

Immunotoxicology: History and Current Advances

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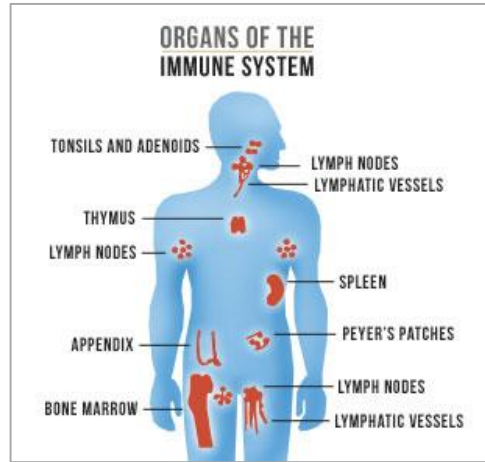
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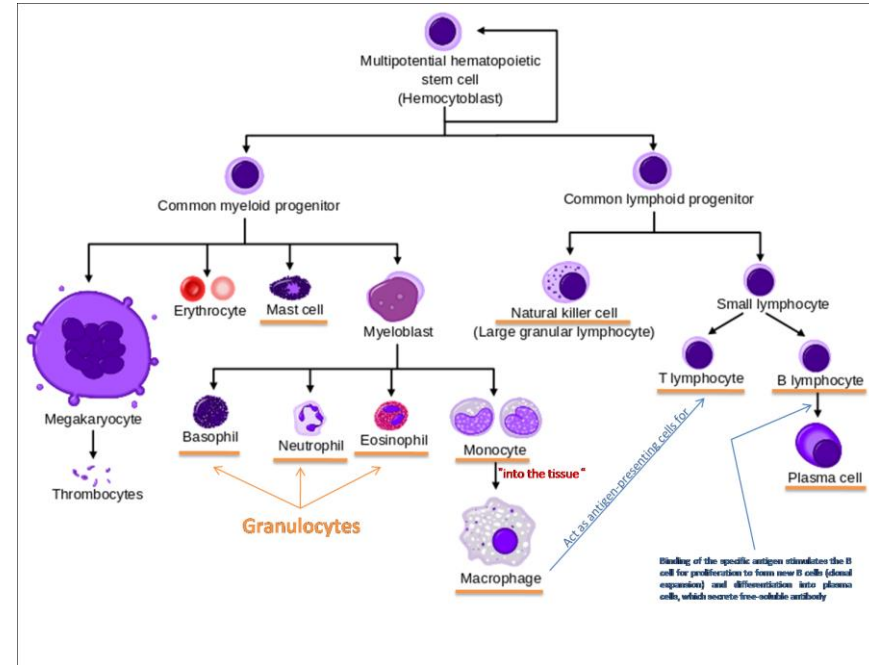
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Immune System

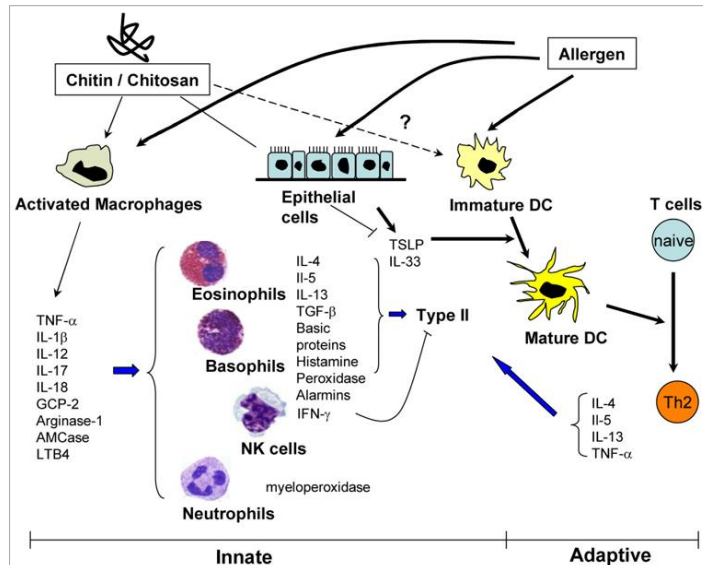
Network of organs/tissues, cells and mediators



