Introduction to Immunology and Immunotoxicology

Dori R. Germolec, Ph.D.
Immunology Discipline Leader
Toxicology Branch, National Toxicology Program
National Institute for Environmental Health Sciences
Research Triangle Park, North Carolina
germolec@niehs.nih.gov
Role of the Immune System in Homeostasis

• Recognition and elimination of pathogenic organisms
  – Bacteria, viruses, fungi, parasites and their products
• Recognition and elimination of neoplastic cells
• Response to foreign proteins
  – Hypersensitivity responses
• Distinguishes self from non-self
  – Breakage of tolerance to self – leads to autoimmunity
• Regulation of the immune response once it has been initiated
A System in Balance

Immunosuppression

Immunostimulation

Altered resistance to Infectious Disease and Neoplasia

Hypersensitivity Autoimmunity
Basics of Immunology

The Immune Response

Innate (Non-specific) Immunity
- Phylogenetically ancient
- First line of defense
- Rapid (minutes – hours)
- Limited recognition
- No cell proliferation required
- Limited memory (? mammals)

Adaptive (Acquired) Immunity
- Cell- and humoral-mediated immunity (T and B lymphocytes)
- Infinite array of specificities
- Slow (days)
- Requires proliferation and differentiation
- Long-lasting memory
Immune System Anatomy

- **Primary Lymphoid Organs**
  - Bone Marrow
  - Thymus

- **Secondary Lymphoid Organs**
  - Spleen
  - Lymph Nodes
  - Peyer’s Patch

- **Tertiary Lymphoid Organs**
  - SALT, BALT, GALT, MALT
Organs of the Immune System

Thymus: source of naive T cells

Thymus size and architecture
- Very sensitive to certain xenobiotics and drugs
- Very sensitive to acute toxicity and stress
Organs of the Immune System

Spleen: Antigen trapping and presentation, clonal expansion, cellular export

Image courtesy of Dr. Jim Faix, Northern Arizona University

Image from NTP atlas of non-neoplastic lesions (http://ntp.niehs.nih.gov/nnl/)
Organs of the Immune System

Lymph nodes: Antigen trapping and presentation, clonal expansion, cellular export

Images from NTP atlas of non-neoplastic lesions (http://ntp.niehs.nih.gov/nnl/)
Organs of the Immune System

Bone Marrow: Primary Source of Immune System Cells
Cells of the Innate Immune System: Granulocytes

Neutrophil (“PMN”)
- First responders
- Phagocytosis and killing of bacteria
- Inflammation

Eosinophil
- Allergy
- Killing parasite larvae

Basophil
- Circulating mast cells
- Allergy/anaphylaxis
- Resistance to intestinal nematodes

Images courtesy of Dr. Michelle Cora, CMPB, NTP, NIEHS
Cells of the Innate Immune System: Monocytes

Monocyte/macrophage
- Phagocytosis and killing of bacteria
- Antigen processing
- Inflammation

Macrophage
Phagocytosis
Inflammatory Mediators

- Eicosanoids
- Hydrolytic Enzymes
- Reactive Oxygen Species
- Reactive Nitrogen Species
- Adhesion Molecules
- Cytokines and Chemokines
<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Source</th>
<th>Target</th>
<th>Action</th>
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<tbody>
<tr>
<td>IFN-γ</td>
<td>T Cells</td>
<td>Lymphocytes, Monocytes</td>
<td>Immunoregulation, Antiviral</td>
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<td>IL-1</td>
<td>Macrophages</td>
<td>T and B Cells</td>
<td>Immunoregulation, Inflammation, Fever</td>
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<td>IL-2</td>
<td>T Cells</td>
<td>T and B Cells, Monocytes</td>
<td>Proliferation, Activation</td>
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<td>IL-4</td>
<td>T Cells</td>
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<td>Division, Differentiation</td>
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<tr>
<td>TNF-α</td>
<td>Macrophages</td>
<td>Fibroblast</td>
<td>Inflammation, Cytotoxicity</td>
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</table>
Cells of the Innate Immune System: Dendritic Cells

- Intersection of innate and adaptive response
- Identify threats via pattern recognition receptors
- Professional antigen presenting cells
Antigen Processing and Presentation

