Emanuela Corsini

IMMUNOTOSSICOLOGY

Monday, April 4, 2016
RELEVANCE OF IMMUNOTOXICOLOGY

- **Industrialized countries** had faced a significant increase over the past few decades of diseases, such as:
  - cancer (i.e. breast, lung and prostate cancer)
  - allergy
  - autoimmunity (i.e. arthritis)
that can be all linked to immune alterations.

- **Environmental factors** are believed to be a major factor responsible for increased prevalence. Operating in genetically predisposed individuals, they may directly initiate, facilitate or exacerbated pathological immune processes.
Objectives

- Overview of the immune system
- Immunotoxicology: definition
- In vivo evaluation
By definition immunology deals with the functioning of the immune system and its malfunctions in immunological disorders (i.e. autoimmune diseases, hypersensitivities, immune deficiency, cancer).

The word **immunity** derives from the Latin word “*immunis*” meaning exempt.

Immunology is the study of how the body fights disease and infection.
Defense against:

- Infection (bacteria, viruses, fungi, parasites).
- Spontaneously arising neoplasm.
- Any foreign material
- Self / non-self discrimination
ORGANIZATION OF THE IMMUNE SYSTEM

A complex, multi-cellular organ system
- granulocytes
- lymphocytes (B, T)
- macrophages
- dendritic cells

Location
- peripheral blood
- lymphatic fluid

Immunocompetent cells, such as T and B lymphocytes, monocytes/macrophages, granulocytes, that originate from the hematopoietic bone marrow and the thymus, are ubiquitous as they constantly screen the blood, lymph, tissues and organs for potential pathogens or neoplastic cells.
<table>
<thead>
<tr>
<th>Tests</th>
<th>Result</th>
<th>FLAGS</th>
<th>Units</th>
<th>Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBC With Differential/Platelet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>x10E3/uL</td>
<td>4.0 -</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>x10E6/uL</td>
<td>4.14 -</td>
<td>5.80</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>g/dL</td>
<td>12.6 -</td>
<td>17.7</td>
<td></td>
</tr>
<tr>
<td>Hematocrit</td>
<td>%</td>
<td>37.5 -</td>
<td>51.0</td>
<td></td>
</tr>
<tr>
<td>MCV</td>
<td>fL</td>
<td>79 -</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>MCH</td>
<td>pg</td>
<td>26.6 -</td>
<td>33.0</td>
<td></td>
</tr>
<tr>
<td>MCHC</td>
<td>g/dL</td>
<td>31.5 -</td>
<td>35.7</td>
<td></td>
</tr>
<tr>
<td>RDW</td>
<td>%</td>
<td>12.3 -</td>
<td>15.4</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>x10E3/uL</td>
<td>140 -</td>
<td>415</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>%</td>
<td>40 -</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Lymphs</td>
<td>%</td>
<td>14 -</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td>%</td>
<td>4 -</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Eos</td>
<td>%</td>
<td>0 -</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Basos</td>
<td>%</td>
<td>0 -</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Neutrophils (Absolute)</td>
<td>x10E3/uL</td>
<td>1.8 -</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>Lymphs (Absolute)</td>
<td>x10E3/uL</td>
<td>0.7 -</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Monocytes (Absolute)</td>
<td>x10E3/uL</td>
<td>0.1 -</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Eos (Absolute)</td>
<td>x10E3/uL</td>
<td>0.0 -</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Baso (Absolute)</td>
<td>x10E3/uL</td>
<td>0.0 -</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Immature Granulocytes</td>
<td>%</td>
<td>0 -</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Immature Grans (Abs)</td>
<td>x10E3/uL</td>
<td>0.0 -</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>
Half life of blood components

* Red cells - 120 days
* Platelets - 10 days
* Granulocytes – 10 h
* Monocytes – 3-4 days
* Lymphocytes – usually they die after having eradicated the infection (with the exception of the memory cells that live months / years)
## SERUM IMMUNOGLOBULINS