<http://www.newhealthguide.org/Immune-System-1.html>

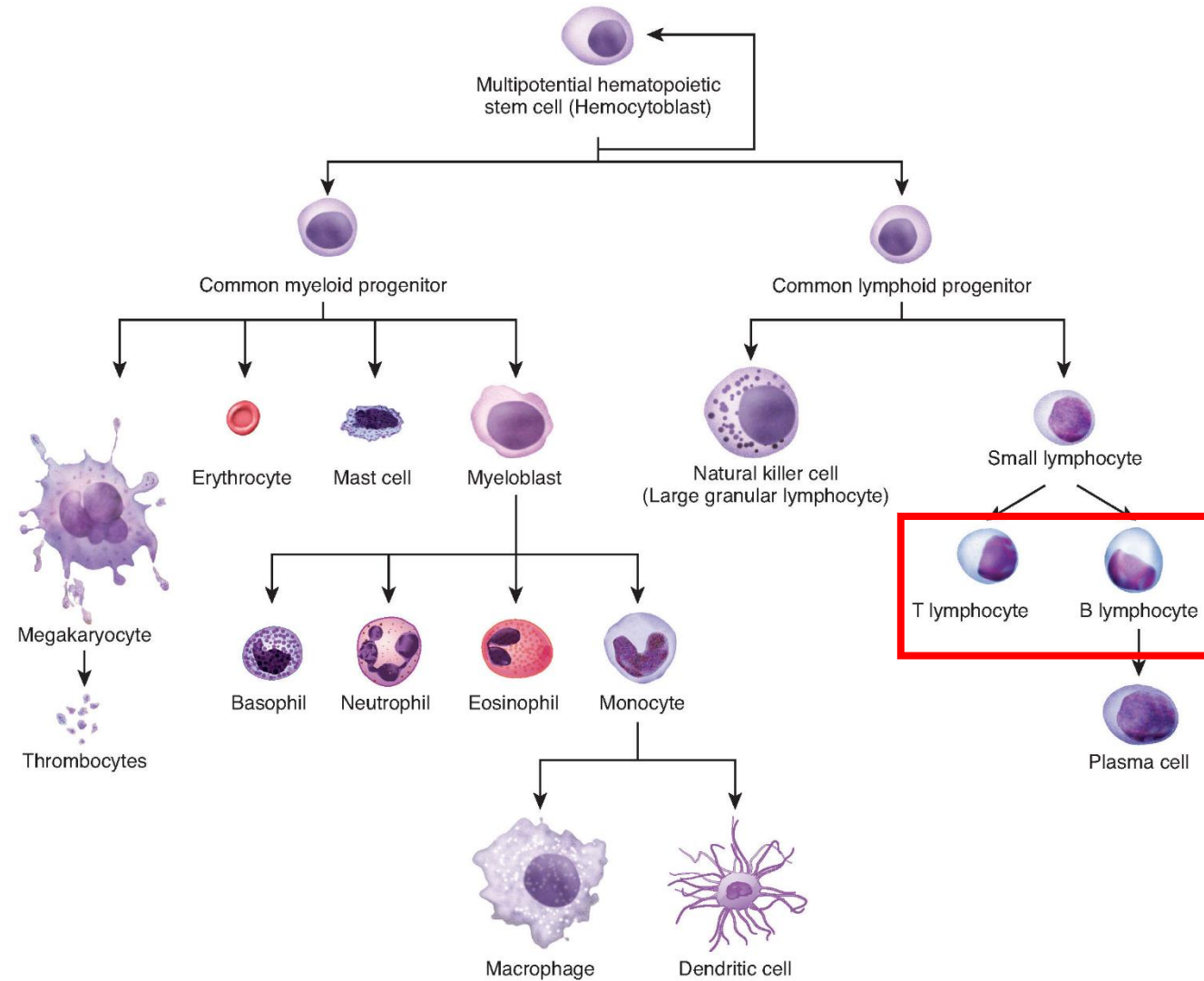


<http://www.theamazingmedicine.blogspot.com/2013/07/immunology-i-physiology-of-immune-system.html>

