Eminent Toxicologist Lecture Series

Immunotoxicology: A Historical Perspective

Society of Toxicology
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Immunotoxicology: A Historical Perspective

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Conflict of Interest Declaration

As presenting author, I certify on behalf of myself and the co-authors of the abstract that:

___X___ Neither myself nor any of my co-authors, including members of our immediate families, have any financial interest or affiliation of the type described above with a commercial organization that has a direct or indirect interest in the subject matter of my presentation.

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Overview/Objectives

• The Formative Period (1977-1990) Drivers:
  – Drivers: Michigan PBB Accident, Immune Suppression & Occupational Allergy
• Methods Development & Validation Period (1980-1988):
  – NIEHS/NTP Promoted Methods Development Contracts, & Student Training
  – Tier Testing Approach was Introduced, Standardized & Interlaboratory Validation
• SOT Acceptance & Organization of Specialty Section (1985)
• Emergence of Regulatory Guidance Period (1996-2005)
• Shift from Descriptive to Mechanistic Studies (1995-Present)
• Why the Introduction of Developmental Immunotoxicology?
• Major Advances in Assessment of Chemical Allergy (LLNA)
• Future & Ongoing Challenges

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The Formative Period (1977-1990) Drivers

• Michigan PBB Accident and Other Environmental Chemical Concerns
• Concern about Immune Suppression
• Understanding of Occupational Allergy
Consequence of Immunomodulation

Immunosuppression

Resistance to Cancer
- Advanced Metastasis
- Overwhelming Infections

Immunostimulation

Autoimmunity
- Minor Symptoms
- Progressive Degeneration

Resistance to Infection
- Limited Neoplasia
- Minor Infections

Hypersensitivity
- Minor Allergy
- Anaphylaxis
Formative Period Meetings

• 1978: Gordon Research Conference on Drug Safety–Immunotoxicology Symposium
• 1980 Williamsburg Workshop: Biological Relevance of Immune Suppression as induced by Genetic, Therapeutic & Environmental Factors
• 1984 CEC & International Programme on Chemical Safety of the World Health Organization: Immuno-toxicology: The Immune System as a Target for Toxic Damage
**Immunotoxicology** is the discipline concerned with studying events that can lead to **undesired effects** as a result of the interactions between xenobiotics and the immune system. These undesired effects may be . . .

- . . . due to direct or indirect actions of the xenobiotic or its metabolite on components of the immune system (e.g., suppression or stimulation); or
- . . . due to an immunologically-based host response to the compound or its metabolite (e.g., hypersensitivity); or
- . . . due to modifications in host (e.g., “self”) antigens caused by the compound or its metabolite (e.g., autoimmunity).
Immunotoxicology’s Founding Fathers

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Methods Development & Validation Period (1979-1988)

- Tier Testing Paradigm Introduced-1979
- NIEHS/NTP Awarded Contracts for Methods Development-1981
- SOT Established Immunotoxicology Specialty Section (1985)
- NIEHS/NTP Sponsored Interlaboratory Validation of Immunosuppression & Host Resistance Assays
### NTP’s Panel of Tests for Detecting Immunotoxicity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Screen (Tier I)</strong></td>
<td></td>
</tr>
<tr>
<td>Immunopathology</td>
<td>Hematology: complete blood count and differential</td>
</tr>
<tr>
<td></td>
<td>Weights: body, spleen, thymus, kidney, liver</td>
</tr>
<tr>
<td></td>
<td>Cellularity: spleen</td>
</tr>
<tr>
<td></td>
<td>Histology: spleen, thymus, lymph node</td>
</tr>
<tr>
<td>Humoral immunity</td>
<td>Enumerate IgM antibody plaque forming cells to T-dependent antigen (e.g., SRBC)</td>
</tr>
<tr>
<td></td>
<td>LPS mitogen response</td>
</tr>
<tr>
<td>Cell-mediated immunity</td>
<td>Lymphocyte blastogenesis to mitogens (Con A)</td>
</tr>
<tr>
<td></td>
<td>and mixed leukocyte response against allogeneic leukocytes (MLR)</td>
</tr>
<tr>
<td>Nonspecific immunity</td>
<td>Natural killer (NK) cell activity</td>
</tr>
</tbody>
</table>
NTP’s Panel of Tests for Detecting Immunotoxicity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Comprehensive (Tier II)</strong></td>
<td></td>
</tr>
<tr>
<td>Immunopathology</td>
<td>Quantitation of splenic B and T cell numbers</td>
</tr>
<tr>
<td>Humoral immunity</td>
<td>Enumeration of IgG antibody response to SRBCs</td>
</tr>
</tbody>
</table>
| Cell-mediated immunity | Cytotoxic T lymphocyte (CTL) cytolysis  
Delayed hypersensitivity response (DHR) |
| Nonspecific immunity | Macrophage function: quantitation of resident peritoneal cells, and phagocytic ability (basal and activated by MAF) |
| Host resistance challenge model | Syngeneic tumor cells:  
- PYB6 sarcoma (tumor incidence)  
- B16F10 melanoma (lung burden)  
Bacterial model:  
- *Listeria monocytogenes* (mortality)  
- *Streptococcus* species (mortality)  
Viral model: *Influenza* (mortality)  
Parasite models: *Plasmodium yoelii* (parasitemia) |
IgM Antibody Forming Cell Assay