- Foreign antigen
- Antigen uptake
- Processing
- Peptide binds MHC
- MHC transport vesicle
- Presentation (surface expression) of MHC and peptide
Cells of the Adaptive Immune System: B Lymphocytes

- B cells differentiate into plasma cells, secrete antibody: IgM, IgG, IgA, IgE
  - IgM: Primary response, efficient agglutination
  - IgG: Recall response, highest serum concentration
  - IgA: Mucosal surfaces, trapping of microbes
  - IgE: Parasitic infections, allergy, anaphylaxis
Cells of the Adaptive Immune System: T Lymphocytes

- **CD4^+ T helper (Th) cells** produce stimulatory and regulatory cytokines
  - Th1-cellular immunity/inflammation: IL-2, IFNγ, TNFβ
  - Th2-humoral immunity, resistance to helminths, allergy: IL-4, IL-5, IL-10, IL-13
  - Th17-inflammation/resistance to infection and autoimmune disease: IL-17

- **CD4^+CD25^+FoxP3^+ T regulatory (Treg)**: downregulate autoreactive cells

- **CD8^+ T cytotoxic/suppressor (Ts/c)**: direct cytotoxicity, cytokine production
Factors Affecting Immunocompetence

Immunocompetence, in the absence of chemical exposure, is complex, dynamic and affected by fixed and variable factors. Therefore, at the population level, the “normal” range is broad.

- Age
- Sex
- Genotype
- Nutritional status
- Life style choices
Mechanisms of Resistance to Infectious Agents

**Humoral Immunity**
- Antibody
  - Neutralization
  - Opsonization
  - Lysis

**Cell Mediated Immunity**
- Cytokine production
  - Activation of intracellular killing
  - Lysis

**Extracellular pathogens**
- *Staphylococcus*, *Streptococcus*, *E. coli*, viruses/parasites, microbial toxins

**Intracellular pathogens**
- *Listeria*, *M. tuberculosis*, *Leishmania*, viruses

Susceptibility to infection is strongly correlated with immunocompetence and the type of immune system defect.
# Nonimmunological Factors Influencing Outcome of Pathogen Encounter

## Host Factors

- **Physical barriers**
  - Skin, mucus lining, intestinal motility

- **Microbicidal products**
  - Fatty acids on skin
  - Lysozyme in tears, sweat
  - Acid environment of stomach

- **Competitive normal flora**
  - Physical space
  - Inhibitory products/metabolites
  - Microbiome

## Pathogen Factors

- **Dose of organism**
  - Few: easily overcome
  - Many: Overwhelms innate defenses

- **Virulence factors**
  - Toxins
  - Adherence factors
  - Evasion of host IR
    - Mimic host proteins
    - Inhibit or disrupt IR
  - Rapid growth
  - Very low infectious dose
    - Norovirus, Giardia
    - Cryptosporidium
What happens when something goes wrong with the immune response?
Adverse Immune Responses

- Immunomodulation
  - Immunosuppression
  - Immunostimulation
- Hypersensitivity
- Autoimmunity
The NTP Immunotoxicology Testing Paradigm

- How do we evaluate for immunosuppression or immunostimulation after chemical exposure?
  - Basic Toxicology
  - Immune Function Assays
  - Host Resistance Assays

- May be assessed following adult or developmental exposures (sometimes both)
Development of a testing battery to assess chemical-induced immunotoxicity: National Toxicology Program’s Guidelines for Immunotoxicity Evaluation in Mice


- **Screen (Tier I)**
  - Immunopathology (Hematology, Organ weights, Spleen Cellularity, Histopathology)
  - Humoral Immunity (IgM TDAR; Proliferative Responses – LPS)
  - Cell-mediated Immunity (Proliferative responses – MLR, ConA)
  - Non-specific Immunity (NK cell assay)