<table>
<thead>
<tr>
<th></th>
<th>IgM</th>
<th>IgG (1-4)</th>
<th>IgA (1-2)</th>
<th>IgE</th>
<th>IgD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heavy chain</strong></td>
<td>μ</td>
<td>γ</td>
<td>α</td>
<td>ε</td>
<td>δ</td>
</tr>
<tr>
<td><strong>MW (Da)</strong></td>
<td>900,000</td>
<td>150,000</td>
<td>385,000</td>
<td>200,000</td>
<td>185,000</td>
</tr>
<tr>
<td><strong>% in serum</strong></td>
<td>6</td>
<td>&gt;80</td>
<td>13</td>
<td>0.002</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>mg/ml in serum</strong></td>
<td>1.5</td>
<td>13.5</td>
<td>3.5</td>
<td>0.00005</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Half life (days)</strong></td>
<td>6-8</td>
<td>20-25</td>
<td>6</td>
<td>6 h</td>
<td>3</td>
</tr>
<tr>
<td><strong>Cross placenta</strong></td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Fix complement</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Function</strong></td>
<td>Main ab of primary responses</td>
<td>Main blood ab of secondary responses, neutralizes toxins, opsonization</td>
<td>Secreted into mucus, tears, saliva colostrum</td>
<td>Ab of allergy and antiparasitic activity</td>
<td>B cell receptor</td>
</tr>
</tbody>
</table>
LYMPHOID ORGANS

Primary lymphoid organs:
- thymus
- bone marrow
- tonsil
- adenoid
- lymph node
- right subclavian vein
- left subclavian vein

Secondary lymphoid organs:
- spleen
- thoracic duct
- large intestine
- small intestine
- Peyer’s patch in small intestine
- kidney
- appendix
- lymphatics
- heart
Primary lymphoid organs or central lymphoid organs (thymus, bone marrow)

- it is where immature lymphocytes differentiate, proliferate and mature into immune competent cells (T and B cells)

Secondary lymphoid organs (i.e. spleen, lymph nodes)

- it is where antigen is brought so that it can be effectively exposed to mature lymphocytes. It is where adaptive immune response initiate.
MATURATION AND DIFFERENTIATION

Rearrange TCR/Ig gene segments and acquire specificity

1 out of 100 cells is successful, the majority of cells are eliminated by apoptosis

CLONAL EXPANSION

A single progenitor cell gives rise to a large number of lymphocytes, each with a different specificity

Removal of potentially self-reactive immature lymphocytes by clonal deletion

Pool of mature naive lymphocytes

Proliferation and differentiation of activated specific lymphocytes to form a clone of effector cells

Effector cells eliminate antigen

© 1999 Elsevier Science/Garland Publishing
### SPECIFICITY

<table>
<thead>
<tr>
<th>Element</th>
<th>Immunoglobulin</th>
<th>(\alpha\beta) receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>(\kappa+\lambda)</td>
</tr>
<tr>
<td>Variable segments (V)</td>
<td>65</td>
<td>70</td>
</tr>
<tr>
<td>Diversity segments (D)</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>D segments read in 3 frames</td>
<td>rarely</td>
<td>–</td>
</tr>
<tr>
<td>Joining segments (J)</td>
<td>6</td>
<td>5(\kappa) .4(\lambda)</td>
</tr>
<tr>
<td>Joints with N and P nucleotides</td>
<td>2</td>
<td>(1)</td>
</tr>
<tr>
<td>Number of V gene pairs</td>
<td>(3.4 \times 10^6)</td>
<td></td>
</tr>
<tr>
<td>Junctional diversity</td>
<td>(~3 \times 10^7)</td>
<td></td>
</tr>
<tr>
<td>Total diversity</td>
<td>(~10^{14})</td>
<td></td>
</tr>
</tbody>
</table>
LYMPHOID ORGANS

**Primary lymphoid organs or central lymphoid organs** (thymus, bone marrow)

- it is where immature lymphocytes differentiate, proliferate and mature into immune competent cells (T and B cells)

**Secondary lymphoid organs** (i.e. spleen, lymph nodes)

- it is where antigen is brought so that it can be effectively exposed to mature lymphocytes. It is where adaptive or specific immune response initiate.
Cellular traffic in the lymph node draining an infection

- **ENTRANCE OF ANTIGENS AND APCs**
- **EXIT OF EFFECTOR MECHANISMS**
Immune Responses

Two major types of immune response:

1. **NATURAL OR INNATE IMMUNE RESPONSE**

2. **SPECIFIC OR ACQUISITE OR ADAPTIVE IMMUNE RESPONSE**
Adaptive Immunity

Adaptive immune system has two arms

**Humoral Immunity**
- Provided by B lymphocytes
- Can recognize protein, polysaccharide, phospholipid and nucleic acid antigens
- Can act against soluble or free antigens
- Provides immunity to extracellular bacteria, viruses and toxins
- Causes Type I, II & III hypersensitivity

