Introduction to the new WHO/IPCS Guidelines for Immunotoxicity Risk Assessment for Chemicals
April 25, 2012

Assessment of Immunotoxicity (Immune System, Immunosuppression, Immunostimulation, Sensitization, and Autoimmunity) – MaryJane Selgrade, ICF International

Immunotoxicity Risk Assessment with Case Studies
- Andrew A. Rooney – NIEHS/DNTP
Immune System - Function

• Defense against
  – infection (bacteria, viruses, fungi, parasites)
  – tumors

• Response to foreign proteins
  – can result in allergy

• Distinguishes self from non-self
  – if tolerance to self breaks down results in autoimmune disease
Leukocytes

- Granulocytes (polymorphonuclear leukocytes)
  - Neutrophils  Eosinophils  Basophils/mast cells*

- Lymphocytes
  - Natural Killer Cells, T cells, B cells

- Monocytes/macrophages*

*found in blood/more activated form found in tissues
Immunotoxicology

The study of undesired effects resulting from interactions of xenobiotics with the immune system

- Suppression
- Infectious
- Neoplastic
- Allergic
- Autoimmune

Stimulations
Test Guideline Availability

- Guidelines Available
  - Immune suppression
  - Contact hypersensitivity (allergic contact dermatitis)

- No guidelines – Wt of Evidence Approach
  - Respiratory hypersensitivity
  - Systemic hypersensitivity
  - Autoimmunity
  - Other types of immune stimulation, e.g. adjuvant effects
IMMUNOSUPPRESSIVE COMPOUNDS

- **Drugs:** cyclophosphamide, cyclosporin A, glucocorticoids (Dexamethazone), azothioprine
- **Heavy metals:** Hg, Cd, Ni, Pb
- **Pesticides:** Chlorodane, DDT, Dieldrin
- **Industrial Compounds:** TCDD, PCBs, PBBs, benzene, PAHs
- **Addictive Substances:** cocaine, ethanol, opiates, cannabinoids, nicotine
- **Air Pollutants:** ETS, O₃, NO₂
- **Radiation:** Ionizing, UV
Immunotoxicity Testing Tier 1

- Immuno-pathology
- Antibody Immunity
- Cell-mediated
- Nonspecific

- Hematology: CBC & Diffs
- Weights: body, spleen, thymus
- Histopath: thymus, spleen, LN
- IgM to SRBC*
- Lymphocyte Prolif B cell mitogens (LPS, STM)
- Lymphoproliferative response: T cell mitogens ConA, PHA, allogeneic leukocytes
- Natural Kill Cell, Phagocytosis
Immunotoxicity Testing Tier 2

- Immuno-pathology
- Humoral
- Cell-mediated
- Non-spec
- Cytokines
- Host resistance

- B & T cell phenotype
- IgG to SRBC, IgM to a T independent Ag
- Cytotoxic T lymphocyte, (CTL), delayed-type hypersensitivity (DTH)
- Phagocytosis, neutrophil chemotaxis, Complement activity
- IIs, IFNs, TNFs, CSFs
- bacterial, parasitic, viral, tumor cell, fungi
IgM Response to SRBCs

IV SRBC

Remove spleen 4d

Or draw blood for ELISA 5-6 d

Assess antibody forming cells
Parallelogram Model
(relating rodent data to human risk)

- **ETS**
  - Epi data \(\text{risk of cancer in offspring of smoking moms}\)
  - \(\text{tumors in rodents exposed in utero & challenged with tumors}\)
  - \(\text{cytotoxic T cell response}\)

- **Arsenic**
  - \(\text{High incidence of tumors in exposed humans}\)
  - \(\text{immune responses in mice}\)
  - \(\text{T cell responses with arsenic in urine in humans}\) (Selgrade, ToxSci 100:328-332,2007)

- **PCB**
  - \(\text{Ab in rodents, infection}\)
  - \(\text{Ab to vaccine quantitatively related to exposure at birth}\)
  - \(\text{Frequency of infection}\)
Hypersensitivity

Definition: Excessive humoral or cellular response to an antigen which can lead to tissue damage
Hypersensitivity: Classification

- **Type 1**: IgE mediated (Immediate type)

- **Type 2**: IgM, IgG, cytolysys of cells

- **Type 3**: IgM, IgG Immune complex mediated

- **Type 4**: T-cell mediated (delayed-type)
Two Stages (Distinguishes from irritation)

Induction
Sensitization (1st exposure)

Elicitation
Challenge (subsequent exposure)
Type I (Immediate) (Atopy)