- **Comprehensive (Tier II)**
  - Cell Quantification (Surface Marker Analysis in spleen)
  - Humoral Immunity (IgG TDAR)
  - Cell-mediated Immunity (CTL, DTH)
  - Non-specific Immunity (Macrophage Function)
  - Host resistance assays
<table>
<thead>
<tr>
<th></th>
<th>Plaque Forming Cells</th>
<th>NK Cell Activity</th>
<th>T Cell Mitogens</th>
<th>MLR</th>
<th>DHR</th>
<th>CTL</th>
<th>Surface Markers</th>
<th>Leukocyte Counts</th>
<th>Thymus/BW Ratio</th>
<th>Spleen/BW Ratio</th>
<th>Spleen Cellularity</th>
<th>LPS Response</th>
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<td>74 (34)</td>
<td>71 (35)</td>
<td>63 (27)</td>
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Why is the T-dependent antibody response highly predictive?
IgM Plaque Forming Cell Assay

Day 4

3 Hour Incubation

500 µl Aliquot

Complement + sRBC in Agar Solution

sRBC around AFC are hemolyzed = PLAQUE

- Antibody Forming Cell (AFC)
- Sheep RBC
Kinetics of the Antibody Response

Primary Antigen Challenge

Secondary Antigen Challenge

Serum Antibody Titer vs. Time (days)

IgM

IgG
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Current testing battery to assess chemical-induced immunotoxicity: National Toxicology Program’s Guidelines for Immunotoxicity Evaluation in Rodents

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  - Cell Quantification (Surface Marker Analysis in spleen)
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  - Cell-mediated Immunity (CTL, DTH)
  - Non-specific Immunity (NK cell assay)

- **Definitive (Tier II)**
  - Humoral Immunity (IgG TDAR)
  - Non-specific Immunity (Macrophage Function)
  - Host resistance assays
<table>
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<th>Challenge Agent</th>
<th>Endpoint Measured</th>
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<tr>
<td>Listeria monocytogenes</td>
<td>Liver, CFU, Spleen CFU, Morbidity</td>
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<tr>
<td>Strep pneumoniae</td>
<td>Morbidity</td>
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<tr>
<td>Plasmodium yoelli</td>
<td>Parasitemia</td>
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<tr>
<td>Influenza Virus</td>
<td>Morbidity, Viral titer/tissue burden</td>
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<tr>
<td>Cytomegalovirus</td>
<td>Morbidity, Viral titer/tissue burden</td>
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<tr>
<td>Trichinella spiralis</td>
<td>Encysted larvae, Adult parasites</td>
</tr>
<tr>
<td>PYB6 Sarcoma</td>
<td>Tumor Incidence (subcutaneous)</td>
</tr>
<tr>
<td>B16F10 Melanoma</td>
<td>Tumor Burden (Lung nodules)</td>
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Decreased Host Resistance: Implications for Human Health

- Most likely adverse outcome in humans is mild to moderate immunosuppression
  - Consequences: decreased resistance to common infections
- Redundancy and reserve capacity compromised
  - At the population level
    - Small but potentially significant increase in incidence or severity of disease
    - Significant economic impact
  - At the individual level
    - Outcome dependent on response phenotype, xenobiotic dose, encounter with infectious agent
Assessment of Immunocompetence in Humans

- Hematology
- Clinical Chemistry
- Serum Immunoglobulins
- Surface Markers
- Proliferation of PBLs
- Macrophage Assays
- Primary or Secondary antibody responses to vaccines
- Health Histories - Self or physician reported infectious disease or neoplasia rates
• A majority of the *in vivo* *ex vivo* tests have an *in vitro* counterpart

• *In vitro* studies often excellent for providing mechanistic or mode of action information

• Have been a number of efforts to validate *in vitro* endpoints with functional immune tests
Adverse Immune Responses

- Immunomodulation
- Hypersensitivity
- Autoimmunity
<table>
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<th>Type</th>
<th>Reaction</th>
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<tr>
<td>I</td>
<td>Immediate (IgE)</td>
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<tr>
<td>II</td>
<td>Antibody-dependent cytotoxic</td>
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<tr>
<td>III</td>
<td>Immune-complexes</td>
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<tr>
<td>IV</td>
<td>Delayed type (DTH)</td>
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Models for Assessing Dermal Sensitization

- Guinea Pig Tests
  - Maximization Test
  - Occlusive Patch Test
  - Respiratory Challenge
  - Systemic Anaphylaxis
- Murine Local Lymph Node Assay
- Mouse Ear Swelling Test
Local Lymph Node Assay