**Cell mediated Immunity**
- Provided by T lymphocytes
- Can recognize only protein antigens
- Recognizes antigens presented by APCs with Class I or Class II MHC molecule
- Provides immunity to intracellular bacteria, viruses, fungi and protozoa
- Causes Type IV hypersensitivity
- Causes acute graft rejection

http://www.slideshare.net/sufihannan/humoral-immune-response
Induced CD4CD25 Treg

Bone Marrow

Inflammatory Cells
- Eos.
- Bas.
- PMNs
- Macrophages

Phagocytic Cells

Treg

Thymus

Tr1 (IL-10)

Tr3 (TGF-β)

Induced CD4CD25 Treg

Ag

Ag Presenting Cell (macrophages, Langerhans, etc.)

Eos., Bas., PMNs, Macrophages

Innate Non-Specific Responses

Acquired Specific Responses

T

Act T

IL-2

CD-8 Cyt.T

CD-4 Helper

TH17

TH1

TH2

IL-2

IFN-γ

IL-12

IL-4

IL-5

IL-6

IL-10

IL-13

Act B

Plasma Cells

Specific Antibodies

IgM, IgG, IgE, IgA, IgD

Humoral immunity

Cellular immunity

NK

Bone Marrow

Innate Non-Specific Responses

Acquired Specific Responses
Objectives

- Overview of the immune system
- Immunotoxicology: definition
- In vivo evaluation
DEFINITIONS

- **IMMUNOTOXICOLOGY** studies the adverse effects of xenobiotics on the immune system.
- **IMMUNOTOXIC COMPOUND** is a compound that can alter one or more immune functions resulting in an adverse effect for the host.
**Immunotoxic adverse effects**

- **Immunosuppression**
  - frequent and severe infections
  - atypical infections (e.g. opportunistic infections)
  - virus-induced neoplasias (e.g. B-lymphomas)

- **Inappropriate immunostimulation**
  - Hypersensitivity
  - Autoimmunity
  - Immunostimulation (i.e therapeutic cytokines, mAbs)
  - Inflammatory responses
    - ROS production initiates inflammation which unless quenched may result in chronic inflammatory disease states, e.g. hepatitis, nephritis, multiple system organ failure, etc.
FACTORS AFFECTING SUSCEPTIBILITY TO IMMUNOTOXICITY
IMMUNOTOXICANT

Conditions of exposure
(dose, frequency, duration, route)

Primary target
(lymphoid tissue)

Secondary target
(non lymphoid tissue)

Host related factors:
- Genetic
- Age/sex
- Nutrition/disease
- Hormonal and CNS status

Chemical related factors:
- Chemical reactivity
- Biotransformation
- Toxicokinetic

ALTERED IMMUNE FUNCTION

IMMUNOSUPPRESSION

IMMUNOENHANCEMENT
SPANISH FLU: MORTALITY RATE BY AGE RANGE

SPANISH FLU WORLDWIDE
MORTALITY IN ONE YEAR:
OVER 20 MILLION PEOPLE
Two properties make the immune system vulnerable to chemical or physical insults:

1. Its late developing in life (prenatal and neonatal) and continuous renewal.

2. The delicate control of the balance between activation, silencing and regulation of immune reactivity after each pathogen attack, as well as during immunosurveillance.
Objectives

- Overview of the immune system
- Immunotoxicology: definition
- In vivo evaluation

Diagram:
- Immune enhancement
- Immune suppression
- Normal range
- Hypersensitivity
  - Autoimmunity
- Susceptibility to disease
Immunotoxicology: area of research

- Immunosuppression
- Immunostimulation
- Developmental immunotoxicology
- Immunosenescence
- Molecular immunotoxicology
- *In vitro/ in silico* immunotoxicology
EXPERIMENTAL MODELS
Toxicity test should be performed with species that will respond to a test chemical in a toxicological manner similar to that anticipate in humans (i.e., equivalent metabolism and target organ).

Rodents, however, still appear to be the most appropriate animal model for examining the immunotoxicity of non-species specific compounds.
CURRENT IN VIVO MODELS

At present, assessment of immunotoxic effects relies on different animal models and several assays have been proposed to characterize immunosuppression and sensitization.

