Colloquium Introduction

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Traverse City, MI
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Colloquium Objectives

● What’s so special about the developing immune system?
  – Why should you be interested in Developmental Immunotoxicology?
  – Historical Perspective Leading to Testing Recommendations

● Current understanding of mammalian immune development and its impact on testing strategies and interpretation

● New science impacting study design and data interpretation
  – Translational impact of environmental/nutritional alterations during human development
  – Prenatal vs postnatal hematopoiesis and its impact on immunity

● Looking toward the Future: early efforts in developing NAMs
<table>
<thead>
<tr>
<th>Time</th>
<th>Title</th>
<th>Speaker</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:10</td>
<td>The Evolution of the Discipline of Developmental Immunotoxicology</td>
<td>Leigh Ann Burns Naas, PhD, DABT, ATS, ERT, Magnolia Toxicology Consulting</td>
<td>Traverse City, MI</td>
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<tr>
<td>10:00</td>
<td>Comparative Developmental Immunology and Implications for Testing and Data Interpretation</td>
<td>Hollie Skaggs, PhD, Horizon Therapeutics</td>
<td>Wilmington, DE</td>
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<td></td>
<td><strong>Break (10 min)</strong></td>
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<td>10:45</td>
<td>The Role of the Metagenome and Microbiome During Pregnancy and Lactation on the Risk of Immune-Related Disease</td>
<td>Kjersti M. Aagaard, MD, PhD, FACOG</td>
<td>Baylor College of Medicine, Houston, TX</td>
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<tr>
<td>11:20</td>
<td>Prenatal Immunity Represents a Functionally Distinct Hematopoietic Lineage</td>
<td>Eliver Ghosn, PhD, Emory University School of Medicine</td>
<td>Atlanta, GA</td>
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<tr>
<td>11:55</td>
<td>Paving the Road Toward the Development and Acceptance of Alternative (In Vitro) Methods to Assess Developmental Immunotoxicity</td>
<td>Fenna Sillé, PhD, Johns Hopkins Bloomberg School of Public Health</td>
<td>Baltimore, MD</td>
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<tr>
<td>12:20</td>
<td>Roundtable Discussion</td>
<td>Moderator: Patrick Crittenden, PhD, US FDA CFSAN</td>
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The Evolution of the Discipline of Developmental Immunotoxicology

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Conflict of Interest Statement

I have no conflicts to declare.
Objectives

KEY CONCEPTS
DEVELOPMENT OF THE STATE OF THE SCIENCE
EARLY TESTING STRATEGIES
KNOWLEDGE GAPS
What Suggests a Cause for Concern?

A Few Examples

- Pesticides in the Diets of Infants and Children

- Rise in incidence of allergic disease (asthma, rhinitis, food allergy, eczema) in pediatric populations in industrialized countries
  - Of interest, maternal environment may influence this: genetic risk of allergy, intra-uterine factors, dietary factors

- Preterm infants on supplemental oxygen
  - Increased risk for respiratory viral infections, airway hyperresponsiveness, asthma, more out-of-school sick days, and rehospitalizations

References:
1. NRC, 1993
What’s So Special About the Developing Immune System?

- It is known that common infectious diseases can **occur more often** and are **usually more severe** in the very young when compared to adolescents and adults.
- Yet, infants can mount a vigorous immune response to tissue and organ allografts (“non-pathogenic” foreign antigens) and to vaccines.
- Infants are easily immunosuppressed; susceptible to immune toxicities and immune manipulations

→ suggests a “malleable” immune system
What’s So Special About the Developing Immune System?

**Basic Tenet**

Children differ significantly from adults in their biological and/or physiological responses to environmental exposures.

**Hypothesis**

The developing immune system demonstrates greater susceptibility to chemical perturbation than the adult immune system.
Why Might Kids Be Different?

- Organogenesis
- “Waves” of cellular proliferation, differentiation, migration
- Complex cell-cell interactions; maturation and “education”
- Growth
- Metabolic maturation
- Behaviors and activities
- Individual genetic susceptibilities
Zooming Out—Influences on Developing Immune System

Ag-Environ Chemicals, Pharmaceuticals, Food Additives, Supplements/Nutraceuticals, Infections, Other Stressors

How Children May Show Greater Sensitivity

**Qualitative**

Chemicals alter developing immune system, but **not** adult.

**Quantitative**

Chemicals alter the developing immune system, but **at lower doses** than the adult.