**Sensitization**
- T cells
- B cells
- Plasma cells
- IgM, IgG, IgA, IgD, IgE

**Elicitation**
- Mediators (as such Histamine)
- Bronchoconstriction

**Mast Cell Degranulation**
- Fc receptor
- IgE

**Example:** Bee Sting
Type 2 (Cytotoxic)

Sensitization

Ag $\rightarrow$ Ab (or autoantibody)
Ag binds to cell surface

Elicitation

Ab bind to cell bound antigen

Cell lysis

Examples:
Goodpastures syndrome
Hemolytic anemia
**Type 3 (Arthus)**

**Sensitization**

\[ \text{Ag} \rightarrow \text{Ab} \]

**Elicitation**

Formation of Ag/Ab complexes; Deposition in tissues

Examples: Late onset asthmatic response, fibrosis, serum sickness

**Activation of macrophages And Complement**

Influx of PMN’s, Eosinophils, Lymphocytes (Inflammation)

Activated macrophage

Polymorphonuclear leukocyte
Inflammatory activity

**Cytokines**

**Activated macrophage**

Example:

**Contact Sensitivity, Berylliosis**

**Type 4 (Cell Mediated)**

**Sensitization**

- Antigen
- Ag presenting cell
- Clonal expansion

**Elicitation**

- Antigen
- Ag presenting cell
- Activated T cell

T cell

- Cytokines
- Activated macrophage
- Inflammatory activity
• In absence of infection TGFβ production by dendritic cells predominates and IL-1 is low.

• T reg prevent responses to commensal organisms, food proteins, & self (tolerance)

• During early infection dendritic cell rapidly start to produce IL-6.

• Th-17 cells cause epithelial cells to secrete chemokines that attract inflammatory cell

• Targets extra-cellular pathogens
## Type IV Responses

<table>
<thead>
<tr>
<th>Immune cell</th>
<th>Th1</th>
<th>Th2</th>
<th>CD8 (CTL) Tc</th>
</tr>
</thead>
<tbody>
<tr>
<td>antigen</td>
<td>Soluble (MHC II)</td>
<td>Soluble (MHC II)</td>
<td>Cell-associated MHC I</td>
</tr>
<tr>
<td>Effector mechanism</td>
<td>Macrophage activation</td>
<td>Eosinophil activation</td>
<td>cytotoxicity</td>
</tr>
<tr>
<td>Examples</td>
<td>Tuberculin Rx Contact dermatitis</td>
<td>Chronic asthma/allergy</td>
<td>Contact dermatitis</td>
</tr>
</tbody>
</table>
CD4+ Regulatory T Cells
(Inhibit T cell responses)

• Natural Regulatory T cells (Central Tolerance)
  – Develop in Thymus
  – CD25+, Express FoxP3 transcription factor
  – Effects mediated by direct contact, & possibly IL-10 & TGFβ

• Adaptive Regulatory T Cells (Peripheral Tolerance)
  – Th3 (mucosal origin)
    • IL-4, IL-10, TGFβ
  – TR1 (origin unclear)
    • TGFβ
  – FOXP3 positive (as yet unnamed)
    • TGFβ
  – Relationship between subtypes unclear
Potential Effects of Chemicals on Allergic Disease

• Can themselves act as antigens
  – Haptens
    • Contact sensitizers
    • Respiratory sensitizers
  – Proteins
    • Respiratory allergens
    • Food Allergens

• Can enhance development or expression of allergic reactions
Potential Contact Sensitizers

- Cosmetics and Fragrances
- Dyes
- Preservatives (formaldehyde)
- Metals (Ni, Co, Be, Cr)
- Pesticides

(Poison ivy-type reaction - delayed type IV)
Contact Hypersensitivity
Allergic Contact Dermatitis (ACD)

**A Induction**
- Allergen
- SC
- Keratinocytes
- Langerhans Cell (LC)
- Cytokines
- LC present Ag
- Process Ag migration to lymph node
- Lymphocyte proliferation
- Effector & memory cells

**B Elicitation**
- EDEMA and ERYTHEMA
- Cytoxic Ag presentation
- CD8
- CD4
- Cytokines chemotaxis
- IL-70
- "Primed" lymphocytes
- INFLAMMATORY CELLS
Guinea Pig
Maximization
Test

ID injection w/ and without FCA plus topical application: Days 5-8
Day 20-22 topical challenge
Read: 48, 72 h after challenge
>30% positive

Guinea Pig
Maximization
Test

Induction

Challenge

Endpoint
erythema

Criteria

> 30% positive

Guinea Pig
Maximization
Test

20 animals/group

Buehler Assay

Topical application - closed patch:
Days 0, 6-8, 13-15
Day 27-28 topical challenge untreated flank, 6 h
Read: 21, 24, 48 h after removing patch
> 15% positive

HRIPT
Human Repeat Insult Patch Test

Topical Application Closed patch, 24 hr
3x/wk, 3wk
Rest 2 wk, apply patch 24 hr
Read 48 hr
Any positive
The Local Lymph Node Assay