Current available animal models and assays are not valid to assess the potential for systemic hypersensitivity and, at this time, autoimmunity is ‘not predictable’, with the RA-PLNA holding promise.
CONDITIONS OF EXPOSURE

- They should reflect the most probable route and level of human exposure.

- Treatment conditions should attempt to establish dose-response curves as well as NOEL.

- To be called immunotoxic a xenobiotic should modulate the immune system at doses below overt toxicity (stress and malnutrition depress immune functions).
Immunosuppression hazards induce

- Significant morbidity and mortality
- Possible unacceptable risks: infections, lymphomas...
Current assessment of immunotoxicity rely on animal tests, which include some immune endpoints in repeated dose tests and call for dedicated tests only when certain alerts indicate a problem.

Different requirements, however, depend on guidelines, i.e. functional tests are required by US-EPA for pesticides; weight of evidence approach for pharmaceuticals (ICH S8).
SIGNS OF IMMUNOTOXICITY

From **standard toxicity studies** the following parameters should be evaluated for signs of immunotoxicity:

- changes in total and differential white blood cell counts
- alterations in immune organ weights and histology
- decreased basal plasma immunoglobulins
- increased incidence of infections
- increased occurrence of tumors, in the absence of genotoxicity, hormonal effects, or liver enzyme induction
- chemical retention in organs/cells of the immune system
## PARAMETER RECOMMENDATION

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>RECOMMENDATION</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Haematology</strong></td>
<td></td>
</tr>
<tr>
<td>Total and differential WBC</td>
<td>All</td>
</tr>
<tr>
<td><strong>Organ weights</strong></td>
<td></td>
</tr>
<tr>
<td>Spleen and Thymus</td>
<td>All</td>
</tr>
<tr>
<td><strong>Histopathology</strong></td>
<td></td>
</tr>
<tr>
<td>Spleen, Thymus</td>
<td>C+H (store all)</td>
</tr>
<tr>
<td>Draining and distant lymph nodes, bone marrow</td>
<td>C+H (store all)</td>
</tr>
</tbody>
</table>

**All** = all dose groups  
**C+H** = control and high dose group

### Table: Specific Component

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specific Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematology</td>
<td>Total leukocyte counts and absolute differential leukocyte counts</td>
</tr>
<tr>
<td>Clinical Chemistry</td>
<td>Globulin levels and A/G ratios</td>
</tr>
<tr>
<td>Gross pathology</td>
<td>Lymphoid organs / tissues</td>
</tr>
<tr>
<td>Organ weights</td>
<td>thymus, spleen, (optional: lymph nodes)</td>
</tr>
<tr>
<td>Histology</td>
<td>thymus, spleen, draining lymph node and at least one additional lymph node, bone marrow, Peyer’s patch</td>
</tr>
</tbody>
</table>
**Hazard identification:**
- histopathology (C+H)
- haematology
- lymphoid organ weights

**Interpretation of TIER I data:**
- dose related toxicity
- other toxicity
- most sensitive parameters
  magnitude of effect

review histopathology of low and middle dose groups

**Conduct Tier II testing** (case by case)

Yes → **Hazard identified** → No → STOP

**NOTE:** to be called immunotoxic a xenobiotic should modulate the immune system at doses below overt toxicity (stress and malnutrition depress immune functions).
Relatively well validated models

Relevance of histological changes in lymphoid organs

One immune function assay absolutely necessary

Host resistance assays as second-line assays
Testing Assays

- General tests
  - Organ weights, plasma/serum enzyme levels
- Nonfunctional tests: status
  - Lymphoid organ weights, lymphoid tissue cellularity, histopathology, immunophenotyping
- Functional tests
METHODS IN IMMUNOTOXICOLOGY

Immunosuppression

- Functional Tests
  - Innate Immunity
  - Humoral-mediated Immunity
  - Cell-mediated immunity
  - Host Resistance Assays
IgM Plaque Forming Cell Assay

