UNIVERSITÀ DEGLI STUDI DI MILANO
FACOLTÀ DI FARMACIA

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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

IMMUNOLOGY

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.

IMMUNE SYSTEM: functions

Defense against:

- Infection (bacteria, viruses, fungi, parasites).
- Spontaneously arising neoplasm.
- Any foreign material

- **Self / non-self discrimination**

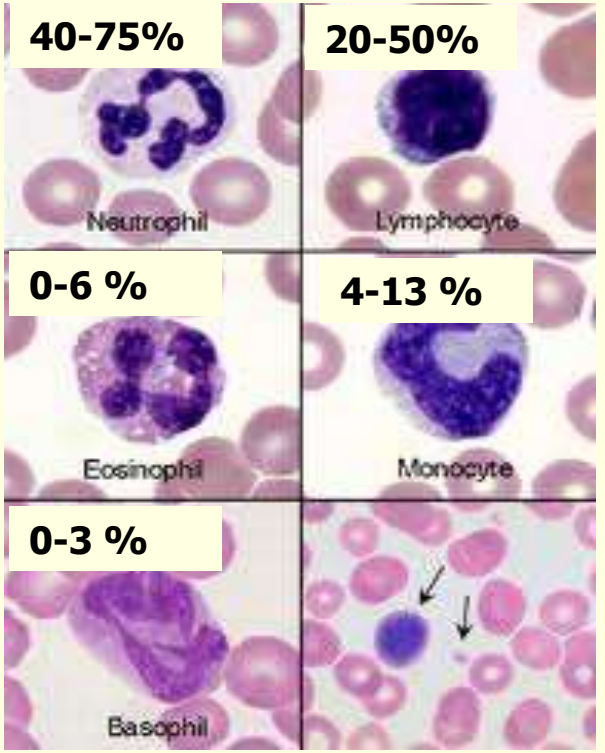
ORGANIZATION OF THE IMMUNE SYSTEM

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**.

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL
CBC With Differential/Platelet				
WBC			x10E3/uL	4.0 - 10.5
RBC			x10E6/uL	4.14 - 5.80
Hemoglobin			g/dL	12.6 - 17.7
Hematocrit			%	37.5 - 51.0
MCV			fL	79 - 97
MCH			pg	26.6 - 33.0
MCHC			g/dL	31.5 - 35.7
RDW			%	12.3 - 15.4
Platelets			x10E3/uL	140 - 415
Neutrophils			%	40 - 74
Lymphs			%	14 - 46
Monocytes			%	4 - 13
Eos			%	0 - 7
Basos			%	0 - 3
Neutrophils (Absolute)			x10E3/uL	1.8 - 7.8
Lymphs (Absolute)			x10E3/uL	0.7 - 4.5
Monocytes(Absolute)			x10E3/uL	0.1 - 1.0
Eos (Absolute)			x10E3/uL	0.0 - 0.4
Baso (Absolute)			x10E3/uL	0.0 - 0.2
Immature Granulocytes			%	0 - 2
Immature Grans (Abs)			x10E3/uL	0.0 - 0.1

Half life of blood components

* Red cells- 120 days

* Platelets - 10 days

* Granulocytes – 10 h

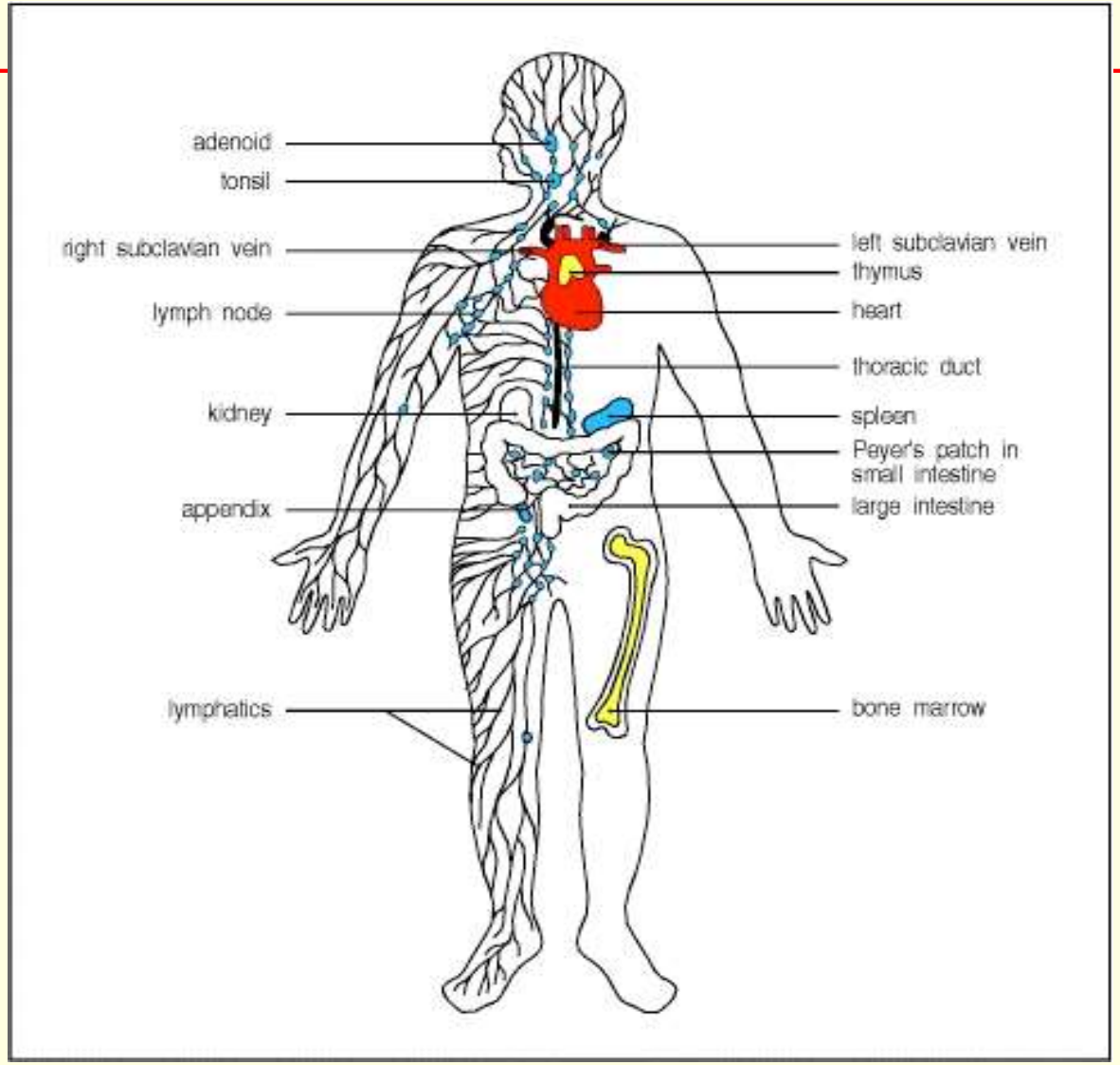
* Monocytes – 3-4 days

* Lymphocytes – usually they die after having eradicated the infection (with the exception of the memory cells that live months / years)

SERUM IMMUNOGLOBULINS

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

LYMPHOID ORGANS



Primary lymphoid organs

Secondary lymphoid organs

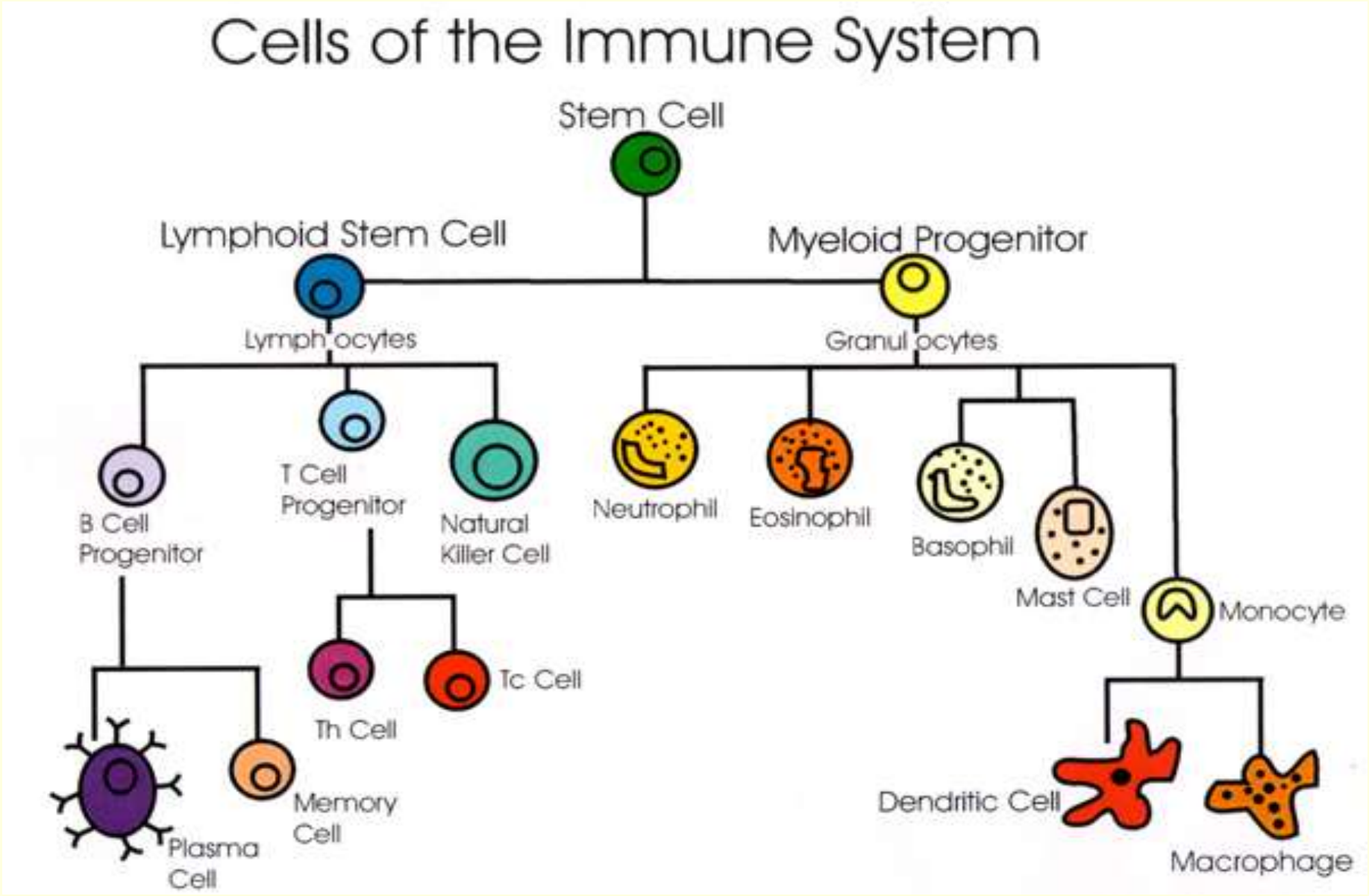
LYMPHOID ORGANS

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)