**Temporal**

Chemicals exhibit **more persistent** effects than in the adult.

**Reality:** Likely that drugs and non-drug chemicals would show a combination of these.

30 Years

Scientific Discussion and Debate

Basic research on DIT and developmental immunology

Legislation, Regulatory, Guidelines

1993 (NRC Workshop)
1995 (EPA ED Workshop)
1996 (ILSI RSI/EPA Workshop)
1999 (EPA Critical Windows Workshop)
2000 (EPA EWG Established)
2001 (EU Workshop)
2001 (ILSI/ESITC Workshop)
2001 (NEHS/NIOSH Workshop)
2003 (SOT Symposium)
2003 (ILSI/IHESI Intl Panel – Test Framework)
2005 (SOT Sunset Session)
2010 (ILSI HESI ITC Pharma DIT Workshop)
2010-2022 (CAAT Alternatives to DIT Workshops)
2023

NRC 1993
1996 (FQPA, SDWA)
1997 (EO 13045)
2002 (FDA Imtox GFI)
2003 (FDA PREA)
2006 (FDA Peds GFI)
2007 (EC 1901/2006 PIP)
2011 (OECD 443)
“Critical Windows” of Exposure and Evidence for Differential Sensitivity
Critical Windows of Exposure for Immune Development

Initiation of hematopoiesis
Colonization of bone marrow and thymus
Migration of stem cells and expansion of progenitor cells
Maturation of immunocompetence
Establishment of immune memory

Developmental Events

Conception
Birth
Sexual Maturity

Critical Windows of Vulnerability During Prenatal Development of the Rodent Immune System

From K.S. Landreth, "Critical windows in development of the rodent immune system," ACT symposium on developmental immunotoxicology, November, 2009
Critical Windows of Vulnerability During Postnatal Development of the Rodent Immune System

From K.S. Landreth, “Critical windows in development of the rodent immune system,” ACT symposium on developmental immunotoxicology, November, 2009
### Critical Windows of Development

<table>
<thead>
<tr>
<th>Critical Window</th>
<th>Proposed Impact of Developmental Immunotoxicants</th>
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<tbody>
<tr>
<td>Hematopoietic stem cell formation from undifferentiated mesenchymal cells</td>
<td>Failure of stem cell formation, abnormal hematopoiesis, partial to complete immune failure</td>
</tr>
<tr>
<td>Migration of hematopoietic stem cells to fetal liver and thymus, early</td>
<td>Thymic atrophy, impaired postnatal T cell function, impaired innate immunity, inflammation in organs where macrophages play a role in development (e.g., brain, testes, lung)</td>
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<tr>
<td>hematopoiesis, and migration of macrophages to tissues</td>
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<tr>
<td>Establishment of the bone marrow as a primary site of hematopoiesis, and the</td>
<td>Increased risk of later-life cancer, autoimmunity, or allergy</td>
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<tr>
<td>bone marrow and thymus as primary lymphopoiesis sites for B and T cell,</td>
<td></td>
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<tr>
<td>respectively</td>
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<tr>
<td>Perinatal functional development and the maturation of immunocompetence</td>
<td>Shifted Th1-Th2 balance which could result in loss of the conceptus during pregnancy, increased incidence of childhood viral infection, reduced response to vaccinations</td>
</tr>
<tr>
<td>Mature immune responses and establishment of immunologic memory</td>
<td>Increased risk of infection (common and opportunistic) and cancer, allergy/atopy</td>
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</table>
Is there Evidence for Differential Sensitivity?

- **Lead**: Quantitative and Qualitative
- **Diazepam (valium)**: Quantitative and Temporal
- **Diethylstilbestrol (DES)**: Temporal
- **TBTO**: Quantitative and Temporal
- **TCDD**: Quantitative and Temporal