End Points

PFC / 10^6 Spleen Cells
PFC / Spleen

Complement + sRBC in Agar Solution

SRBC around AFC are hemolyzed = PLAQUE

- Antibody Forming Cell (AFC)
- Sheep RBC

500 µl Aliquot

3 Hour Incubation

Magnified

Day 4
METHODS IN IMMUNOTOXICOLOGY

Immunomodulation

- Functional Tests
  - Innate Immunity
  - Humoral-mediated Immunity
  - Cell-mediated immunity
  - Host Resistance Assays
Host Resistance Assays

Challenge Model

*Streptococcus pneumoniae*

Antibody, Complement, PMNs

*Listeria monocytogenes*

Macrophages, T Cells

B16F10 Melanoma Tumor

Natural Killer Cells, Macrophages, T Cells
Risk Assessment in Immunotoxicology

I. Sensitivity and Predictability of Immune Tests

The early work from these groups, in terms of immunotoxicology assay development, evaluation and implementation, played a critical role in shaping the development of immunotoxicology guidelines for both pharmaceuticals and environmental chemicals.

II. Relationships between Immune and Host Resistance Tests

Michael J. Luster,∗ Christopher Portier, † D. Gayla Pait,∗,1 Gary J. Rosenthal,∗ Dori R. Germolec,∗ Emanuela Corsini,‡ Benny L. Blaylock,∗ Pam Pollock,∗ Yasuhide Kouchi,§ William Craig,∗ Kimber L. White,§ Albert E. Munson,## and Christine E. Comment∗

∗Environmental Immunology and Neurobiology Section, Laboratory of Integrative Biology and †Statistics and Biomathematics Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709. ‡Department of Toxicology, University of Milan, Milan, Italy; §Taisei Pharmaceutical, Kawauchi-cho, Kokushima 771-01, Japan; and ##Departments of Biostatistics and Pharmacology/Toxicology, Medical College of Virginia/Virginia Commonwealth University, Richmond, Virginia 23298
Risk assessment in immunotoxicology

- **DATABASE**: 50 compounds (NIEHS, CIIT)

- **OBJECTIVES**: to improve future testing strategies, to provide information to aid in risk assessment and to examine the relationship between the immune function and host resistance tests
FIG. 2. Individual and pairwise concordance to establish predictability using the immune panel. Values are presented as percentage concordance which is the sum of specificity (−/−) and sensitivity (+/+). Individual concordance values are shown in boldface on the diagonal of the matrix and combinations, using two tests on the off-diagonal element. Values in parenthesis are the number of chemicals tested for the assay. Since the individual tests were also used to establish the “immunotoxic classification,” the frequency of concordance will obviously increase as the number of tests included for the analysis is increased. (−) No overlapping studies were performed. P values are given for individual concordance only.
CONCLUSIONS

Fund Appl Toxicol 18: 200, 1992

- The performance of only 3 immune tests is sufficient to predict immunotoxic compounds in rodents (100% concordance)

<table>
<thead>
<tr>
<th>Surface marker</th>
<th>No. of tests</th>
<th>Significance of association (Fisher’s Exact test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thy 1.2</td>
<td>24</td>
<td>p = 0.019</td>
</tr>
<tr>
<td>slg⁺</td>
<td>22</td>
<td>p = 0.101</td>
</tr>
<tr>
<td>CD4⁺</td>
<td>3</td>
<td>p = 0.467</td>
</tr>
</tbody>
</table>
CONCLUSIONS

Fund Appl Toxicol 21: 71, 1993

- good correlation between changes in the immune tests and altered host resistance
- no instances where host resistance was altered without affecting an immune tests. However, in some instance immune changes occurred without corresponding changes in host resistance
Initial review of STS to select the most appropriate species (rat or mouse) and gender for immunotoxicity testing. Based on WOE: (1) structural changes in immune system, (2) general toxicity, or (3) default female mouse.

Perform TDAR Assay (SRBC) in selected species.

Dose-related positive at < MTD

IMMUNOTOXIC

Some evidence for immunotoxic potential

Perform NK cell activity assay

Positive at doses < MTD

Not immunotoxic potential

No evidence for immunotoxic potential

NOT IMMUNOTOXIC

Reconsider WoE for potential immunotoxicity

Negative

NOT IMMUNOTOXIC
Normal immune system comprises complementary and compensatory mechanisms (functional reserve).

Failure to identify alterations in host resistance in face of significant changes in functional ability does not necessarily mean the absence of risk to man.

Due to genetic polymorphisms, the response to immunotoxicants vary in human beings.