AGENT

IV $^3$H-thymidine

PROLIFERATION
Characteristics of Allergic Asthma

- **Immediate Response**
  - Bronchoconstriction
  - IgE mediated (IL-4)

- **Late phase**
  - Hyperresponsive to non-specific stimuli (methacholine)
  - Eosinophilic Inflammation
  - Th2 mediated (IL-5, IL-13)
Protein allergens

- Detergent Enzymes
- Molds and spores
- Latex
- microbial pesticides
- animal dander

*Not all proteins are equally allergenic; as yet no particular amino acid sequences has been associated with allergenicity
Guinea Pig Intratracheal Test & Detergent Enzymes Assessment

- IT dose response - cytophilic Ab response to unknown
- IT dose response to Alcalase (subtilisin B)
- Test protein/Reference protein = relative potency
- Relative potency $\leq 1$ set exposure level same as alcalase
- Relative potency $> 1$ adjust by appropriate factor
Low Molecular Wt (<3000 molecular weight) Compounds

- Toluene diisocyanate
- Diphenylmethane diisocyanate
- Phthalic anhydride
- Trimellitic anhydride
- Platinum salts
- Reactive dyes
- 50 or so known allergens (asthmagens)
Air Pollutants That Appear to Be Adjuvants

• **Rodent models**
  – Nitrogen dioxide ($\text{NO}_2$)
  – Residual oil fly ash (ROFA)

• **& Human**
  – Diesel exhaust

• **& Rhesus Monkey**
  – Ozone
Systemic Hypersensitivity

• Reactions distant from the site of challenge
  – Skin
  – Respiratory tract
  – Systemic anaphylaxis
    • Common features: urticaria, angioedema, respiratory distress, and shock
    • Can lead to: asphyxia, cardiovascular collapse, cardiac arrest, and death

• Common Toxicology problems
  – Drug reactions
  – Hymenoptera stings
  – Food Allergy
Autoimmune Disease

- Over 25 diseases
- Distinct organs
- Different mechanisms
- Strongly influenced by genetic & epigenetic factors
- A number of chemical modulate

<table>
<thead>
<tr>
<th>Autoimmune Syndrome</th>
<th>Compounds</th>
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<tbody>
<tr>
<td><strong>Hemolytic Anemia</strong></td>
<td>Methyldopa, Penicillin</td>
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<tr>
<td></td>
<td>Sulfa Drugs</td>
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<tr>
<td><strong>Hepatitis</strong></td>
<td>Halothane, Interferon-α</td>
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<tr>
<td></td>
<td>Ethanol, Phenobarbital, Lipid-lowering drugs</td>
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<tr>
<td><strong>Myositis</strong></td>
<td>Estrogens, Ultraviolet radiation</td>
</tr>
<tr>
<td></td>
<td>Tryptophan</td>
</tr>
<tr>
<td><strong>Rheumatoid arthritis</strong></td>
<td>Interferon-α, PCBs, Silica, Tobacco smoke</td>
</tr>
<tr>
<td></td>
<td>Organochlorine pesticides, Quinidine, Tetracylines</td>
</tr>
<tr>
<td><strong>Scleroderma</strong></td>
<td>Vinyl Chloride, Trichloroethylene, Spanish Toxic Oil, Tryptophan</td>
</tr>
<tr>
<td></td>
<td>Silicone, Interleukin-2, Diphenylhydantoin</td>
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<tr>
<td><strong>Systemic lupus erythematosus</strong></td>
<td>Trichloroethylene, Silicone, Aromatic Amines, Formaldehyde, Hydralazine, Interferon-α</td>
</tr>
<tr>
<td></td>
<td>Procanimide, Chlorpromazine, Silica, Isoniazid, Penicillamine</td>
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<tr>
<td><strong>Thrombocytopenia</strong></td>
<td>Interferon-α, Gold Salts, Rifampin</td>
</tr>
<tr>
<td></td>
<td>Acetzolamide, Quinine, Iodine</td>
</tr>
<tr>
<td><strong>Thyroiditis</strong></td>
<td>Iodine, Lithium</td>
</tr>
<tr>
<td></td>
<td>PCBs, PBBs, Penicillamine</td>
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Mechanisms of Chemically Induced Autoimmunity

- Formation of neoantigens
- Cross reactive with self
- Release of non-tolerant epitopes from immunologically privileged sites
- Interference with the induction of central tolerance
- Interference with the induction of peripheral tolerance (balance of T regs)
Methods for Assessing Potential Autoimmune Effects of Chemicals

- Epidemiology
- Assessing effect of chemical in autoimmune prone rodents (either spontaneous or induced)
- Identifying immunoglobulin complexes or immunoglobulin deposits by immunohistology
- Monitoring increased levels of serum autoantibodies
- Popliteal Lymph Node Assay (PLNA)
Case Studies