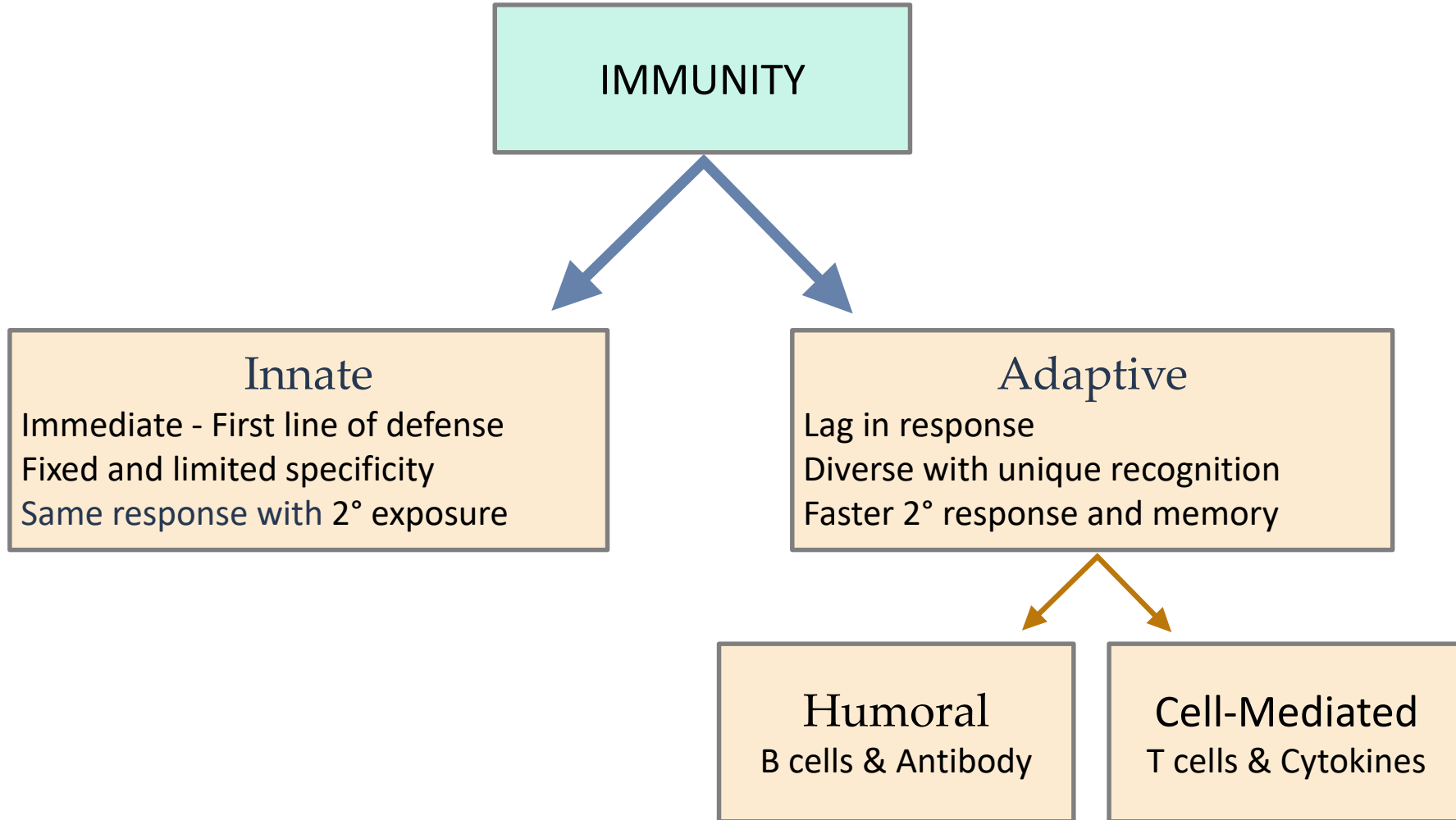


Lee, C G. Chitin, Chitinases and Chitinase-like Proteins in Allergic Inflammation and Tissue Remodeling. *Yonsei Med J.* 2009 Feb;50(1):22-30. <http://dx.doi.org/10.3349/ymj.2009.50.1.22>