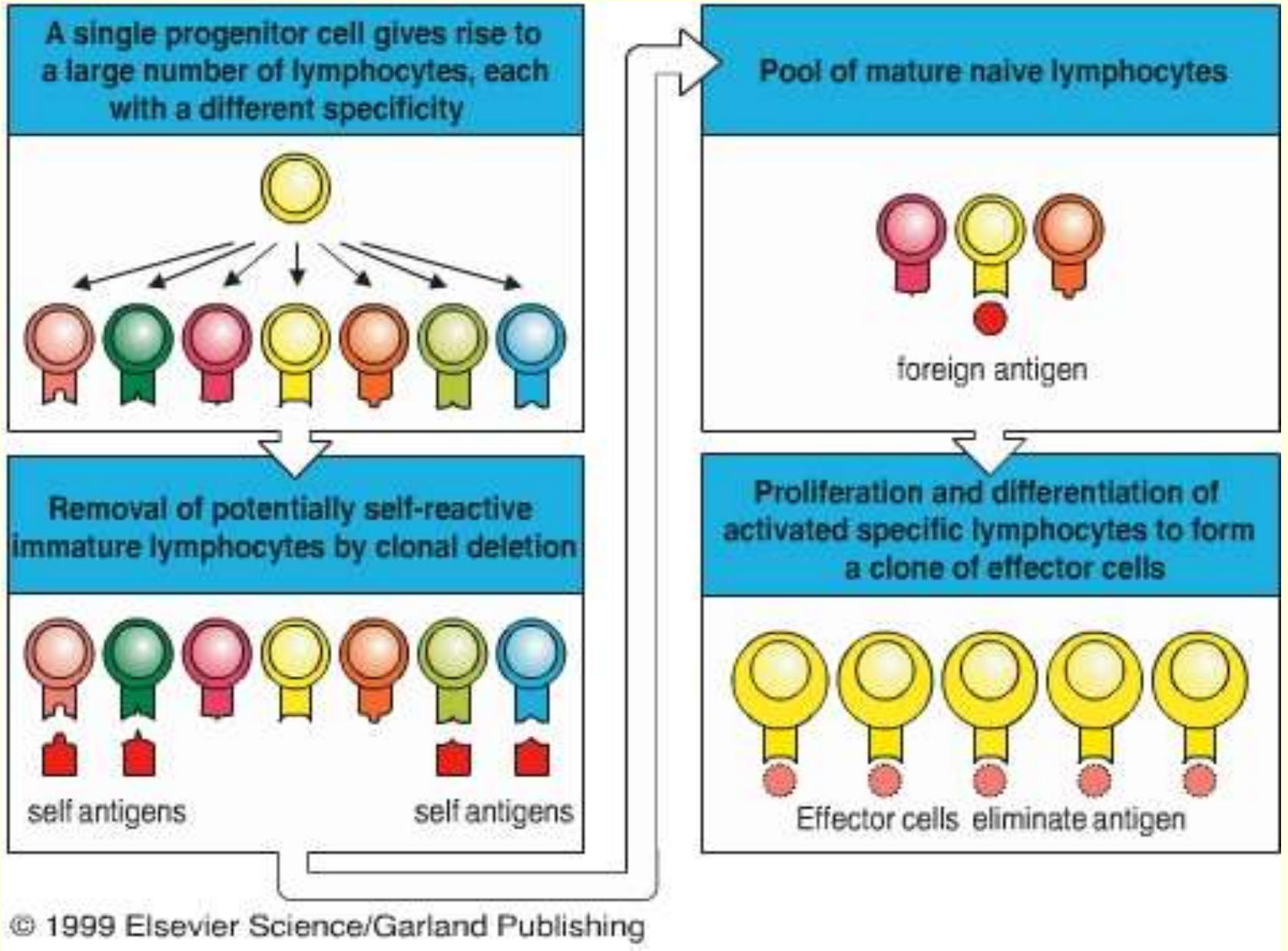
- it is where antigen is brought so that it can be effectively exposed to mature lymphocytes. It is where adaptive immune response initiate.


Cells of the Immune System
Stem Cell
Lymphoid Stem Cell
Myeloid Progenitor
Lymphocytes
Granulocytes
B Cell Progenitor
T Cell Progenitor
Natural Killer Cell
Neutrophil
Eosinophil
Basophil
Mast Cell
Monocyte
Th Cell
Tc Cell
Plasma Cell
Memory Cell
Dendritic Cell
Macrophage

MATURATION AND DIFFERENTIATION CLONAL EXPANSION

Rearrange TCR/Ig gene segments and acquire specifity

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



SPECIFICITY

Element	Immunoglobulin		αβ receptors	
	H	κ+λ	β	α
Variable segments (V)	65	70	52	~70
Diversity segments (D)	27	0	2	0
D segments read in 3 frames	rarely	–	often	–
Joining segments (J)	6	5(κ) 4(λ)	13	61
Joints with N and P nucleotides	2	(1)	2	1
Number of V gene pairs	3.4×10^6		5.8×10^6	
Junctional diversity	$\sim 3 \times 10^7$		$\sim 2 \times 10^{11}$	
Total diversity	$\sim 10^{14}$		$\sim 10^{18}$	

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)

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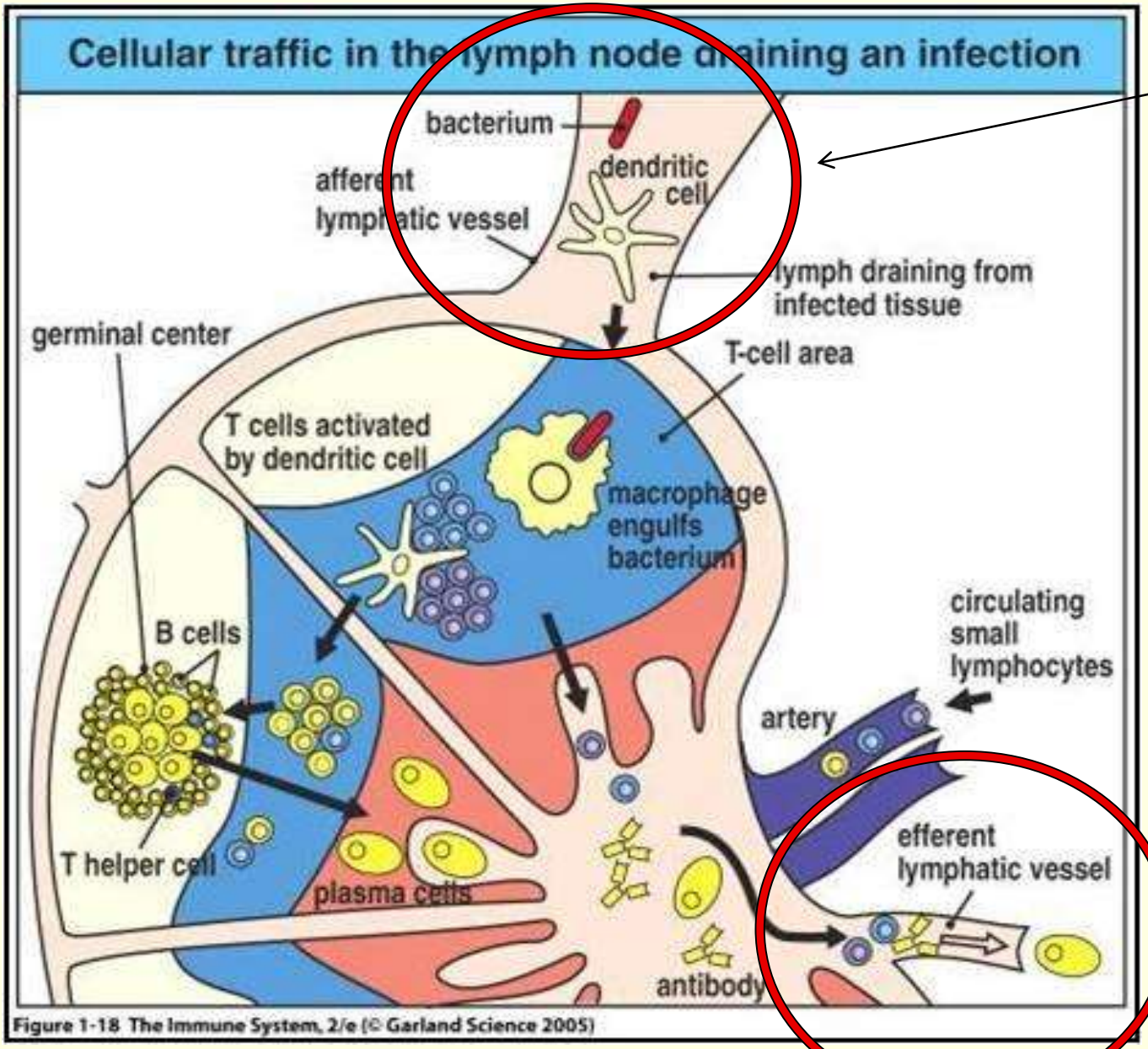


ENTRANCE OF ANTIGENS AND APCs

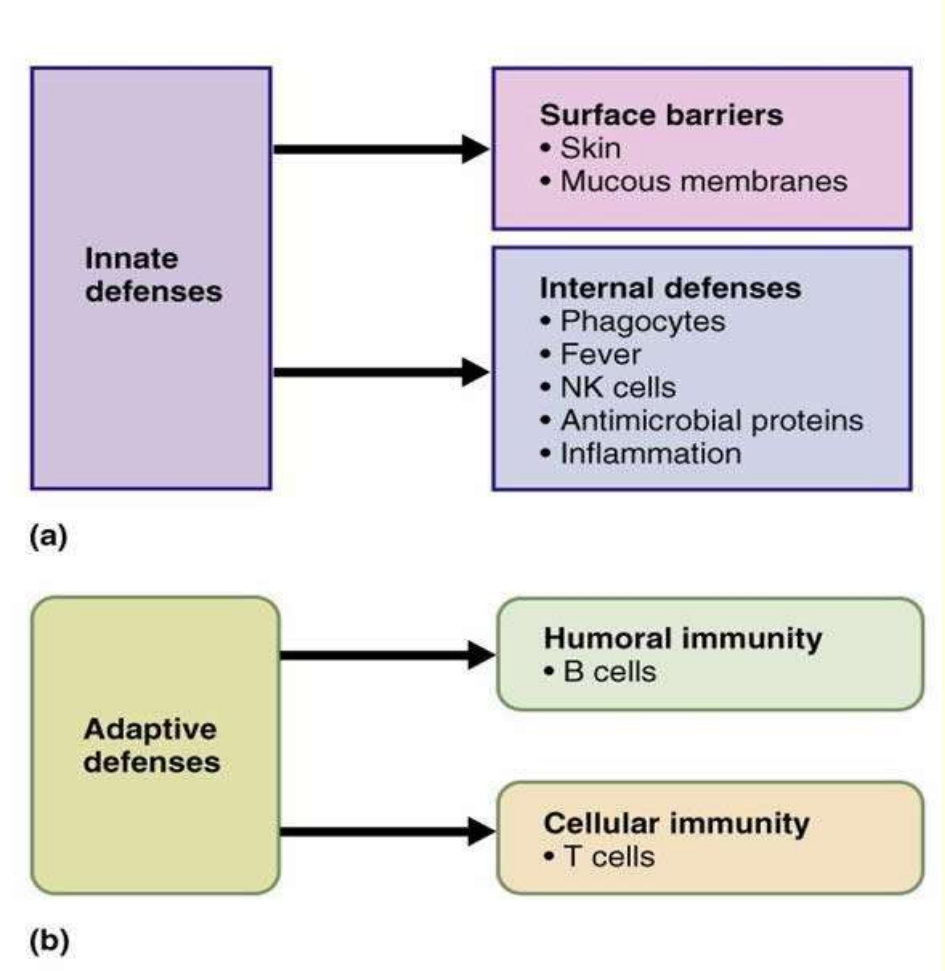
EXIT OF EFFECTOR MECHANISMS

Immune Responses

Two major types of immune response:

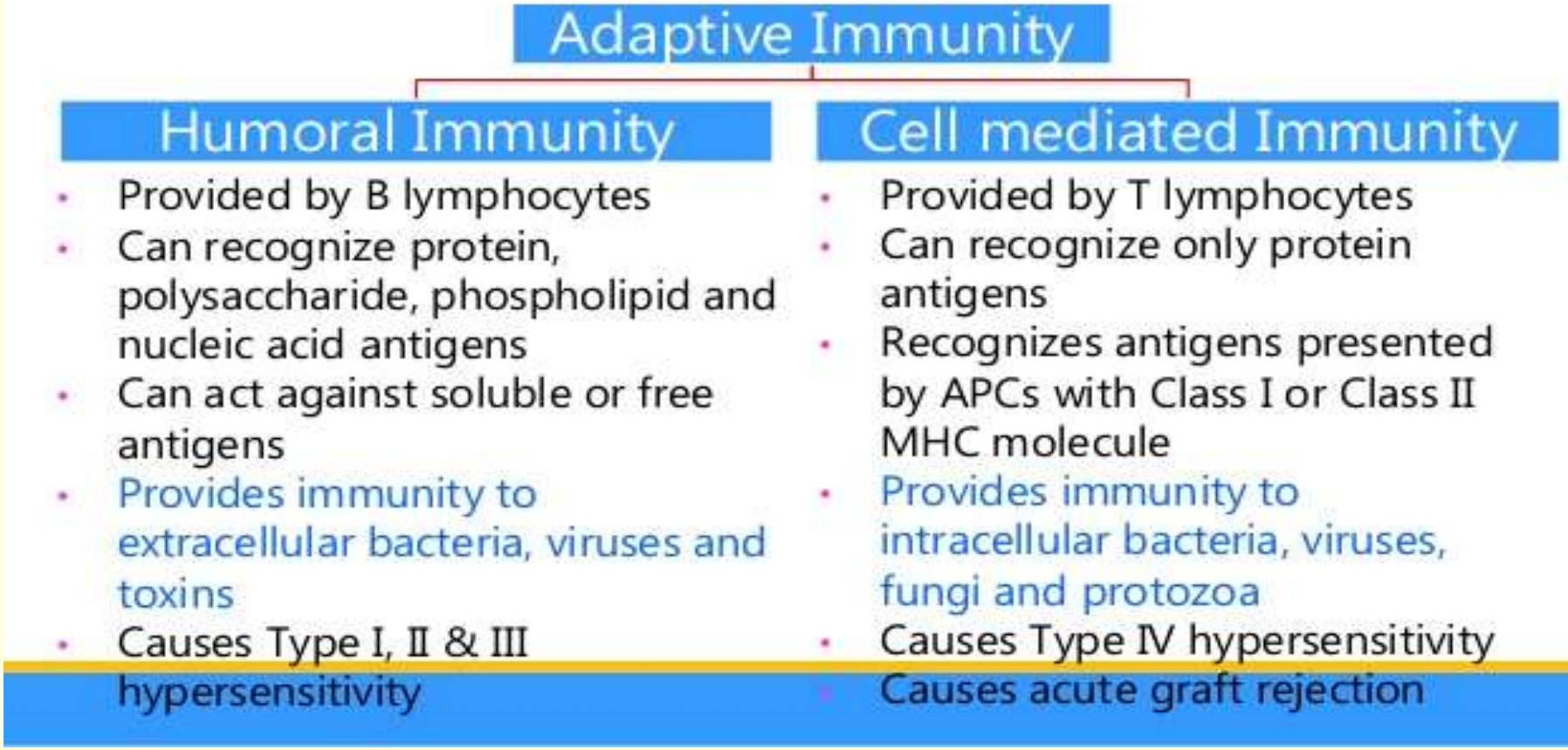
1. NATURAL OR INNATE IMMUNE RESPONSE

2. SPECIFIC OR ACQUISITE OR ADAPTIVE IMMUNE RESPONSE

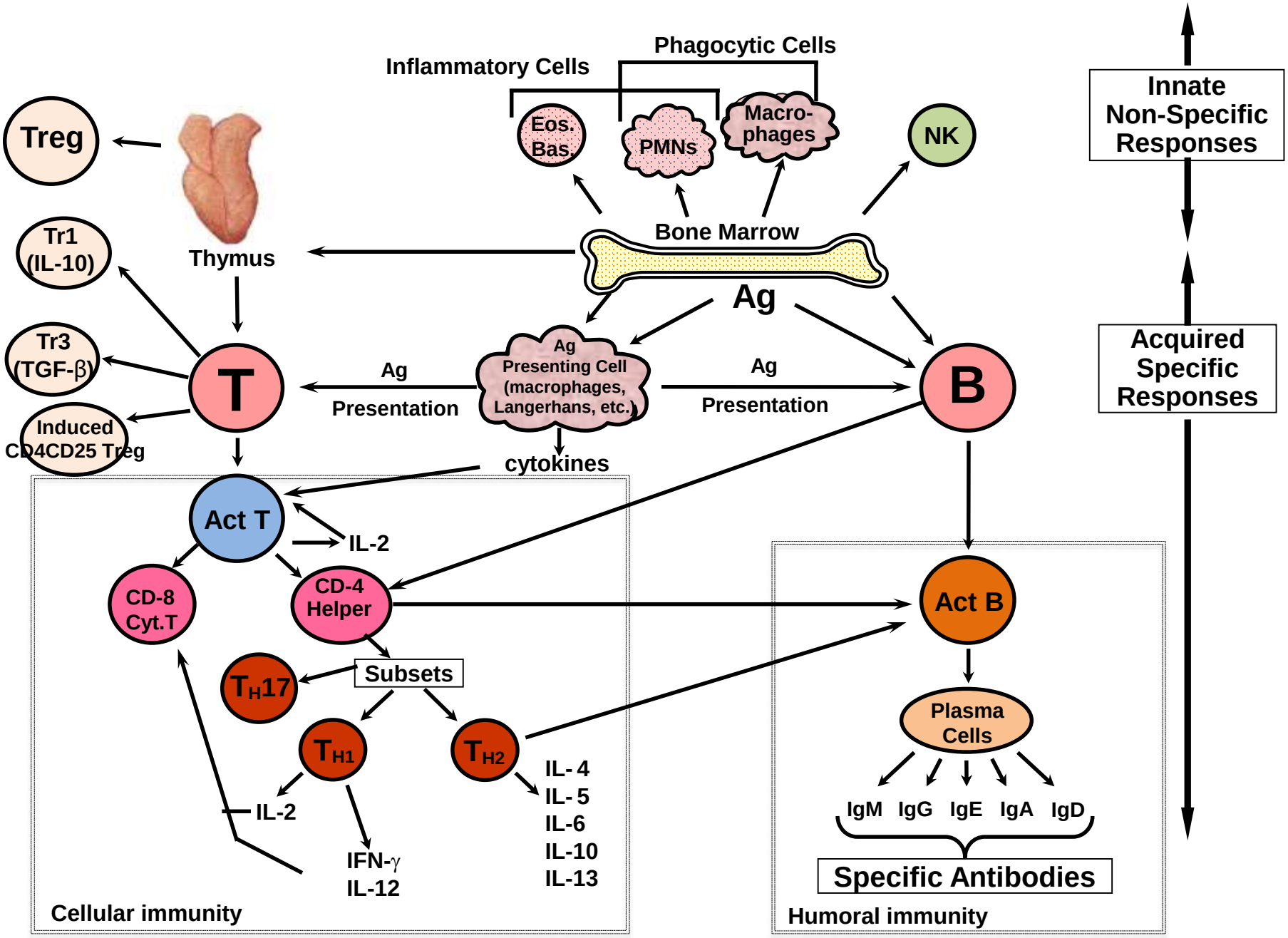


Adaptive Immunity

Adaptive immune system has two arms



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

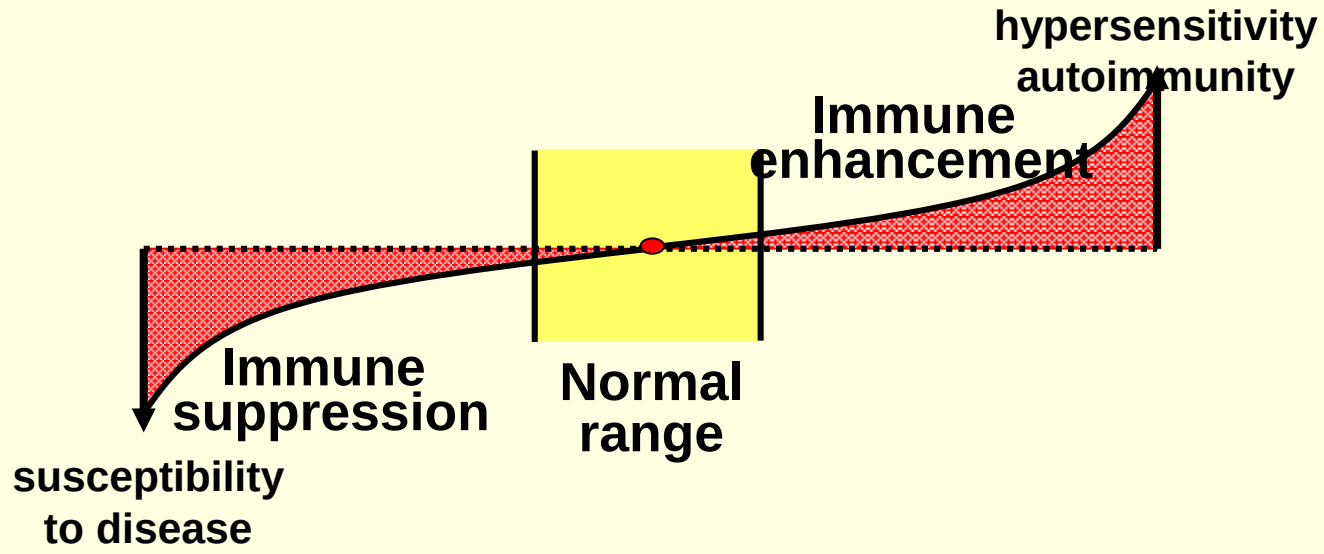

Innate
Non-Specific
Responses
Acquired
Specific
Responses
Phagocytic Cells
Inflammatory Cells
Eos.
Bas.
PMNs
Macro-
phages
NK
Treg
Thymus
Tr1
(IL-10)
Bone Marrow
Ag
Tr3
(TGF-β)
T
Ag
Presenting Cell
(macrophages,
Langerhans, etc.)
B
Ag
Presentation
Ag
Presentation
Induced
CD4CD25 Treg
cytokines
Act T
IL-2
CD-8
Cyt.T
CD-4
Helper
Act B
Subsets
TH17
TH1
TH2
Plasma
Cells
IL-2
IFN-γ
IL-12
IL-4
IL-5
IL-6
IL-10
IL-13
IgM IgG IgE IgA IgD
Specific Antibodies
Cellular immunity
Humoral immunity

Objectives

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

DEFINITIONS

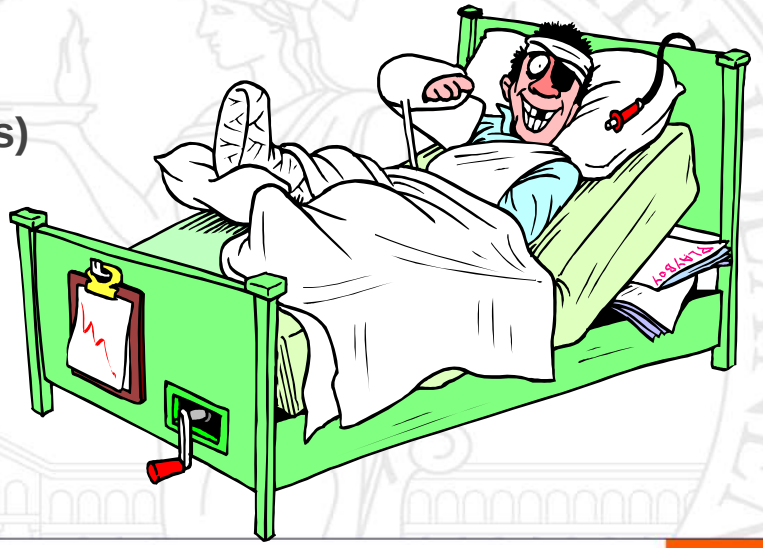
- IMMUNOTOXICOLOGY studies the adverse effects of xenobiotics on the immune system.
- IMMUNOTOXIC COMPOUND is a compound that can alter one or more immune functions resulting in an adverse effect for the host.