End Points:
#AFCs / 10^6 spleen cells
#AFCs / spleen
(spleen cell count)
(spleen weight)

(add complement + sRBC in agar solution)

sRBC around AFC are hemolyzed by complement (e.g., a “plaque”)

Antibody Forming Cell (AFC)
Sheep RBC (sRBC)

Day 4
(3 Hour Incubation)

(500 µl Aliquot)

(expose animals)

(remove spleen)

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NTP’s Immunotoxicology Panel Validation

- NTP Database Developed & Assays Validated--started ~1984; through the collaborative effort of Drs. Luster, Dean, and Munson, a testing panel to assess immunotoxicity in animals was developed and validated.

- Results were described in a series of papers by M. Luster et al., 1988, 1992, 1993.

- 51 chemicals in database--catalysts, solvents, dyes, lubricants, pesticides, disinfectants, drugs, food additives, natural products, etc.

- Multiple endpoints--functional parameters, host resistance, organ weights, differentials.
NTP Interlaboratory Validation Study: “Best” Concordance when Two Tests Combined

- Ab response + NK 94% (34)
- Ab response + thymus wt. 92% (38)
- Flow + T-cell mitogens 92% (24)
- Ab response + Flow 91% (23)
- Flow + NK 90% (21)
- Flow + thymus wt. 90% (21)

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Contribution of Luster’s Laboratory

- With assistance of the EPA and other regulatory agencies, established a framework to allow quantitative risk assessment of immunotoxicology data.
- Developed and validated animal models for occupation asthma and allergic rhinitis using isocyanates as test chemical model.
- Explained the role of genetic variability in humans in developing occupational immune-related diseases including irritant & allergic contact dermatitis, asthma, chronic beryllium disease, and silicosis.
- Elucidated the processes by which xenobiotics produce inflammatory mediated damage and repair (e.g., the role of TNF in chemical induced liver damage and TNF and TGF in skin wound repair).
Luster’s Laboratory Family & Students

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Contribution of Munson’s Laboratory

• Advisor to 17 PhD students and 15 Postdoctoral trainees (MCV)
• Identified Delta 9- tetrahydrocannabinol as an immunosuppressant
• Contributed to NTP’s validation studies
• Chemicals studied: dioxin (Holsapple & Kaminski); polycyclic aromatic hydrocarbons (White); gallium arsenide (Burns); silicones (Wilson); and latex hypersensitivity at NIOSH (Stern & Meade)
Munson’s Laboratory Family

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Contributions of My Laboratory at NIEHS & CIIT

• Advisor to 2 PhD students (Hastings & House) and 6 Postdoctoral trainees (Pallardy, Cornacoff, Murray, Thurmond, Babiuk, & Ward)
• Introduced tiered testing paradigm (1979), tumor host resistance models, and the use of human lymphocytes in immunotoxicology
• Contributed to NTPs Tier Panel Interlaboratory Validation
• Demonstrated altered viral resistance following TCDD exposure
• Chemicals studied: diethylstilbestrol, cyclophosphamide, phorbol myristate acetate, benzo[a]pyrene, 7,12-dimethylbenz[a]anthracene, ethylene glycol monomethyl ether, 1,3-butadiene, TCDD, formaldehyde, and aflatoxin B-2
Dean’s Laboratory Family & Students

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# Xenobiotics Shown to be Immunotoxic (1)

<table>
<thead>
<tr>
<th>Class</th>
<th>Example</th>
<th>Immune Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rodent</td>
</tr>
<tr>
<td>Polyhalogenated Aromatic Hydrocarbons</td>
<td>2,3,7,8-tetrachlorodibenzo-p-dioxin; polybrominated biphenyls; polychlorinated biphenyls; hexachlorobenzene</td>
<td>+</td>
</tr>
<tr>
<td>Metals</td>
<td>Lead, Cadmium</td>
<td>+</td>
</tr>
<tr>
<td>Aromatic Hydrocarbons</td>
<td>Benzene</td>
<td>+</td>
</tr>
<tr>
<td>Polycyclic Aromatic Hydrocarbons</td>
<td>Dimethylbenzanthracene, benzopyrene methylcholanthrene</td>
<td>+</td>
</tr>
<tr>
<td>Pesticides</td>
<td>Trimethyl phosphorothioate, Carbofuran, Chlordane</td>
<td>+</td>
</tr>
</tbody>
</table>

N S = Not Studied

1 = at least one of the chemicals in class shown to modulate immune response in humans
## Xenobiotics Shown to be Immunotoxic (2)