Thus, alterations in immune functions which may be tolerated well in normal individuals could have more serious consequences for those who are chronically sick, malnourished, or those immune system has yet to develop or is in decline.
The ability to resist pathogen challenge is dependent upon the **degree** of immunosuppression and the **quantity** of pathogen administered.
## AGENTS WHICH INHIBIT IMMUNE FUNCTION AND HOST RESISTANCE

<table>
<thead>
<tr>
<th>CLASS</th>
<th>EXAMPLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyhalogenated aromatic hydrocarbons</td>
<td>TCDD, PCB, PBB</td>
</tr>
<tr>
<td>Metals</td>
<td>Lead, Cadmium, Arsenic</td>
</tr>
<tr>
<td>Aromatic hydrocarbons</td>
<td>Benzene, Toluene</td>
</tr>
<tr>
<td>Polycyclic aromatic hydrocarbons</td>
<td>DMBA, B(a)P, MCA</td>
</tr>
<tr>
<td>Pesticides</td>
<td>Carbofuran, chlordane</td>
</tr>
<tr>
<td>Organotins</td>
<td>Dibutyltin chloride</td>
</tr>
<tr>
<td>Aromatic amines</td>
<td>DMN, benzidine, AAF</td>
</tr>
<tr>
<td>Oxidant gases</td>
<td>NO$_2$, O$_3$, SO$_2$</td>
</tr>
<tr>
<td>Particulates</td>
<td>Asbestos, silica, beryllium</td>
</tr>
<tr>
<td>Ultraviolet light</td>
<td>UV-B</td>
</tr>
<tr>
<td>Mycotoxins</td>
<td>T-2, ochratoxin</td>
</tr>
<tr>
<td>Drugs of abuse</td>
<td>Cocaine, marijuana, alcohol</td>
</tr>
</tbody>
</table>
Potential Mechanisms of Immune Suppression

- Structural alterations in immune tissues and organs
- Alterations in immune cells maturation and differentiation
- Alterations in immune cells activation:
  - Cytotoxicity
  - Inhibition of cell proliferation (prevent clonal expansion)
  - Interference with receptor/ligand interaction or transduction of signals to the nucleus
  - Interference with transcription, translation and release

**Antigen recognition**  **Clonal expansion**  **Effectors**

Antigen → APC → IL-1 → IL-2 → Th CD4+ → proliferation → IL-2, IL-3, IFN-γ, IL-12, TNF-β → Th activated

Th activated → IL-4, IL-5, IL-10, IL-13 → B cells → Humoral immunity

Cell mediated Immunity
**SPECIFIC IMMUNITY**

**Antigen recognition**

- Antigen
  - APC
  - MHC-II

**Clonal expansion**

- CD4
  - IL-1
  - IL-2
  - proliferation
  - TCR

- Th CD4+

**Effectors**

- IL-2, IL-3, IFN-γ, IL-12, TNF-β
- T CD8+
- Th activated
- B cells
  - IL-4, IL-5, IL-6
  - IL-10, IL-13

**Humoral immunity**

**Cell mediated Immunity**

---

**Siti d’azione degli agenti immunosoppressori:**

- a. Anticorpo anti Rh
- b. Corticosteroidi
- c. Globuline anti-timociti, OKT3, anti CD4
- d. Ciclosporina, tacrolimus
- e. Azatioprina, metotrexato, ciclofosfamide, rapamicina, micofenolato mofetil, corticosteroidi
Immunotoxic adverse effects

- **Immunosuppression**
  - frequent and severe infections
  - atypical infections (e.g. opportunistic infections)

Inappropriate immunostimulation can cause more frequent autoimmune diseases, allergic reactions, flu-like syndromes and inhibition of hepatic metabolism.

- **Inflammatory responses**
  - ROS production initiates inflammation which unless quenched may result in chronic inflammatory disease states, e.g. hepatitis, nephritis, multiple system organ failure, etc.
Hypersensitivity reactions are often considered a major health problem in relation to environmental chemical exposure. As a consequence chemical allergy is of considerable importance to the toxicologist, who has the responsibility of identifying and characterizing the skin and respiratory potential of chemicals, and estimating the risk they pose to human health. Regulatory authorities worldwide require testing for ACD potential and appropriate hazard labeling to minimize exposures.
Hypersensitivity