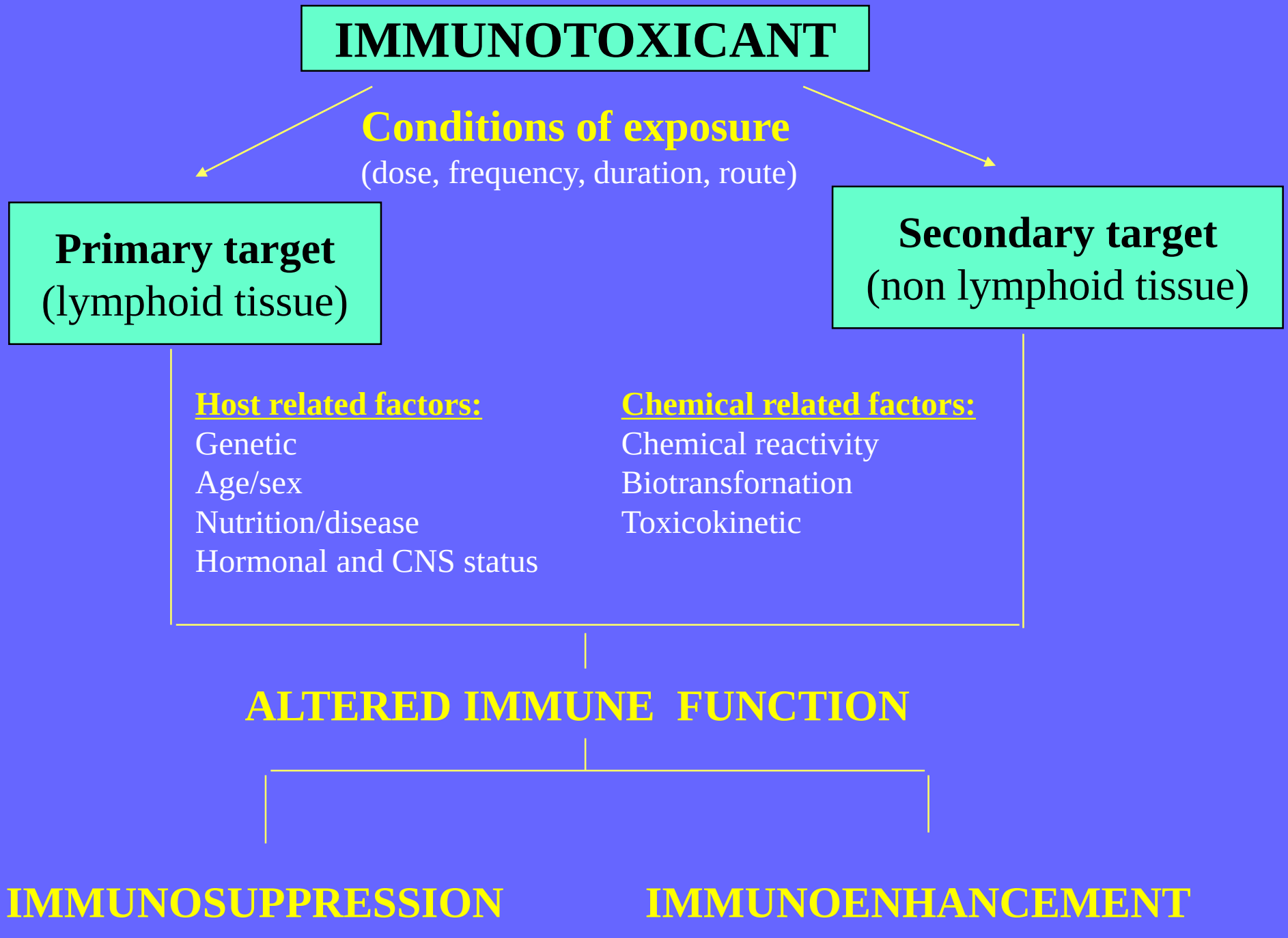
Immunotoxic adverse effects

- **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

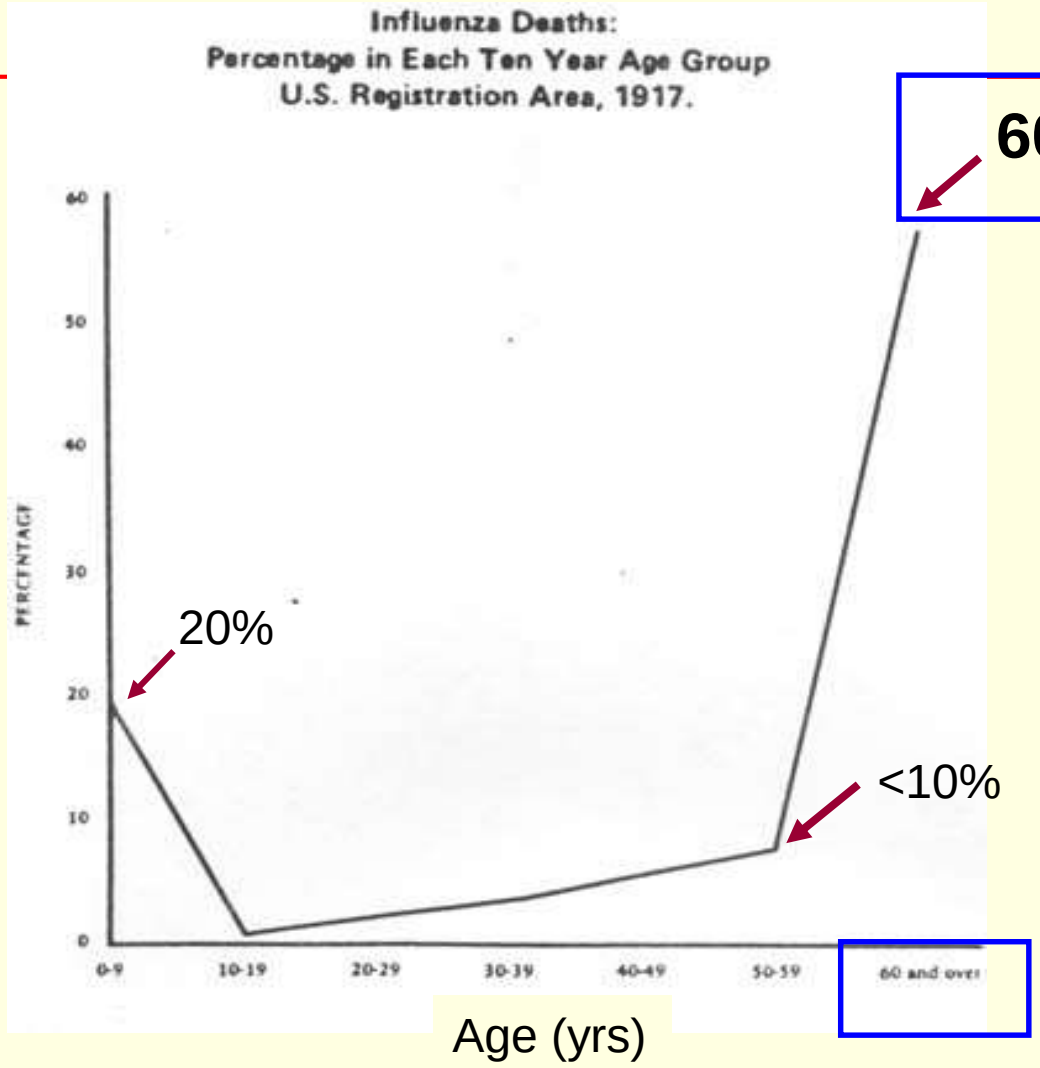
    ROS production initiates inflammation which unless quenched may result in chronic inflammatory disease states, e.g. hepatitis, nephritis, multiple system organ failure, etc.



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
Biotransfornation
Toxicokinetic
ALTERED IMMUNE FUNCTION
IMMUNOSUPPRESSION
IMMUNOENHANCEMENT

SPANISH FLU: MORTALITY RATE BY AGE RANGE



SPANISH FLU WORDLWIDE MORTALITY IN ONE YEAR:

OVER 20 MILION PEOPLE

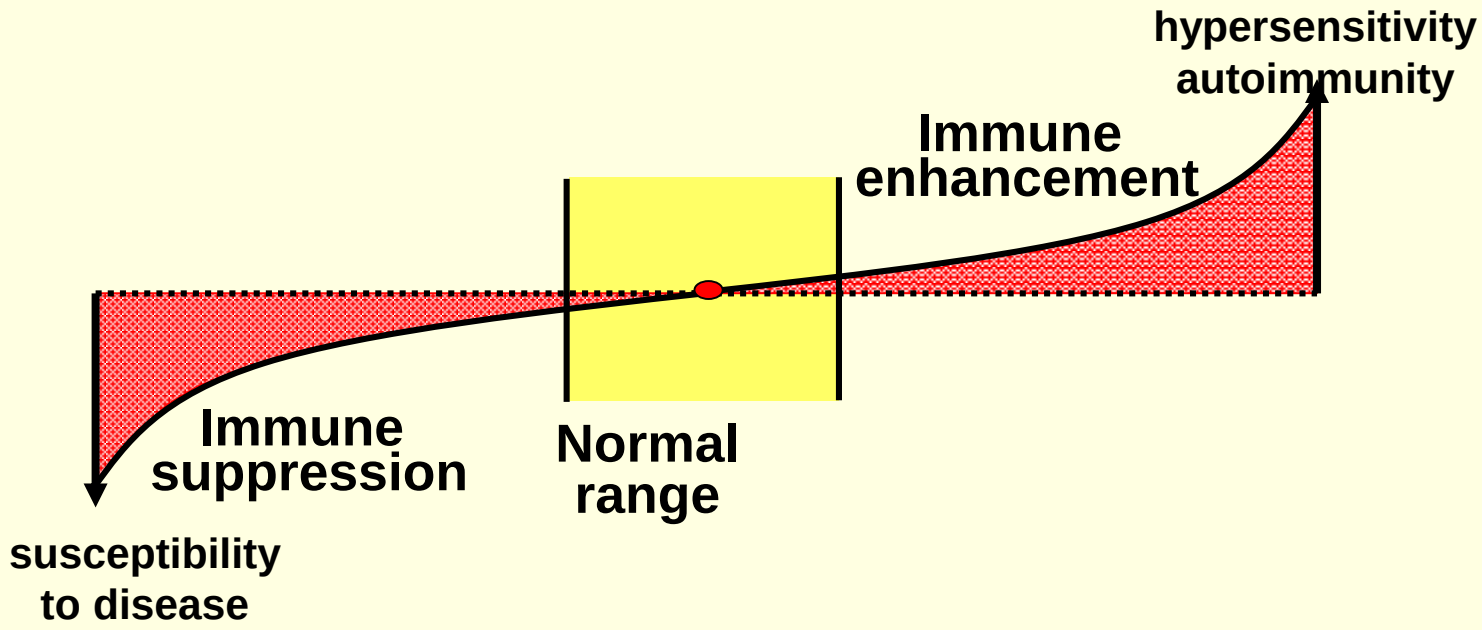
IMMUNE SYSTEM VULNERABILITY

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

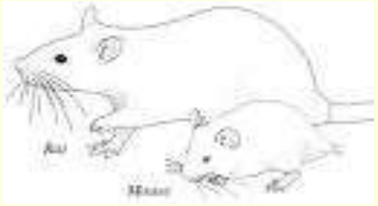
- Overview of the immune system
- Immunotoxicology: definition
- In vivo evaluation



Immunotoxicology: area of research

- Immunosuppression
- Immunostimulation
- Developmental immunotoxicology
- Immunosenescence
- Molecular immunotoxicology
- *In vitro/ in silico* immunotoxicology

EXPERIMENTAL MODELS



ANIMAL 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...**

ASSESSMENT OF IMMUNOTOXICITY

- 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:

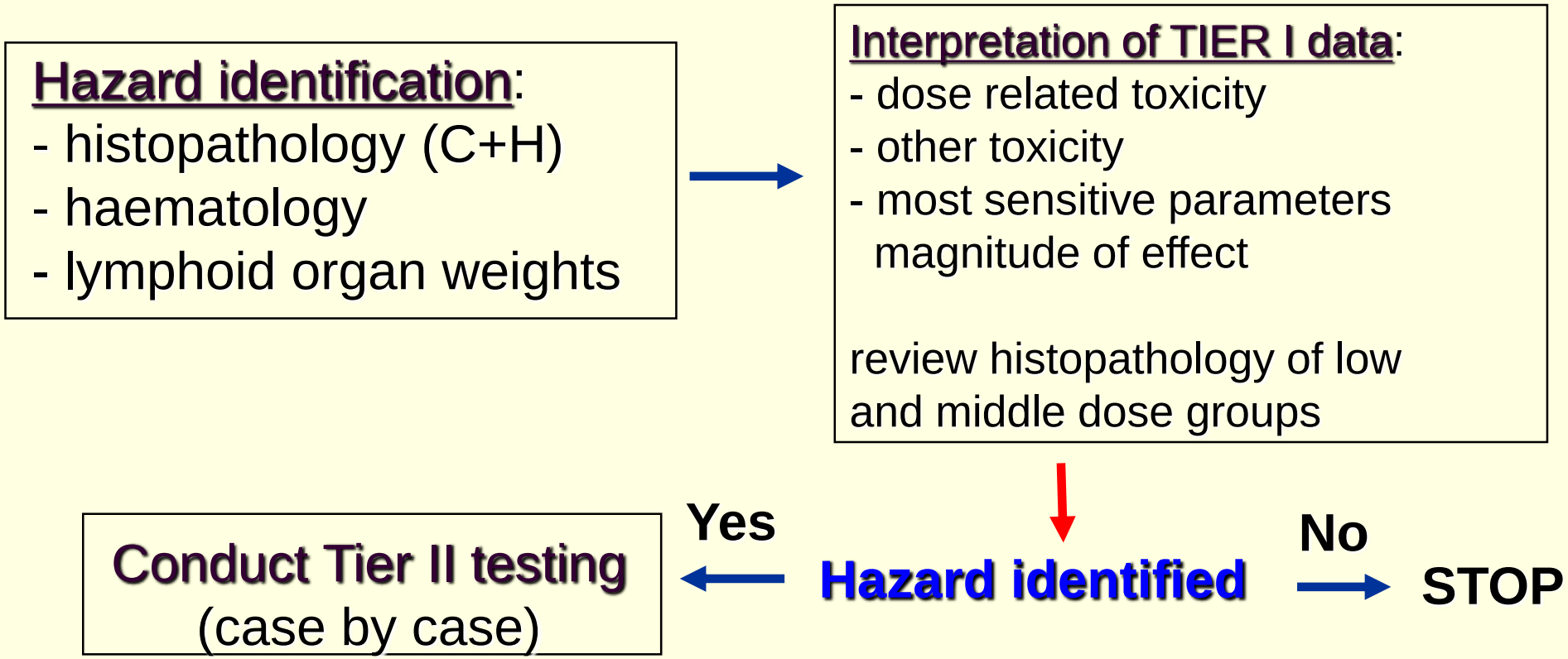
- changes in total and differential white blood cell counts
- alterations in immune organ weights and histology
- decreased basal plasma immunoglobulins
- increased incidence of infections
- increased occurrence of tumors, in the absence of genotoxicity, hormonal effects, or liver enzyme induction
-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

ECETOC Monograph No. 21 (1994)

PARAMETER	RECOMMENDATION
Haematology	
Total and differential WBC	All
Organ weights	
Spleen and Thymus	All
Histopathology	
Spleen, Thymus	C+H (store all)
Draining and distant lymph nodes, bone marrow	C+H (store all)

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

Parameter	Specific Component
Hematology	Total leukocyte counts and absolute differential leukocyte counts
Clinical Chemistry	Globulin levels and A/G ratios
Gross pathology	Lymphoid organs / tissues
Organ weights	thymus, spleen, (optional: lymph nodes)
Histology	thymus, spleen, draining lymph node and at least one additional lymph node, bone marrow, Peyer's patch



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

METHODS IN IMMUNOTOXICOLOGY
Immunosuppression

- Relatively well validated models
- Relevance of histological changes in lymphoid organs
- One immune function assay absolutely necessary
- Host resistance assays as second-line assays

METHODS IN IMMUNOTOXICOLOGY
Immunosuppression

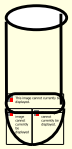
- Testing Assays
  - General tests
    - Organ weights, plasma/serum enzyme levels
  - Nonfunctional tests: status
    - Lymphoid organ weights, lymphoid tissue cellularity, histopathology, immunophenotyping
  - Functional tests

METHODS IN IMMUNOTOXICOLOGY
Immunosuppression

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

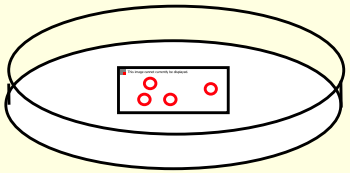
IgM Plaque Forming Cell Assay

Day 4

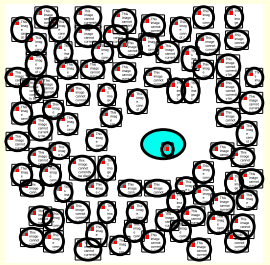


Complement + sRBC
in Agar Solution

500 µl Aliquot



3 Hour Incubation



Magnified

End Points

PFC / 10^6 Spleen Cells

PFC / Spleen

SRBC around AFC are hemolyzed = PLAQUE

- Antibody Forming Cell (AFC)
- Sheep RBC

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

FUNDAMENTAL AND APPLIED TOXICOLOGY 18, 200–210 (1992)

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 I. 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*

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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

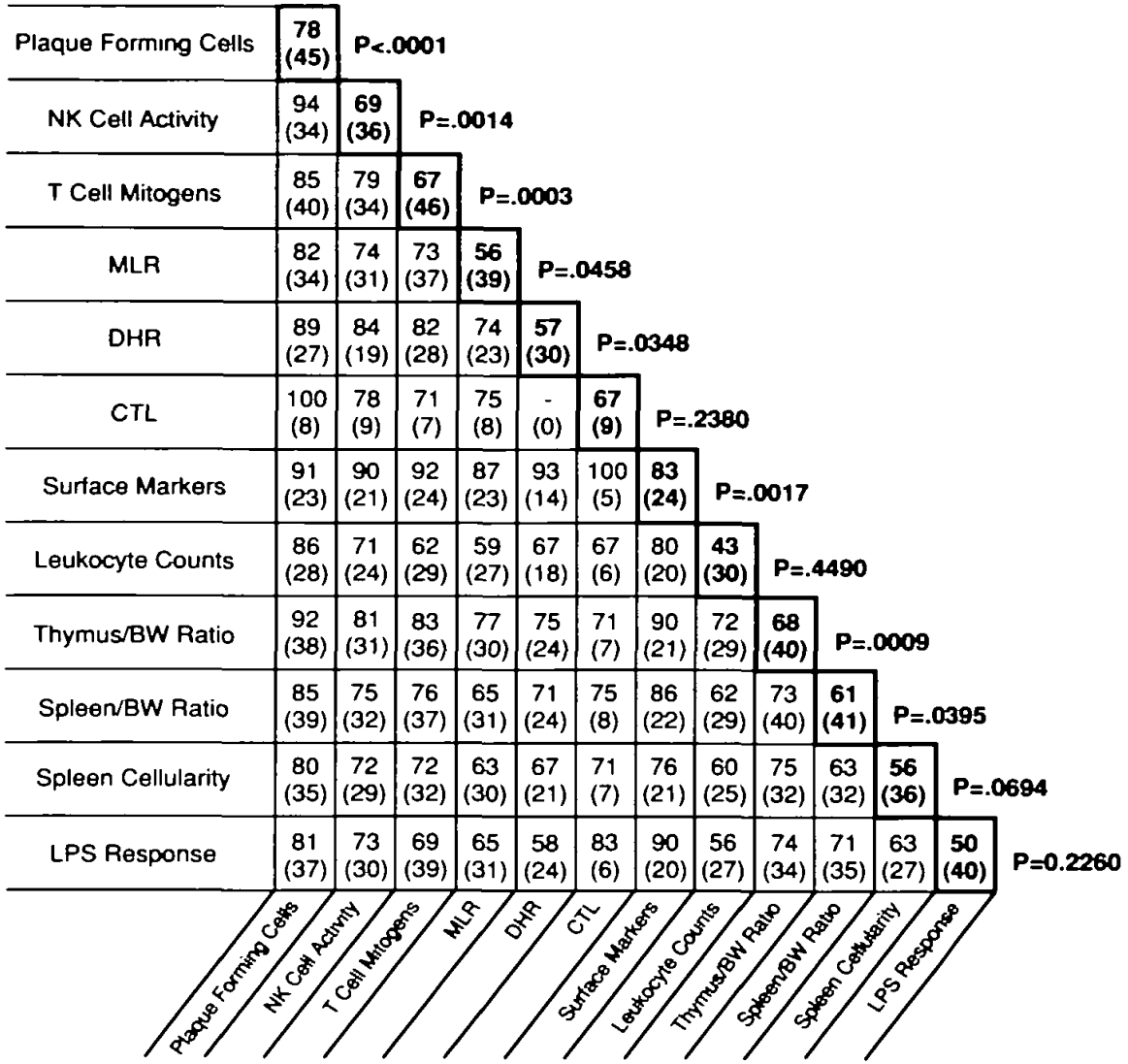


FIG. 2. Individual and pairwise concordance to establish predictability using the immune panel. Values are presented as percentage concordance which is the sum of specificity (−/−) and sensitivity (+/+). Individual concordance values are shown in boldface on the diagonal of the matrix and combinations, 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.

Risk assessment in immunotoxicology

CONCLUSIONS

Fund Appl Toxicol 18: 200, 1992

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

TAE

Operational Characteristics of Each of the Cell Surface M

Surface marker	No. of tests	Significance of association (Fisher's Exact test)
Thy 1.2	24	$p = 0.019$
sIg^+	22	$p = 0.101$

