistance.

References

1. Luster MI, Faith RE, Kimmel CA (1978) Depression of humoral immunity in rats following chronic developmental lead exposure. *J Toxicol Environ Pathol* 1:397–402
2. Barnett JB, Holcomb D, Menna JH, Soderberg LS (1985) The effect of prenatal chlordane exposure on specific anti-influenza cell-mediated immunity. *Toxicol Lett* 25:229–238
3. Dietert RR, Qureshi MA, Nanna UC, Bloom SE (1985) Embryonic exposure to aflatoxin-B1: mutagenicity and influence on development and immunity. *Environ Mutagen* 7:715–725
4. Holladay SD (2005) Developmental immunotoxicology. CRC Press, Boca Raton, FL
5. Barnett JB (1996) Developmental immunotoxicology. In: Smialowicz RJ, Holsapple MP (eds) *Experimental Immunotoxicology*. CRC Press, Boca Raton, FL, pp 47–62
6. Smialowicz RJ, Brundage KB, Barnett JB (2007) Immune system ontogeny and developmental immunotoxicology. In: Luebke R, House R, Kimber I (eds) *Immunotoxicology and Immunopharmacology*, 3rd edn. CRC Press, Boca Raton, FL, pp 327–346
7. Dietert RR (2009) Developmental immunotoxicology. In: Ballantyne B, Marrs T, Syversen T (eds) *General and applied toxicology*, 3rd edn. Wiley, Chichester, UK, pp 1977–1991
8. Dietert RR, Burns-Naas LA (2008) Developmental immunotoxicity in rodents. In: Herzyk DJ, Bussiere JL (eds) *Immunotoxicology strategies for pharmaceutical safety assessment*. Wiley, Hoboken, NJ, pp 273–297
9. Holsapple MP, van der Laan JW, van Loveren H (2008) Development of a framework for developmental immunotoxicity (DIT) testing. In: Luebke R, House R, Kimber I (eds) *Immunotoxicology and Immunopharmacology*, 3rd edn. CRC Press, Boca Raton, FL, pp 327–346
10. Luster MI, Dietert RR, Germolec DR, Luebke RW, Makris SL (2008) Developmental immunotoxicology. In: Sonawane B, Brown R (eds) *Encyclopedia of environmental health*. Elsevier, Oxford
11. Dietert RR, Piepenbrink MS (2006) Perinatal immunotoxicity: why adult exposure assessment fails to predict risk. *Environ Health Perspect* 114:477–483
12. Lu YC, Wu YC (1985) Clinical findings and immunological abnormalities in Yu-Cheng patients. *Environ Health Perspect* 59:17–29
13. Nakanishi Y, Shigematsu N, Kurita Y, Matsuba K, Kanegae H, Ishimaru S, Kawazoe Y (1985) Respiratory involvement and immune status in Yusho patients. *Environ Health Perspect* 59:31–36
14. Yu ML, Hsin JW, Hsu CC, Chan WC, Guo YL (1998) The immunologic evaluation of the Yucheng children. *Chemosphere* 37:1855–1865
15. Dewailly E, Ayotte P, Bruneau S, Gingras S, Belles-Isles M, Roy R (2001) Susceptibility to infections and immune status in Inuit infants exposed to organochlorines. *Environ Health Perspect* 108:205–211