# Putative Impact of Xenobiotics on the Developing Rodent Immune System during Critical Windows of Development

<table>
<thead>
<tr>
<th>Critical Window Events</th>
<th>Xenobiotics</th>
<th>Reported Effects a</th>
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<tbody>
<tr>
<td>Macrophage seeding in tissues</td>
<td>lead</td>
<td>altered regulation of regional macrophages; fewer colony-forming units; enhanced inflammatory-related response</td>
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<tr>
<td></td>
<td>chlordane</td>
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<td></td>
<td>lipopolysaccharide</td>
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<td></td>
<td>ozone</td>
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<tr>
<td>Thymic seeding, maturation and clonal selection</td>
<td>polycyclic aromatic hydrocarbons</td>
<td>thymic atrophy; apoptosis; altered thymic selection</td>
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<tr>
<td></td>
<td>2,3,7,8-tetrachlorodibenzo-p-dioxin</td>
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<td></td>
<td>cyclophosphamide</td>
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<tr>
<td></td>
<td>diethylstilbestrol</td>
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<tr>
<td></td>
<td>nicotine</td>
<td></td>
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<tr>
<td></td>
<td>polychlorinated biphenyls</td>
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<tr>
<td>Development of Tregs and seeding</td>
<td>bisphenol-A</td>
<td>fewer regulatory T cells; altered T-reg function</td>
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<tr>
<td></td>
<td>cyclophosphamide</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cyclosporine A</td>
<td></td>
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<tr>
<td>Maturation and initiation of function of dendritic cells and monocyte/macrophage function</td>
<td>lead</td>
<td>delayed maturation of Th1 response/maintenance of Th2-skewing</td>
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<tr>
<td></td>
<td>dexamethasone</td>
<td></td>
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<tr>
<td></td>
<td>tobacco Smoke</td>
<td></td>
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<tr>
<td></td>
<td>alcohol</td>
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<tr>
<td>Functional increase in Th1 activity at birth</td>
<td>lead</td>
<td>Th2-skewing (e.g., decreased cytotoxic T cell activity; enhanced IgE)</td>
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<tr>
<td></td>
<td>mercury</td>
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Methods to Assess Developmental Immunotoxicology
Parameters

- **Standard Toxicology Measurements**
  - Organ Weights—spleen, thymus
  - Clinical Pathology
    - Total white cell count, differential leukocyte count (priority when sample volume is limited)
    - Serum albumin, globulin; total protein; and A:G ratio
  - Anatomic Pathology (Immunopathology)

- **Immunophenotyping**

- **Functional Evaluation**
  - T-Dependent Antibody Response (TDAR)—Gold Standard
  - Cell-Mediated Immunity
  - Innate Immunity
The Word on Immunopathology in Developing Rat

- Current early data suggests there is little potential for histopathologic detection of chemically-mediated alterations in the developing immune system of rats at or prior to GD 15

- Major perturbations (e.g., failure to form) detectable by GD 20

- Immune system is intact by PND 22, but histologic features suggest relative inactivity (e.g., lack of stimulation by exogenous stimuli)

- For consideration: Historically speaking, most pathologists have far less experience with fetal and neonatal immune histology compared with adult immune histology

Splenic Lymphocyte Populations

10-Day Old Rats

21-Day Old Rats

Ladics et al., Tox. Methods 10:283-311, 2000
Humoral Response in Rats of Various Ages

- TDAR can be sub-optimally assessed at PND21 and optimally PND42-49 onward.
- B cells are at an adult level in the spleen at PND21, but T cells are only ~50% of adult levels.

Ladics et al., *Toxicol Methods* 10:238-311, 2000
Cell-Mediated Response in Rats of Various Ages

- CMI can be assessed at PND21, depending on the assay used

# Age-Dependent Development of the Immune System

**Human**

<table>
<thead>
<tr>
<th>Age: System:</th>
<th>Birth</th>
<th>1st Solid Food</th>
<th>Weaning</th>
<th>Puberty</th>
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<tbody>
<tr>
<td>Premature to Full Term</td>
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<tr>
<td>Neonate (Term – 27 d)</td>
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<tr>
<td>Infant/Toddler (28 d – 23 mo)</td>
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<td>Child (2-11 yr)</td>
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<tr>
<td>Adolescent (12-18 yr)</td>
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<tr>
<td>Adult (&gt;18 yr)</td>
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**Immune**
- Neonatal structural expansion of primary and secondary immune tissues
- Progressive population of secondary immune tissues and development of memory as a function of time and environment

**Rat**

<table>
<thead>
<tr>
<th>System</th>
<th>General Considerations</th>
<th>Neonate (~PND 1-10)</th>
<th>1st Solid Food (~PND 15)</th>
<th>Weaning (~PND 21-25)</th>
<th>Puberty (M: PND 42) (F: PND 35)</th>
<th>Adult (~PND 70)</th>
</tr>
</thead>
</table>
| Immune | • Progressive population of secondary immune tissues and development of memory as a function of time and environment  
• TDAR typically assessed after PND 45 | | | | | |

**Critical period of structural & functional growth and development**

**Active period of structural and/or functional maturation**

**Slow continued growth and/or refinement of function**

**Structurally and functionally fully mature**

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Details and impact will be addressed in the next talk by Dr. Skaggs.