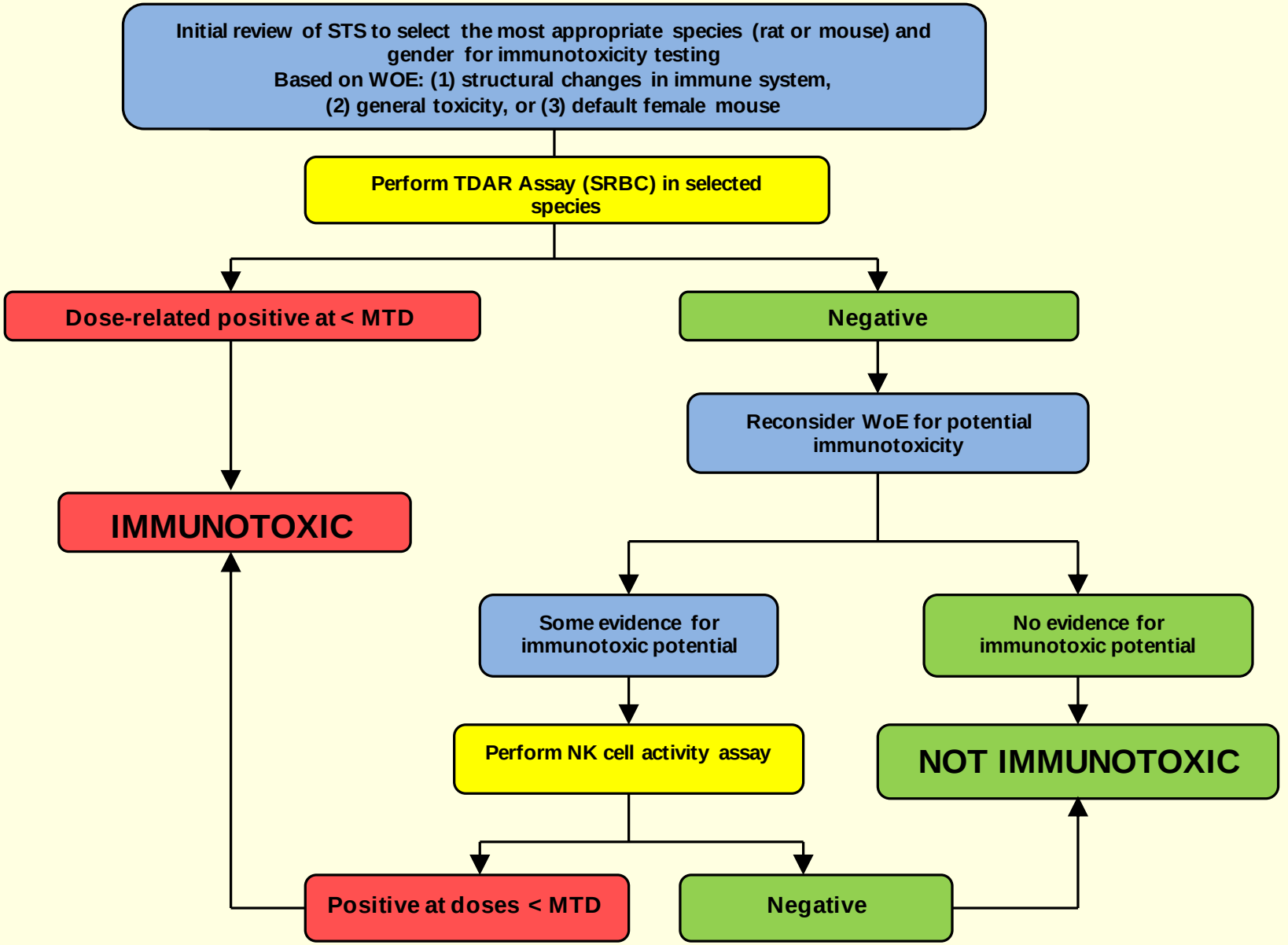
Risk assessment in immunotoxicology

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

US-EPA: ASSESSMENT OF PESTICIDE IMMUNOTOXICITY

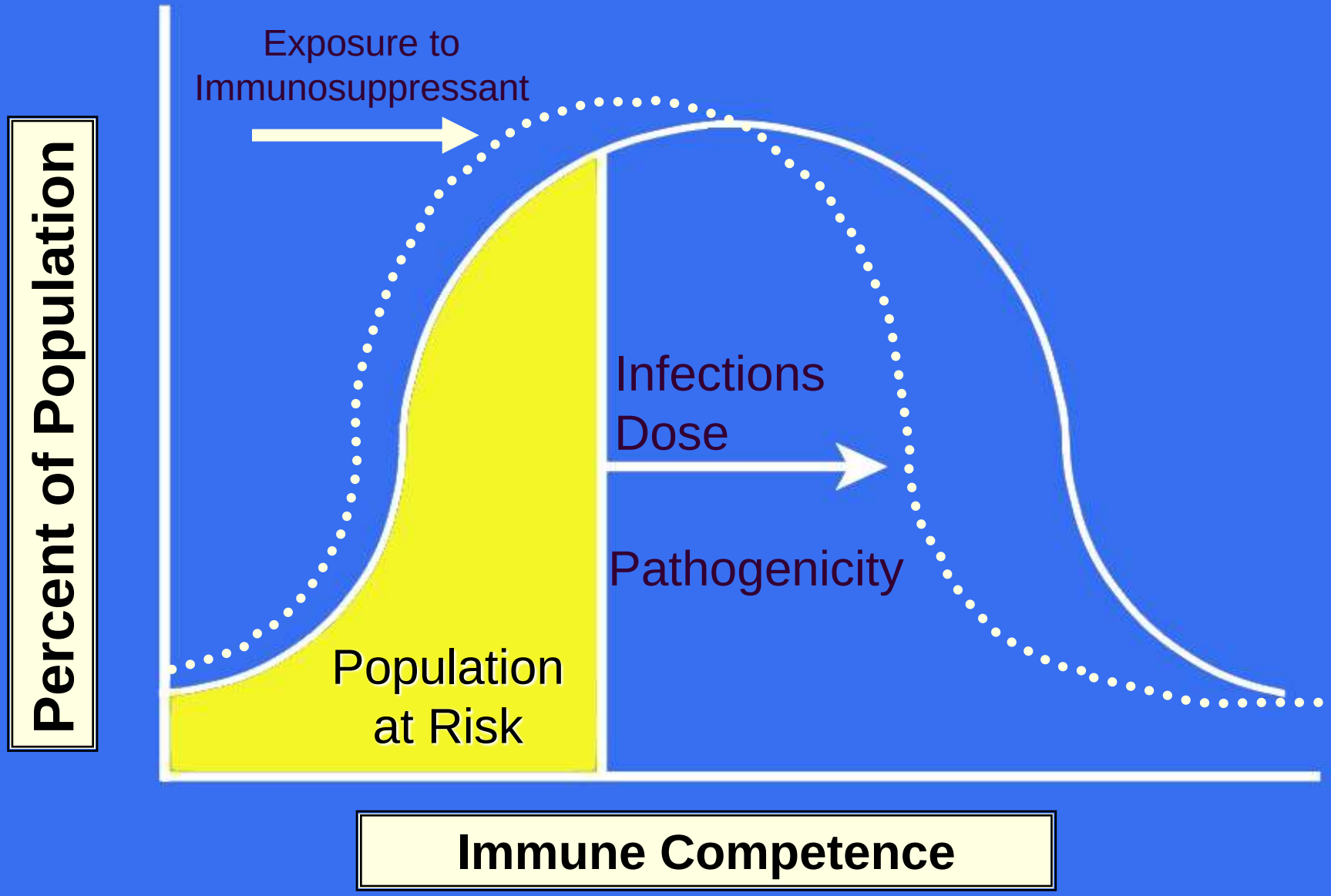


ILSI-HESI WORKSHOP SUMMARY 2012

INTERPRETATION

- 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 necesseraly mean the absence of risk to man.
- Due to genetic polymorphims the response to immunotoxicants vary in human beings.
- Thus, alterations in immune functions which may be tollerated well in normal individuals could have more serious consequences for those who are cronically sick, malnourisehd, 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.

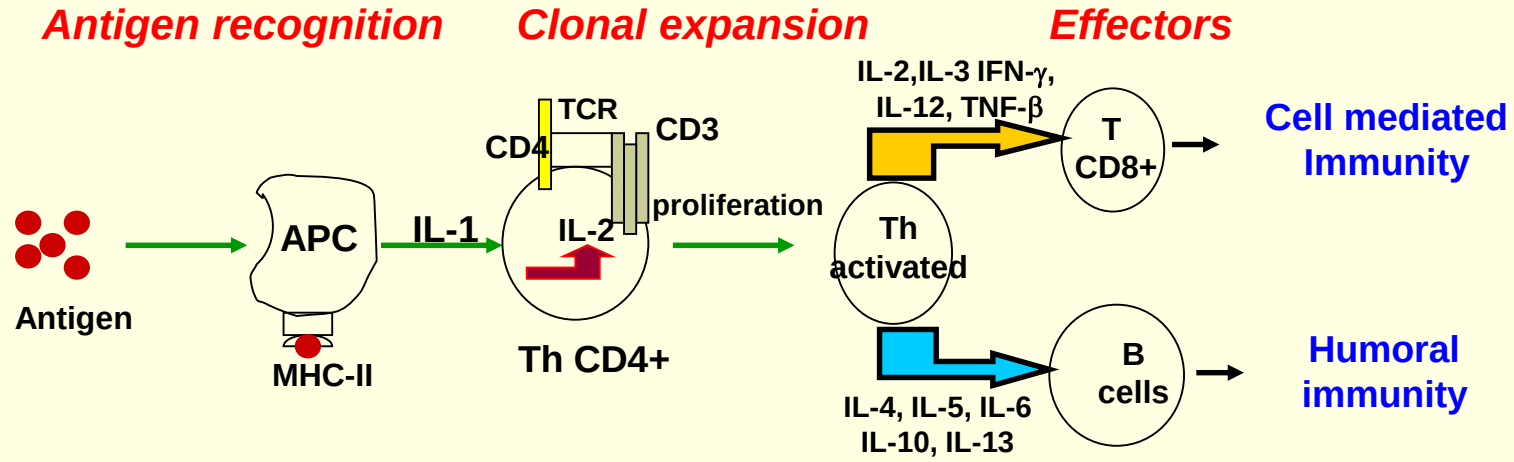


AGENTS WHICH INHIBIT IMMUNE FUNCTION AND HOST RESISTANCE

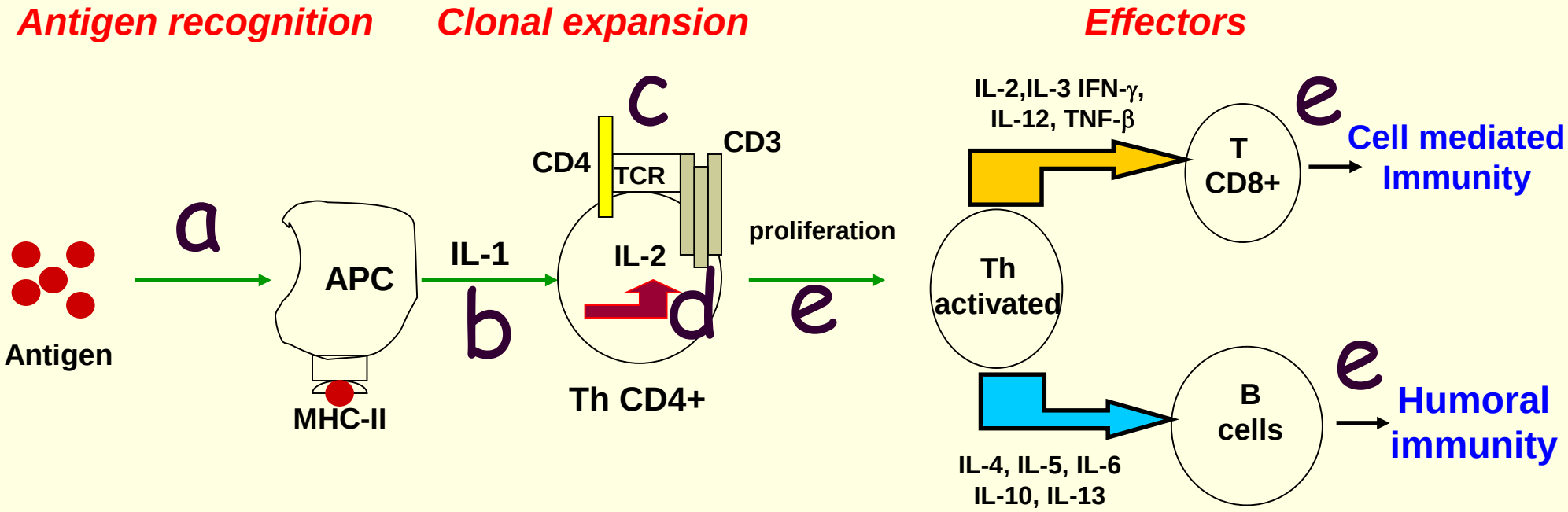
CLASS	EXAMPLE
Polyhalogenated aromatic hydrocarbons	TCDD, PCB, PBB
Metals	Lead, Cadmium, Arsenic
Aromatic hydrocarbons	Benzene, Toluene
Polycyclic aromatic hydrocarbons	DMBA, B(a)P, MCA
Pesticides	Carbofuran, chlordane
Organotins	Dibutyltin chloride
Aromatic amines	DMN, benzidine, AAF
Oxidant gases	NO_2, O_3, SO_2
Particulates	Asbestos, silica, beryllium
Ultraviolet light	UV-B
Mycotoxins	T-2, ochratoxin
Drugs of abuse	Cocaine, marijuana, alcohol

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



SPECIFIC 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.

CHEMICAL ALLERGY

- **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

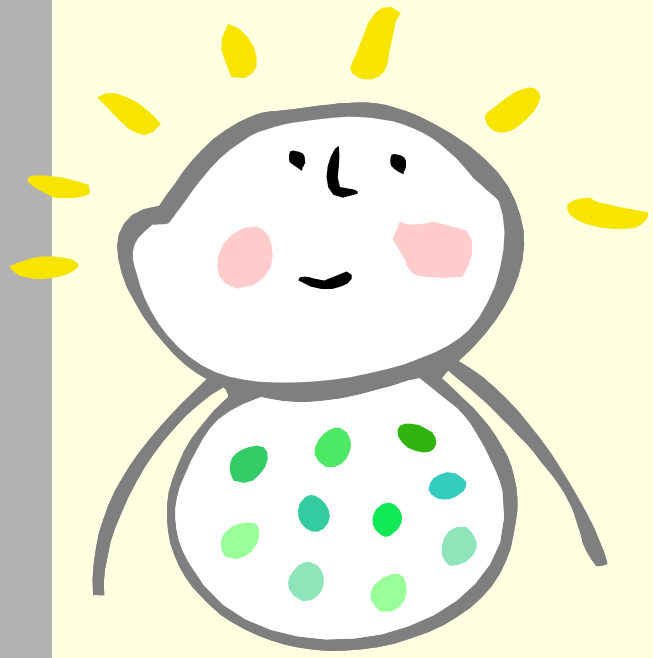
Definition: excessive humoral or cellular response to an antigen which can lead to tissue damage.