1. DNFB
2. 125IUDR
3. Rest 2 Days
4. Rest 5 Hours
5. Excise Lymph Nodes
6. Process Nodes
7. Count DPMs
Adverse Immune Responses

- Immunomodulation
- Hypersensitivity
- Autoimmunity
Autoimmunity is an inappropriate immune response against self-antigens
Spectrum of Autoimmune Diseases and Putative Autoantigens

Organ Specific

Hashimoto’s Thyroiditis
Thyrotoxicosis
Pernicious anemia
Autoimmune Atrophic Gastritis
Addison’s Disease
Insulin-Dependent Diabetes Mellitus
Goodpasture’s Syndrome
Myasthenia Gravis
Male Infertility (isolated cases)
Sympathetic Ophthalmia
Multiple Sclerosis
Autoimmune Hemolytic Anemia
Ulcerative Colitis
Rheumatoid Arthritis
Scleroderma
Systemic Lupus Erythematosus (SLE)

Thyroglobulin
Thyroid-stimulating hormone (TSH)
H+/K+-ATPase
Intrinsic factor
21-hydroxylase
Glutamic acid decarboxylase 65
Type IV collagen
Acetyl choline receptor
Epididymal glycoprotein, FA-1
Interphotoreceptor retinol binding protein
Myelin basic protein
X-antigen, glycophorin
Catalase; a-enolase
Rheumatoid factor
Topoisomerase 1; laminins
DNA nucleotides and histones

Non-Organ Specific
Modulation of genetic or experimentally-induced autoimmunity can be measured:

- In humans and experimental animals
  - Quantitation of autoantibody levels
  - Measurement of tissue cytokine and cytokine receptor levels
  - Measurement of appropriate and serum or urinary parameters
- In experimental animals only
  - Histologic evaluation of tissue damage
  - Popliteal lymph node assay
Methods to Study Autoimmune Disease

Animal Models

- Genetic Predisposition
  - Insulin-Dependent Diabetes Mellitus
    - NOD (m), BB (r), BN (r)
  - Systemic Lupus Erythematosus
    - MRL+/+ (m), MRL/lpr (m), NZB/NZW (m)

- Autoimmunization
  - Multiple Sclerosis
    - CFA + myelin basic protein (m,mo)

- Organic or Chemical Induction
  - Systemic Lupus Erythematosus
    - Mercury (m,r,mo)
    - Penicillamine (m,r)
    - Procainamide (m,r)
How do we evaluate the data once we have obtained it?
Challenges

- Exposure to a single agent or class of chemicals is very unlikely
- Long latency period between exposure and onset of disease
- “No effects” tough to prove
  - Must distinguish no response in individual vs. no effects in the population
  - Small numbers of subjects
  - Determining true dose is difficult
The NTP has long employed specific conclusion statements, that are approved by the NTP BSC, for its “Toxicology and Carcinogenesis” studies.

The NTP has developed similar conclusion statements to represent a “level of evidence” with regard to evaluating immune system toxicity:
- Clear evidence
- Some evidence
- Equivocal evidence
- No evidence
- Inadequate study

Such an approach allows for comparisons of different studies on the same test substance and for comparisons of conclusions across studies, to ensure similar criteria are employed uniformly.

The NTP has developed guidance notes as to how these criteria should be applied.

http://ntp.niehs.nih.gov/testing/types/criteria/index.html
Weight of Evidence Approach to Hazard Identification

- Guidance Contains Distinct Flow Charts for Immunosuppression, Immunomodulation, Hypersensitivity and Autoimmunity
- Questions prioritized from most predictive to least
- Vary slightly depending on what risk is being considered

From the WHO Harmonization Project – GUIDANCE FOR IMMUNOTOXICITY RISK ASSESSMENT FOR CHEMICALS. Available on the WHO website: http://www.who.int/ipcs/en/
Case studies illustrating adverse immune responses

- Immunomodulation – Dr. Jamie DeWitt, Immunomodulatory Effects of Perfluoroalkyl Substances in Rodents and Humans

- Hypersensitivity – Toxicology and Food Allergy: Case study of the food preservative, tBHQ

- Autoimmunity – Dr. Prakash Nagarkatti, Dietary Supplement Modulation of Autoimmune Disease