Taken from ICH S11, Appendix A, 2020
Study Design Considerations….

Different Designs for Different Questions!
and/or
The Data Reflects the Study Design
Dosing Paradigms in Historical DIT Studies

- ~GD 6
- Birth
- Weaning
- Young Adult (PND 42-49)
The Effects of Perinatal/Juvenile Methoxychlor Exposure on Adult Rat CNS, Immune, and Reproductive Function

Slide compliments of Ralph Smialowicz, US EPA
Factors to Consider in Study Design and Interpretation

Because of the extensive DART database, the rat is preferred model.

Evaluation is generally case-by-case.

“Triggers” for assessing DIT may include SAR info, results from other toxicity studies and intended use.

“Cause for Concern”

Immunogenicity of proteins/biotherapeutics may require segmented dosing regimens.

Incorporate into existing protocols, to the extent possible (rather than stand-alone).

Any protocol should be based on current validated assays.

Sex differences occur—both should be evaluated.

Holsapple et al., Tox. I Sci. 83:18-24, 2005
Factors to Consider in Study Design and Interpretation

**Dose Selection**

- Maternal toxicity may influence endpoints
- Over-exposure from poor high dose selection may confound the ability to determine primary vs. secondary effects
- Consider a dose range-finding study, especially if stand-alone

**Dosing/Exposure**

- Animals should be exposed to the agent through the period of immune assessment to avoid recovery of potential early effects; recovery can be assessed at that point, if necessary
- Understanding of exposure during gestation, lactation, and juvenile life stages is important
- If dosing during gestation/lactation, best to understand placental transfer and secretion into breast milk
- A “negative” DIT study in the absence of exposure is useless
Stand-Alone DIT Study

- MATERNAL DOSING PHASE
  - ~GD 6
  - Birth

- F₁ DOSING PHASE
  - Weaning
  - Young Adult (PND 42-49)

Immunopathology* and/or
Cell-Mediated Immunity


SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety
Extended One-Generation Reproductive Toxicity Study (OECD 443)

- Mating
- Birth
- Weaning
- Pup Selection to F₁ Sets 1-3
- Adult (8-13 Weeks)

Set 1: Hematology, OW, Histology, Phenotyping
Set 3: TDAR (8 wk)

Cooper, RL et al., Crit Rev Toxicol 36(1):69-98, 2006
Add-On to Rat Peri- and Postnatal Development

A.

- MATERNAL DOSING PHASE
  - ~GD 6
  - Birth
  - Weaning
  - Young Adult (PND 42-49)
  - Immunopathology*

B.

- MATERNAL DOSING PHASE
  - ~GD 6
  - Birth
  - Weaning
  - Young Adult (PND 42-49)
  - Immunopathology*
  - Cell-Mediated Immunity
  - TDAR

Culling

histologic assessment feasible on pups

OPT. F1 DOSSING PHASE

NO DOSING / RECOVERY
Add-On to Rat Juvenile Toxicology

- Mating
- Birth
- Weaning

F₁ DOSING PHASE

- Hematology, OW, Histology, Phenotyping, HI Innate, CMI, other

Adult (PND 42-63)

Let the Data Do The Talking
A Weight of Evidence Approach to Testing

Pharmaceutical, Food/Ingredient or Environmental/Occupational Chemical Toxicant

- Exposure potential/intended population
- Epidemiological data and disease relevance
- Potential for risk reduction

Existing General Toxicology Data
- Developmental and reproductive toxicity
- Subchronic/chronic toxicity

Mechanism / Mode of Action

Existing Adult Immunotoxicity Data

Structure – Activity Relationships

Evaluation of Developmental Immunotoxicology

Modified from vonderEmbse and Dewitt, In Immunotoxicity Testing: Methods and Protocols, JC Dewitt, CE Rockwell, and CC Bowman, Eds., 2018
Knowledge Gaps
Summary

- Children are not small adults; several ways kids may be different
  - Development is a continuous process and thus a “moving target”
  - Neonates ≠ toddlers ≠ children ≠ adolescents
- There is evidence for differential sensitivity
- Interpretation of study results relies heavily on study design and animal model used for testing
  - Important! Mammalian immune systems mature at different rates/times during development (Dr. Skaggs)
  - Ability to assess immune system depends on the animal model and relative development of the immune system, and the availability of test methods in that species