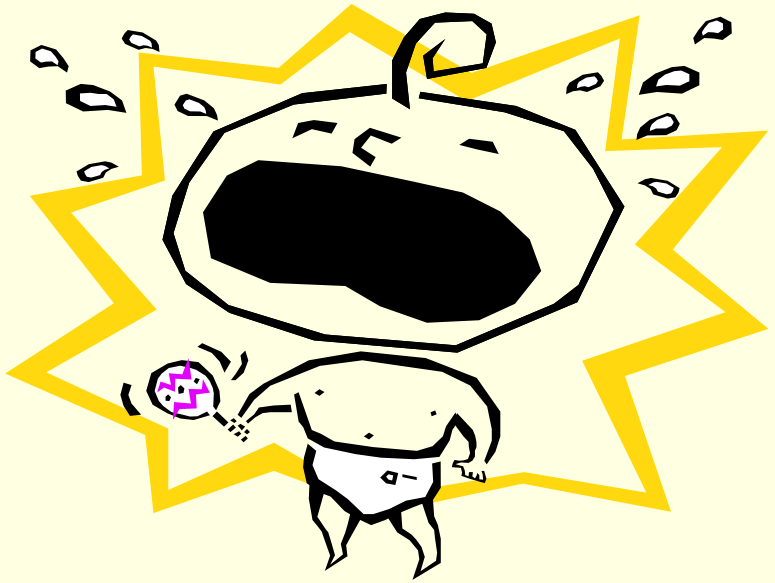
Hypersensitivity reactions are the result of normally beneficial immune responses acting inappropriately.

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)



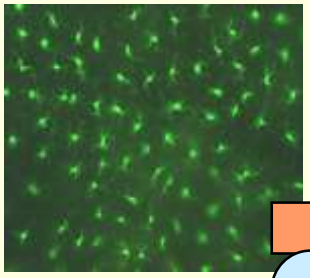


Induction
Sensitization
(1st exposure)

Elicitation
Challenge
(subsequent exposure)

CHEMICAL ALLERGY

- Classification
- **Mechanisms**
- Experimental models
- Examples



MHC class II
Chemical

STRATUM CORNEUM

EPIDERMIS

KC

LC

IL-1
IL-6
IL-7
IL-8
IL-10
IL-12
IL-15
IL-18
GM-CSF
TGF-α
TGF-β
MIP-2
IP-10
TNF- α
IFN-γ

IL-1β
GM-CSF

TNF- α
RANTES
MCP-1

MIGRATION

MATURATION

LYMPH NODE

CD4+

Th0

IL-12, INF- γ, TNF- β

IL-4, IL-5, IL-6, IL-10

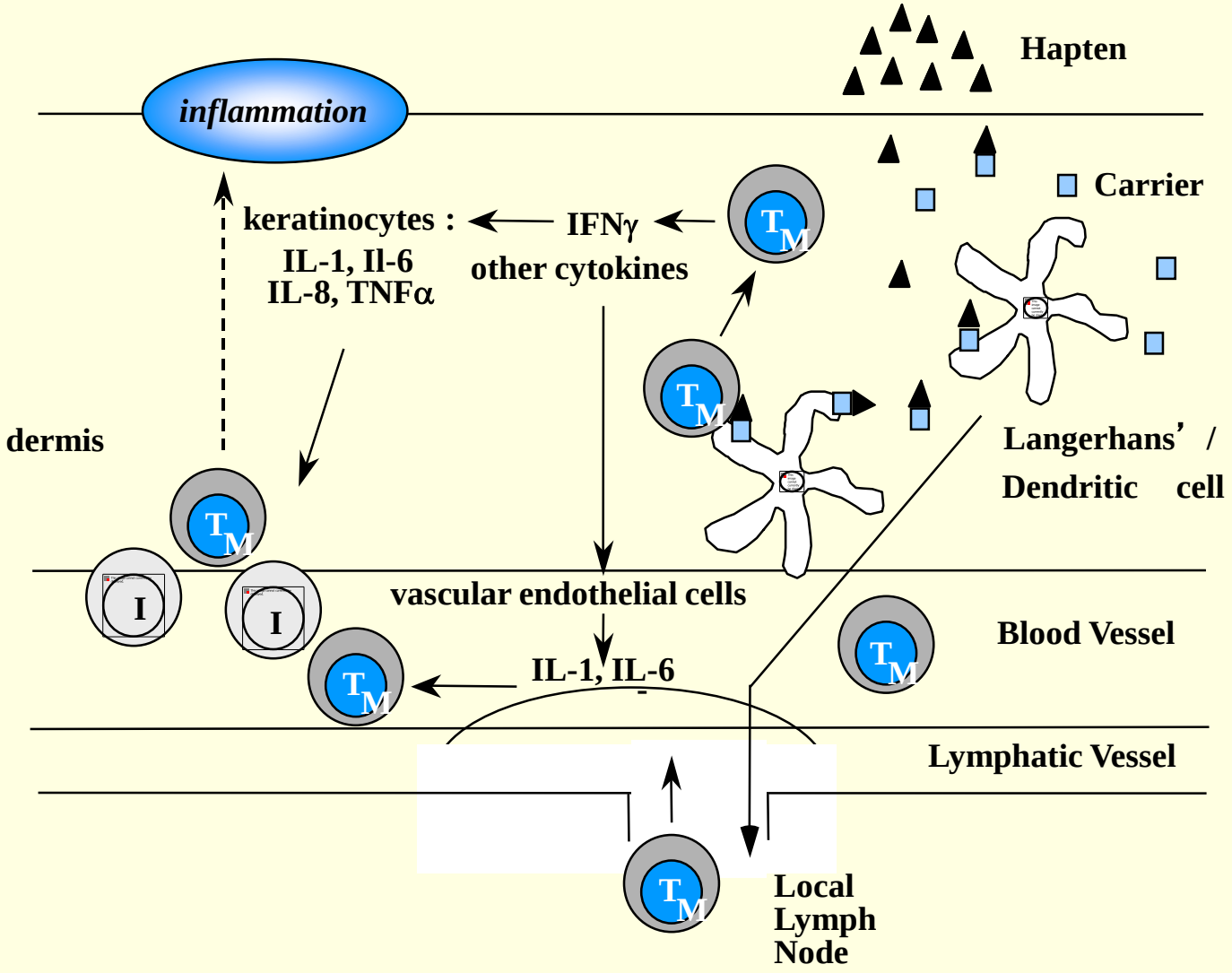
Allergic contact dermatitis
(Type IV)

Atopic dermatitis (Type I)

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: Elicitation



- 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

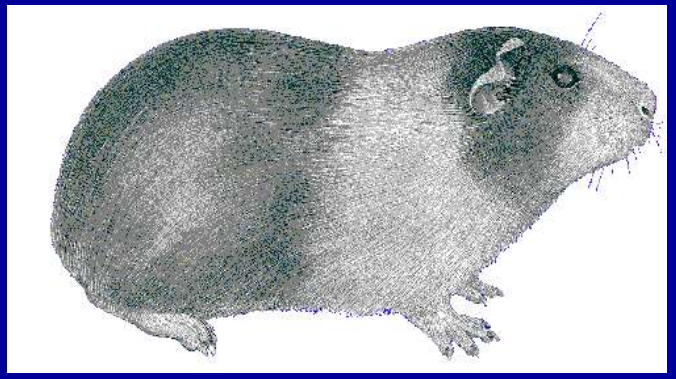
- **Well established methods for contact hypersensitivity.**
- **Current models and assays as inadequate predictors for system hypersensitivity reaction.**

METHODS IN IMMUNOTOXICOLOGY
Hypersensitivity Testing

- **Mouse Tests:**
  - Local lymph node assay
  - Mouse Ear Swelling Test
- **Guinea Pig Tests:**
  - Maximization Test
  - Occlusive Patch Test
  - Respiratory Challenge
  - Systemic Anaphylaxix

GUINEA PIG MODELS

Guinea Pig Maximization Test



20 animals/ group

Buehler Assay

ID injection w/ and without FCA plus topical application:

Days 5-8

Topical application - closed patch:

Days 0, 6-8, and 13-15

Induction

Day 20-22 topical challenge

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

Challenge

Endpoint ***erythema***

Read: 48,72 h after challenge

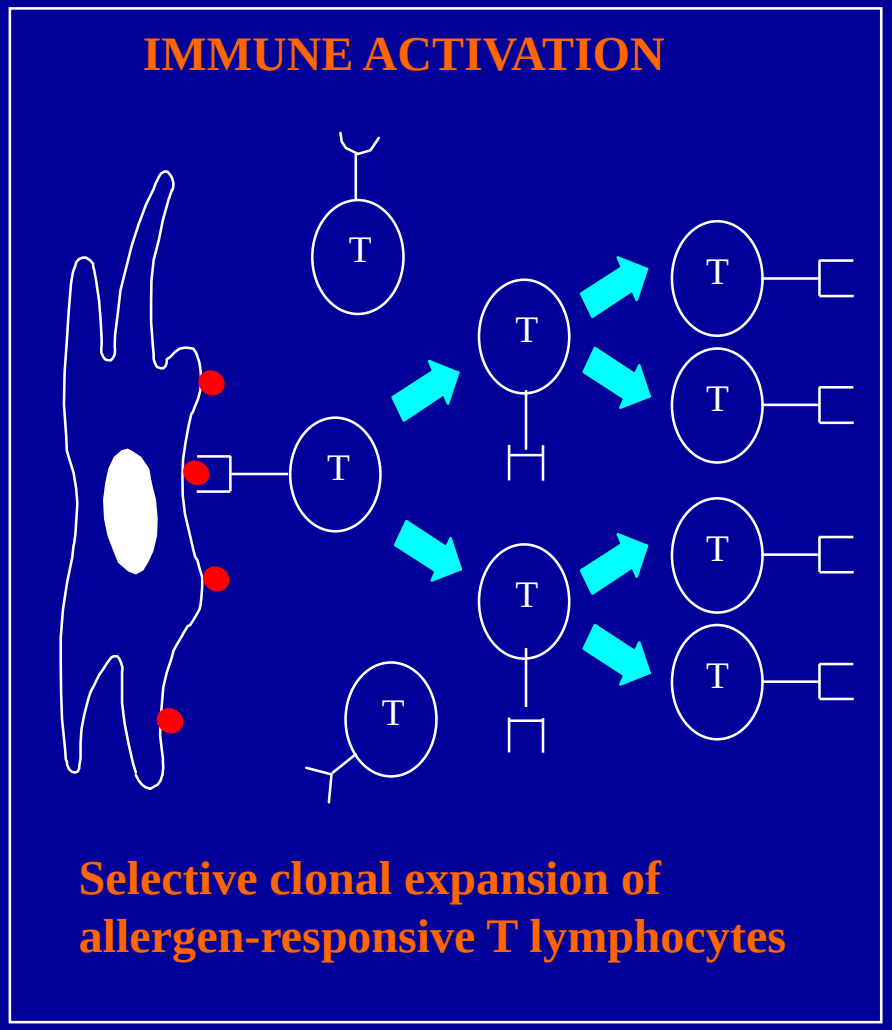
Read: 21, 24, 48 h after removing patch

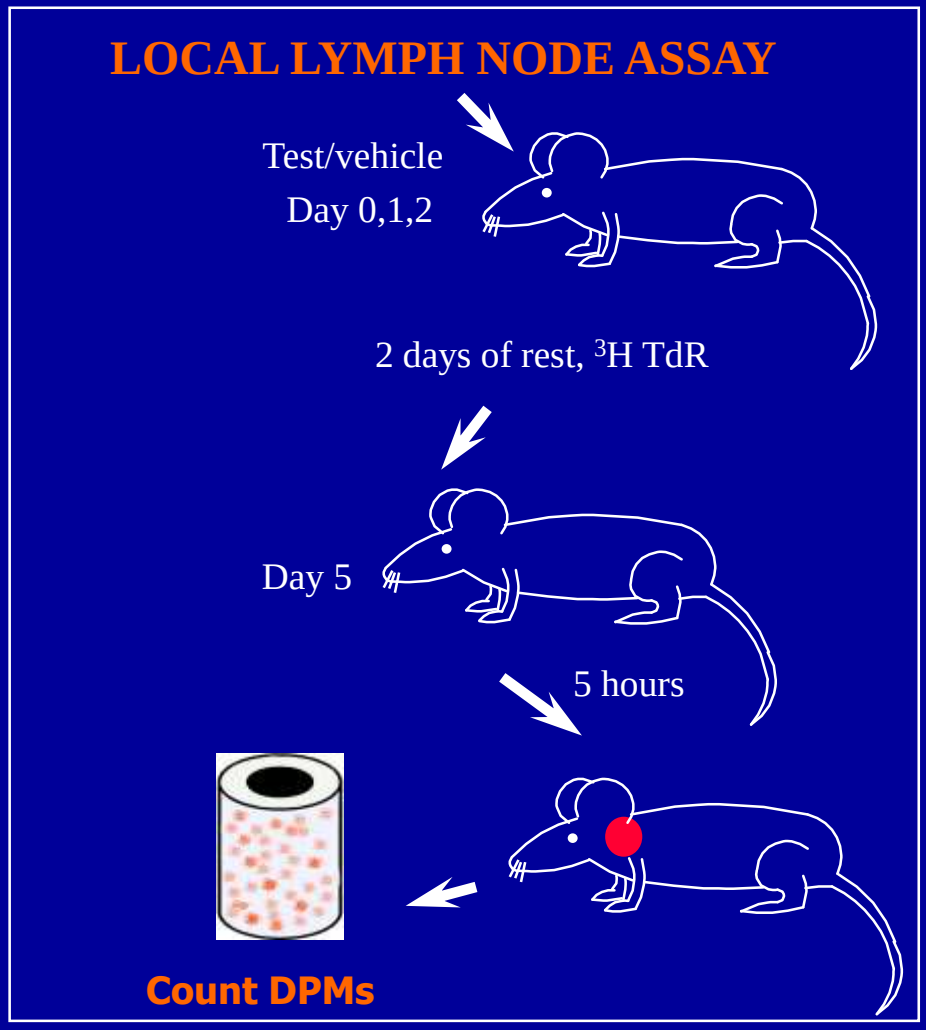
Criteria

>30% positive

> 15% positive

The mouse local lymph node assay (LLNA)