<table>
<thead>
<tr>
<th>Class</th>
<th>Example</th>
<th>Immune Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rodent</td>
</tr>
<tr>
<td>Organotins</td>
<td>Bis(tri-n-butyltin)oxide</td>
<td>+</td>
</tr>
<tr>
<td>Aromatic Amines</td>
<td>Benzidine, Acetyl aminofluorene</td>
<td>+</td>
</tr>
<tr>
<td>Oxidant Gases (Air Pollutants)</td>
<td>NO2, O2, SO2</td>
<td>+</td>
</tr>
<tr>
<td>Natural Products</td>
<td>Selected Antibiotics, Fungal products, Vinca alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Abused Drugs</td>
<td>Ethanol, Cannabinoids, Cocaine, Opioids</td>
<td>+</td>
</tr>
<tr>
<td>Therapeutics</td>
<td>Diphenylhydantoin, Diethylstilbestrol</td>
<td>+</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Asbestos, butylated hydroxyanisole</td>
<td>+</td>
</tr>
</tbody>
</table>

N S = Not Studied
^1 = at least one of the chemicals in class shown to modulate immune response in humans
Drugs Associated With Immune Suppression (1)

Alkylating Agents – Interfere with normal mitosis and cell division: chlorambucil, cyclophosphamide, isophosphamide, and BNCU.

Antimetabolites – Interfere with the synthesis of folic acid and purine and pyrimidine nucleotides: methotrexate, fluorouracil (5-FU) and mercaptopurine.

Antibiotics – create single and double strand DNA breaks: actinomycin D, adriamycin, daunorubicin and bleomycin.
Mitotic Inhibitors—block mitosis and produce metaphase arrest: vinblastine and vincristine.

Antiviral Agents—Inhibit viral replication by blocking viral DNA synthesis: zidovudine, ribavirin, AZT, ganciclovir, and acyclovir.

Immunosuppressive Agents—Suppress the immune system through various mechanisms including the inhibition of T-cell activity and phagocytosis: cyclosporine A and azathioprine.
ISS’s 25th Anniversary (1985-2010)

- 50 to >400 members
- Major contributor to SOT’s annual meeting program

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Emergence of Regulations


• 2000, Committee for Proprietary Medicinal Products (CPMP), *Note for Guidance on Repeated Dose Toxicity (CPMP/SWP/1042/99)*


• 2002, OECD Guidelines for the Testing of Chemicals 429: Skin Sensitization: Local Lymph Node Assay
Developmental Immunotoxicology

• Recognized that during organogenesis & maturation the immune system has increased sensitivity to toxicants (Faith & Moore, 1977).


• Consensus workshops held on methods to evaluate (2001-2003)
Allergic Contact Dermatitis

Contact dermatitis is the most common occupational skin disease as well as a major non-occupational, environmental problem

• 5.7 million physician visits per year
• 20% of cases are allergic type
• 80% of cases are irritant type
Kimber & Dearman’s Laboratory:
Developed the Local Lymph Node Assay (LLNA) that demonstrated discrimination between contact allergens and non-sensitising chemicals that became the gold standard for hazard identification.
Local Lymph Node Assay

**DNCB**

- Concentration (%w/v)
- Stimulation Index

**PABA**

- Concentration (%w/v)
- Stimulation Index

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Cytokine Induction of Langerhans Cell Migration

Initiation of Langerhans cell migration from the skin is dependent upon the action of TNF-α and IL-1β.
Contact allergens (Th1) and chemical respiratory allergens (Th2) induce different cytokine expression.
### Materials Associated With Contact, Food or Respiratory Allergy

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmaceuticals</td>
<td>Phenylglycine acid chloride, Piperazine, Amprolium hydrochloride, Antihistamines, Anesthetics</td>
<td>Ampicilline, Spiramycin, Antibiotic dust, Quinidine, Plasma substitutes</td>
</tr>
<tr>
<td>Foodstuffs</td>
<td>Castor bean, Green coffee bean, Papain, Tree nuts, Peanuts</td>
<td>Pancreatic extracts, Grain and flour, Molds, Shell fish</td>
</tr>
<tr>
<td>Industrial Chemicals</td>
<td>Ethylenediamine, Diisocyanates (TMI, HDI, MDI), Metallic salts</td>
<td>Phthalic anhydride, Trimellitic anhydride</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Wood dusts, Latex proteins, Flour</td>
<td>Animal products, Fragrance components, Detergent enzymes (subtisilin)</td>
</tr>
</tbody>
</table>
Future & Ongoing Challenges

• Risk Assessment using Immune Function Measurements
• Sensitivity of the Developing Immune System to Toxic Insult
• Identifying Susceptible Populations
• Immunotoxicity Risk Assessment using Human Lymphocytes or Stem Cells
• Safety Evaluation of Immunotherapeutics, Immuno-modulators, micro-RNA, T Cell Education, etc.
References (1)

- Dean, J.H., Padarathsing, M.L. and Jerrells, T.R., Assessment of immunobiological effects induced by chemicals, drugs or food additives. I. Tier testing and screening approach, *Drug Chem. Toxicol.* 27, 23, 1979
References (2)


Thank You

Thanks to Drs. Germolec, Burns Naas, Luster, Munson, & Kimber for their contributions

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