16. Weisglas-Kuperus N, Patandin S, Berbers GA, Sas TC, Mulder PG, Sauer PJ, Hooijkaas H (2000) Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. *Environ Health Perspect* 108:1203–1207
17. Karmaus W, Kuehr J, Kruse H (2001) Infections and atopic disorders in childhood and organochlorine exposure. *Arch Environ Health* 56:485–492
18. Luebke RW, Chen DH, Dietert R, Yang Y, King M, Luster MI (2006) The comparative immunotoxicity of five selected compounds following developmental or adult exposure. *J Toxicol Environ Health B Crit Rev* 9:1–26
19. Dietert RR, Lee JE, Bunn TL (2002) Developmental immunotoxicology: emerging issues. *Hum Exp Toxicol* 21:479–485
20. Selevan SG, Kinnel CA, Mendola P (2000) Identifying critical windows of exposure for children's health. *Environ Health Perspect* 108(Suppl. 3):451–455
21. Daston G, Faustman E, Ginsberg G, Fenner-Crisp P, Olin S, Sonanwane B, Bruckner J, Breslin W, McLaughlin TJ (2004) A framework for assessing risks to children from exposure to environmental agents. *Environ Health Perspect* 112:238–256
22. Landrigan PJ, Kimmel CA, Correa A, Eskenazi B (2004) Children's health and the environment: public health issues and challenges for risk assessment. *Environ Health Perspect* 112:257–265
23. Kimmel CA, Collman GW, Fields N, Eskenzi B (2005) Lessons learned for the National Children's Study from the National Institute of Environmental Health Sciences/U.S. Environmental Protection Agency Centers for Children's Environmental Health and Disease Prevention Research. *Environ Health Perspect* 113:1414–1418
24. Landrigan PJ, Trasande L, Thorpe LE, Gwynne C, Lioy PJ, D'Alton ME, Lipkind HS, Swanson J, Wadhwa PD, Clark EB, Rauh VA, Perera FP, Susser E (2006) The National Children's Study: a 21-year prospective study of 100,000 American children. *Pediatrics* 118:2173–2186
25. World Health Organization. International Programme on Chemical Safety (2006) Principles for evaluating health risks in children associated with exposure to chemicals. WHO Publications, Geneva
26. Dietert RR (2008) Developmental immunotoxicity (DIT) in drug safety testing: matching DIT testing to adverse outcomes and childhood disease risk. *Curr Drug Saf* 3:216–226
27. Holladay SD, Smialowicz RJ (2000) Development of the murine and human immune system: differential effects of immunotoxicants depend on time of exposure. *Environ Health Perspect* 108(Suppl. 3):463–473
28. Dietert RR, Etzel RA, Chen D, Halonen M, Holladay SD, Jarabek AM, Landreth K, Peden DB, Pinkerton K, Smialowicz RJ, Zoetis T (2000) Workshop to identify critical windows of exposure for children's health: immune and respiratory systems work group summary. *Environ Health Perspect* 108(Suppl. 3):483–490
29. Luster MI, Dean JH, Germolec DR (2003) Consensus workshop on methods to evaluate developmental immunotoxicity. *Environ Health Perspect* 111:579–583
30. Holsapple MP, Paustenbach DJ, Charnley G, West LJ, Luster MI, Dietert RR, Burns-Naas L (2004) Symposium summary: children's health risk-What's so special about the developing immune system? *Toxicol Appl Pharmacol* 199:61–70
31. Luster MI, Johnson VJ, Yucesoy B, Simeonova PP (2005) Biomarkers to assess potential developmental immunotoxicity in children. *Toxicol Appl Pharmacol* 206:229–236
32. Dietert RR, Holsapple M (2007) Methodologies for developmental immunotoxicity (DIT) testing. *Methods* 41:123–131
33. Selgrade MK (2007) Immunotoxicity: the risk is real. *Toxicol Sci* 100:328–332
34. Burns-Naas LA, Hastings KL, Ladics GS, Makris SL, Parker GA, Holsapple MP (2008) What's so special about the developing immune system? *Int J Toxicol* 27:223–254
35. Dietert RR (2009) Developmental immunotoxicology: focus on health risks. *Chem Res Toxicol* 22(1):17–23
36. Dietert RR, Zelikoff JT (2008) Early-life environment, developmental immunotoxicology, and the risk of pediatric allergic disease including asthma. *Birth Defects Res B Develop Reprod Toxicol* 83(6):547–560

Immunotoxicity Testing

Methods and Protocols

Dietert, R.R. (Ed.)

2010, X, 430 p. 43 illus., Hardcover

ISBN: 978-1-60761-400-5

A product of Humana Press