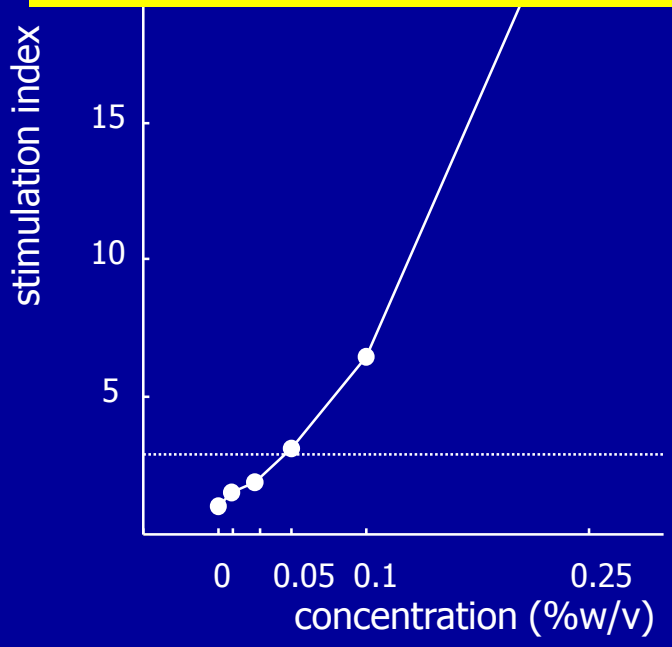


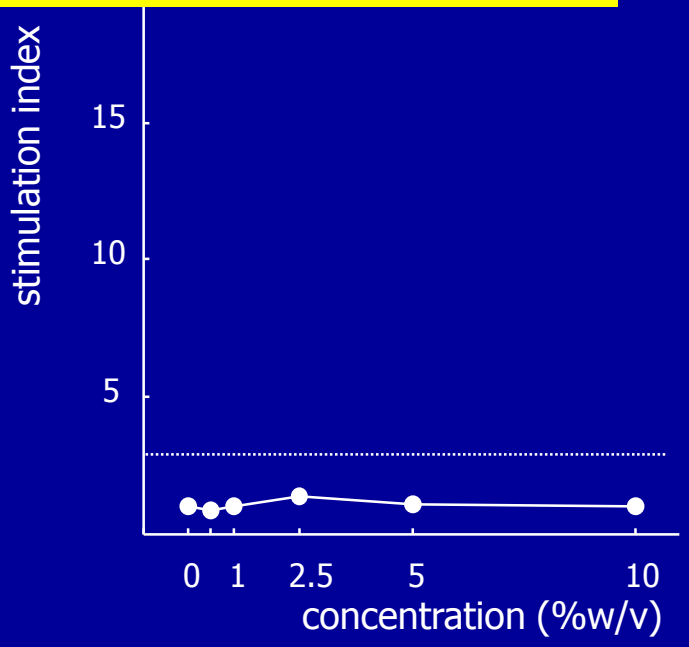


LLNA : illustrative results

DNCB **PABA**

The proliferation is proportional to the dose and chemical reactivity.

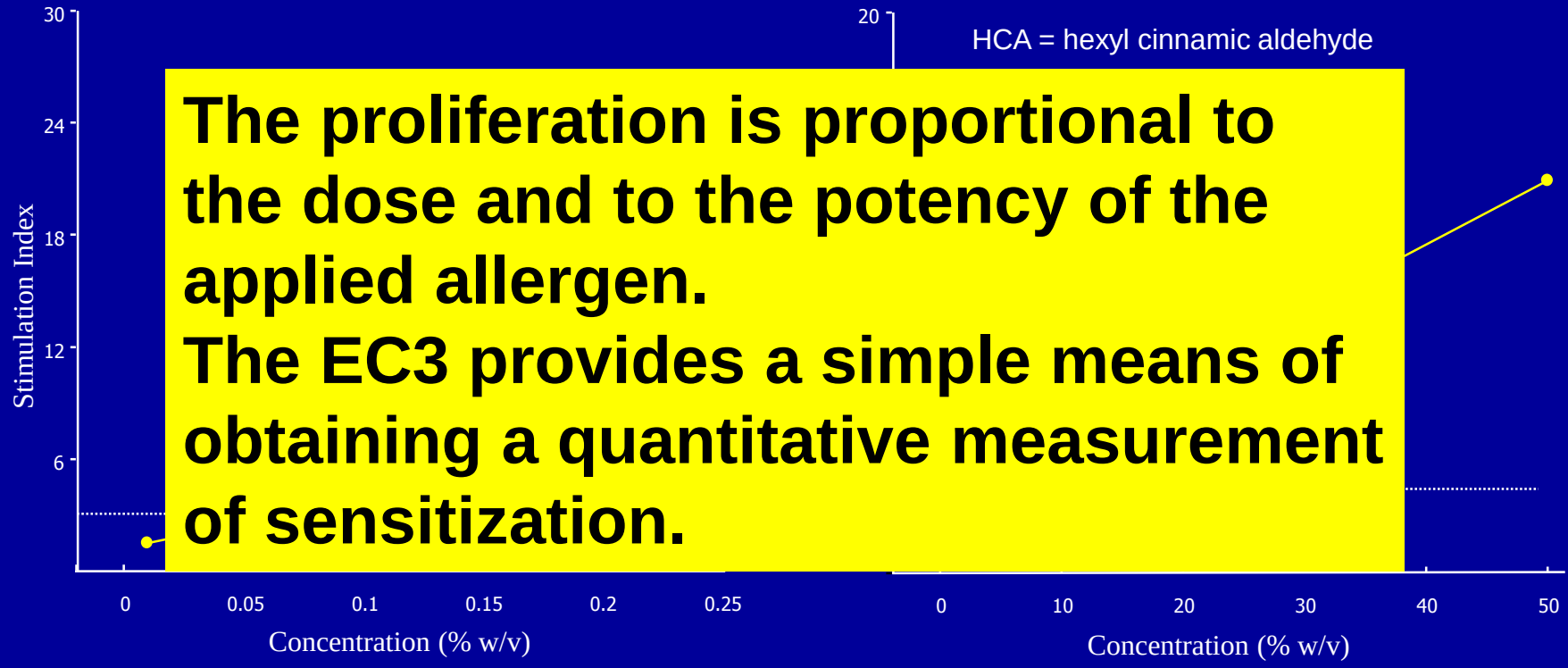




RELATIVE SKIN SENSITIZATION POTENCY

DNCB

HCA



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

Potency Category	EC3 Value µg/cm^2	EC3 Value % Concentration
Extreme	≤ 25	< 0.1
Strong	25 - 250	≥ 0.1 - < 1
Moderate	250 - 2500	≥ 1 - < 10
Weak	>2500	≥ 10

1% dose in a LLNA = 250 µg/cm^2

ECETOC 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

NEW OECD GUIDELINES

- **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 functionall and mechanistically similar to the LLNA.

- **OECD 442A - Skin Sensitization: LLNA DA**
- **OECD 442B - Skin Sensitization: LLNA BrdU-ELISA**

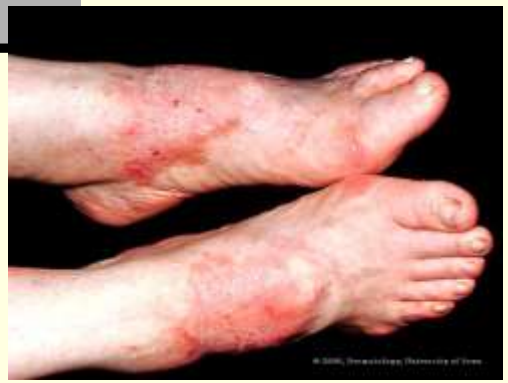
All adopted 22nd July 2010

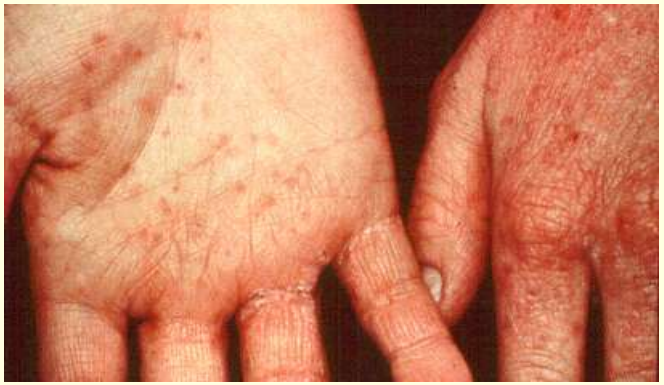
Summary

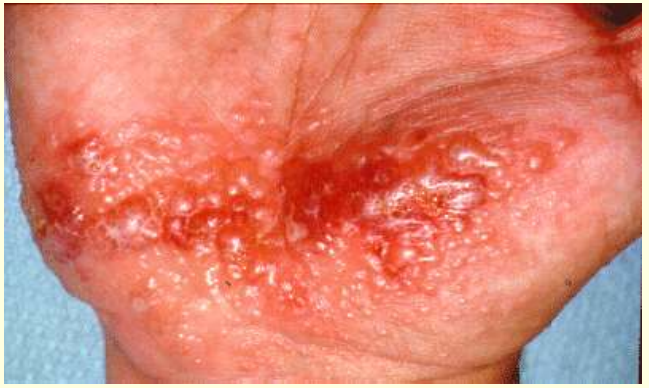
- The LLNA has gained widespread adoption and use internationally in the past 10+ years, providing for significant reduction and refinement
- Updated LLNA protocol reduces animal use by 20% and rLLNA can further reduce animal use by 40%
- Nonradioactive LLNA methods now allow for broad use, with reduced 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

Autoimmunity

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	Thyroglobulin
Thyrotoxicosis	Thyroid-stimulating hormone (TSH)
Pernicious anemia	H+/K+-ATPase
Autoimmune Atrophic Gastritis	Intrinsic factor
Addison's Disease	21-hydroxylase
Insulin-Dependent Diabetes Mellitus	Glutamic acid decarboxylase 65
Goodpasture's Syndrome	Type IV collagen
Myasthenia Gravis	Acetyl choline receptor
Male Infertility (isolated cases)	Epididymal glycoprotein, FA-1
Sympathetic Ophthalmia	Interphotoreceptor retinol binding protein
Multiple Sclerosis	Myelin basic protein
Autoimmune Hemolytic Anemia	X-antigen, glycophorin
Ulcerative Colitis	Catalase; a-enolase
Rheumatoid Arthritis	Rheumatoid factor
Scleroderma	Topoisomerase 1; laminins
Systemic Lupus Erythematosus (SLE)	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

Autoimmunity: multifactorial diseases

- 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

METHODS TO STUDY AUTOIMMUNE DISEASE

- 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

EXOGENOUS FACTORS ASSOCIATED WITH AUTOIMMUNITY

- 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

CONCLUSION

➔ 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.**