**Definition:** excessive humoral or cellular response to an antigen which can lead to tissue damage.

*Hypersensitivity reactions are the result of normally beneficial immune responses acting inappropriately.*
Hypersensitivity: classification

- Type 1: IgE mediated (Immediate type)
- Type 2: IgM, IgG, (Cytolysis of cells)
- Type 3: IgM, IgG (Immune complex mediated)
- Type 4: T-cell mediated (delayed-type)
- Pseudoallergic reaction
Two Stages (Distinguishes from irritation)

Induction
Sensitization
(1st exposure)

Elicitation
Challenge
(subsequent exposure)
CHEMICAL ALLERGY

- Classification
- Mechanisms
- Experimental models
- Examples
Key passages:
- Absorption
- Local trauma – proinflammatory cytokine production
- Protein binding
  - Antigen processing
  - Langerhans cells maturation and migration
- Antigen presentation to Th cells and the generation of memory T cells (immunogenicity)

Allergic contact dermatitis (Type IV)
Atopic dermatitis (Type I)
Upon subsequent contact, some LDC migrate to local lymph node as before. Other LDC present processed hapten-carrier to memory T cells in skin.

Activated memory T cells secrete cytokines that induce release of inflammatory cytokines from other cell types.

Memory T cells and inflammatory cells are recruited to the epidermis from circulation via chemoattractant cytokines and expression of adhesion molecules.
CHEMICAL ALLERGY

- Classification
- Mechanisms
- Experimental models
- Examples
EXPERIMENTAL MODELS

Mice                     Guinea Pigs
Hypersensitivity

- Well established methods for contact hypersensitivity.
- Current models and assays as inadequate predictors for systemic hypersensitivity reaction.
Mouse Tests:
- Local lymph node assay
- Mouse Ear Swelling Test

Guinea Pig Tests:
- Maximization Test
- Occlusive Patch Test
- Respiratory Challenge
- Systemic Anaphylaxis
GUINEA PIG MODELS

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

Buehler Assay

Topical application - closed patch:
Days 0, 6-8, and 13-15

Day 27-28 topical challenge of the untreated flank for 6 h

Read: 21, 24, 48 h after removing patch
> 15% positive

Induction

Challenge

Endpoint erythema

Criteria

20 animals/group
The mouse local lymph node assay (LLNA)

**IMMUNE ACTIVATION**

Selective clonal expansion of allergen-responsive T lymphocytes

**LOCAL LYMPH NODE ASSAY**

- Test/vehicle
  - Day 0, 1, 2
- 2 days of rest, $^3$H TdR
- Day 5
- 5 hours
- Count DPMs
LLNA: illustrative results

The proliferation is proportional to the dose and chemical reactivity.
The proliferation is proportional to the dose and to the potency of the applied allergen. The EC3 provides a simple means of obtaining a quantitative measurement of sensitization.

**EC3 = 0.05%**  
EC3 = 7.9%

Application concentration of test chemical required to provoke a 3-fold increase in LNC proliferative activity compared with concurrent vehicle treated controls.
# Classification of Relative Skin Sensitization Potency Using LLNA EC3 Values

<table>
<thead>
<tr>
<th>Potency Category</th>
<th>EC3 Value µg/cm²</th>
<th>EC3 Value % Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extreme</td>
<td>( \leq 25 )</td>
<td>( &lt; 0.1 )</td>
</tr>
<tr>
<td>Strong</td>
<td>25 - 250</td>
<td>( \geq 0.1 - &lt; 1 )</td>
</tr>
<tr>
<td>Moderate</td>
<td>250 - 2500</td>
<td>( \geq 1 - &lt; 10 )</td>
</tr>
<tr>
<td>Weak</td>
<td>&gt;2500</td>
<td>( \geq 10 )</td>
</tr>
</tbody>
</table>

1% dose in a LLNA = 250 µg/cm²

ECETOCP technical report #87
• The LLNA is suited well for potency information whereas Guinea pigs methods are more challenging due to the inherent design of the study:
  • Measures the elicitation phase
  • Not practicable to examine in detail multiple induction concentrations of a chemical
  • Subjective assessment of the frequency of responses rather than the vigor of the responses

Kimber et al., Toxicology 93: 13, 1994
- Update OECD 429 - Skin sensitization: reduced LLNA

It also includes the Performance Standards that can be used to evaluate the validation status of new and/or modified test methods that are functionally and mechanistically similar to the LLNA.

- OECD 442A - Skin Sensitization: LLNA DA

- OECD 442B - Skin Sensitization: LLNA BrdU-ELISA

All adopted 22nd July 2010
Summary

- The LLNA has gained widespread adoption and use internationally in the past 10+ years, providing for significant reduction and refinement.
- Updated LLNA protocol reduces animal use by 20% and rLLNA can further reduce animal use by 40%.
- Nonradioactive LLNA methods now allow for broad use, with reduced hazards for the environment and lab workers.
- Appropriate use of the newly adopted and updated LLNA protocols are expected to support both continued protection of people and improved animal welfare.
Potential Contact Sensitizers

- Fragrances
- Dyes
- Preservatives (kathon CG, CHOH)
- Metals (Ni, Co, Be, Cr)
Low Molecular Wt (<3000 Da) respiratory sensitizers

- Toluene diisocyanate
- Diphenylmethane diisocyanate
- Phthalic anhydride
- Trimellitic anhydride
- Platinum salts
- Reactive dyes
Protein allergens

- Detergent Enzymes
- Molds and spores
- Latex
- Microbial pesticides
- Animal dander
- Food proteins
Definition: Autoimmunity is an inappropriate immune response against self-antigens, which can lead to chronic inflammation, tissue destruction and/or dysfunction.
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 Erythematous (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

More than 60 diseases!
AUTOIMMUNITY

- Classification
- Mechanisms
- Experimental models
- Examples
POTENTIAL MECHANISMS OF AUTOIMMUNITY

1. Failure to remove potentially autoreactive cells
2. Loss of peripheral tolerance
3. Altered self
4. Molecular mimicry (i.e. infections)
5. Exposure of cryptic antigens
6. Altered regulatory protein production
While genotype and gender (> F) appear to be a critical factor in development of autoimmune disease, contributions by other host factors and exposure to exogenous agents may induce or exacerbate autoimmune diseases.

Occupational exposure to silica, solvents, pesticides has been associated with autoimmune diseases. Three different effects of occupational chemical exposures have been suggested:

1. Enhanced proinflammatory response
2. Modification of endogenous proteins and subsequent autoantibody formation
3. Altered production of regulatory factors.
AUTOIMMUNITY

- Classification
- Mechanisms
- Experimental models
- Examples
Autoimmunity

- Poor understanding of basic mechanisms.
- No reliable models or general strategy and assays (including the popliteal lymph node assay) are at present available (or validated).

Possible strategy:
- tier 1: PLNA or RA-PLNA (hazard identification)
- tier 2: suitable animal models (relevant route of exposure, relevant clinical outcomes)
METHODS IN IMMUNOTOXICOLOGY
Autoimmunity

- Animal Models
  - Genetic Predisposition
  - Autoimmunization
  - Organic or Chemical Induction
Animal Models

Genetic Predisposition

- Autoimmune Thyroiditis
  - MRL (m), BB (r), OS (ch)
- Insulin-Dependent Diabetes Mellitus
  - NOD (m), BB (r), BN (r)
- Rheumatoid Arthritis
  - MRL/lpr (m), SCID (m), HLAB27 (r)
- Systemic Lupus Erythematosus
  - MRL+/+ (m), MRL/lpr (m), NZB/NZW (m)
- Scleroderma
  - TSK (m)
AUTOIMMUNITY

- Classification
- Mechanisms
- Experimental models
- Examples
EXOGENOUS FACTORS ASSOCIATED WITH AUTOIMMUNITY

- Drugs
- Infectious Agents
- Metals
- Particulates (silica)
- Pesticides
- Solvents
- Vaccines
Particulates

- Silica
  - Strong association between silica exposure (from “dusty trades”) and SLE, rheumatoid arthritis, ANCA-associated vasculitis and glomerulonephritis, and scleroderma
  - Some individuals appear to develop fibrogenic responses while others develop immunologic responses
  - Acts as an adjuvant by:
    - Increasing the longevity of APCs
    - Increasing antigen processing in APCs
    - Increasing cytokine production
    - Inducing a polyclonal activation of B and T cells
While it is evident that occupational exposures contribute in some measure to the overall risk for specific autoimmune diseases, improved exposure assessment, better coordination between experimental models and epidemiological studies (priori hypothesis) are needed to define these risks more precisely.