Immune Cell Types

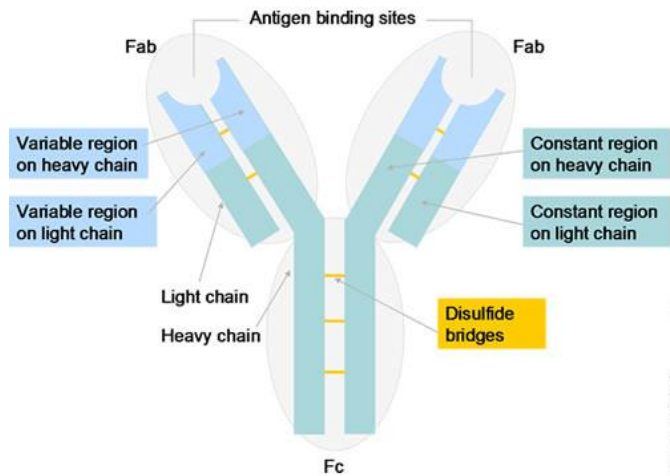


Immune System

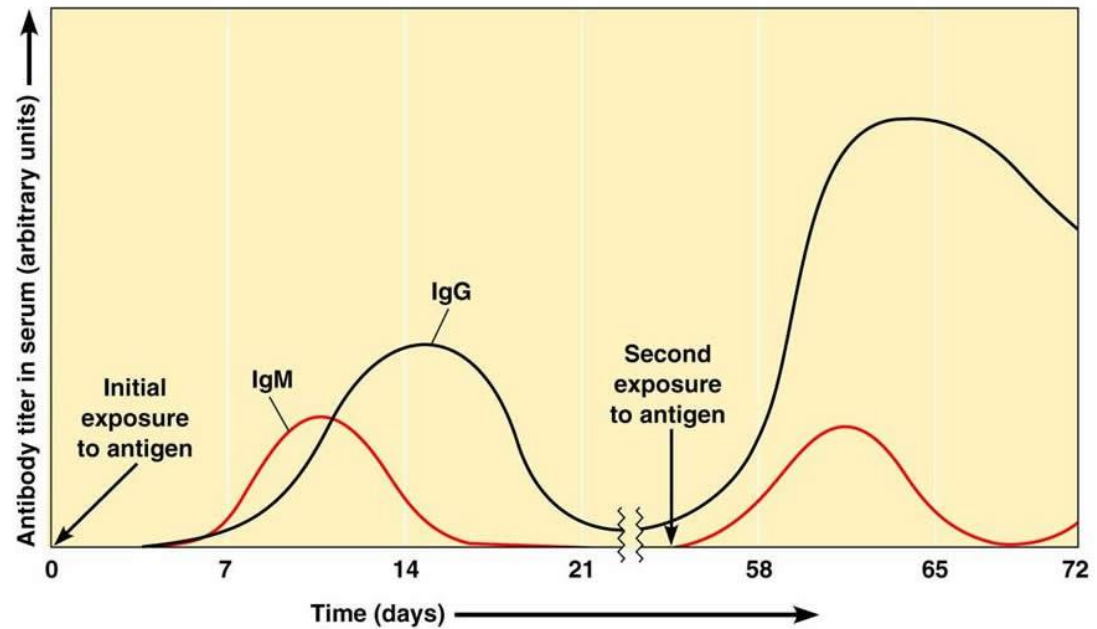
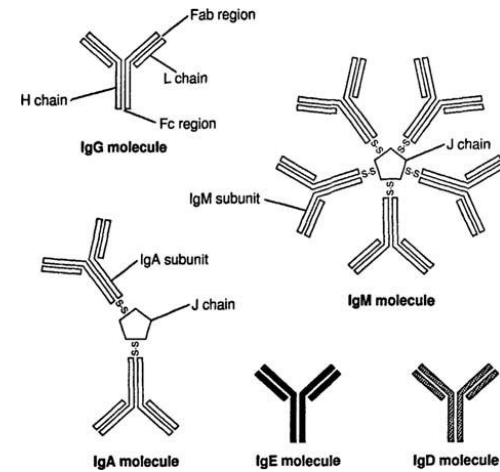


Immune System

Antibody Response



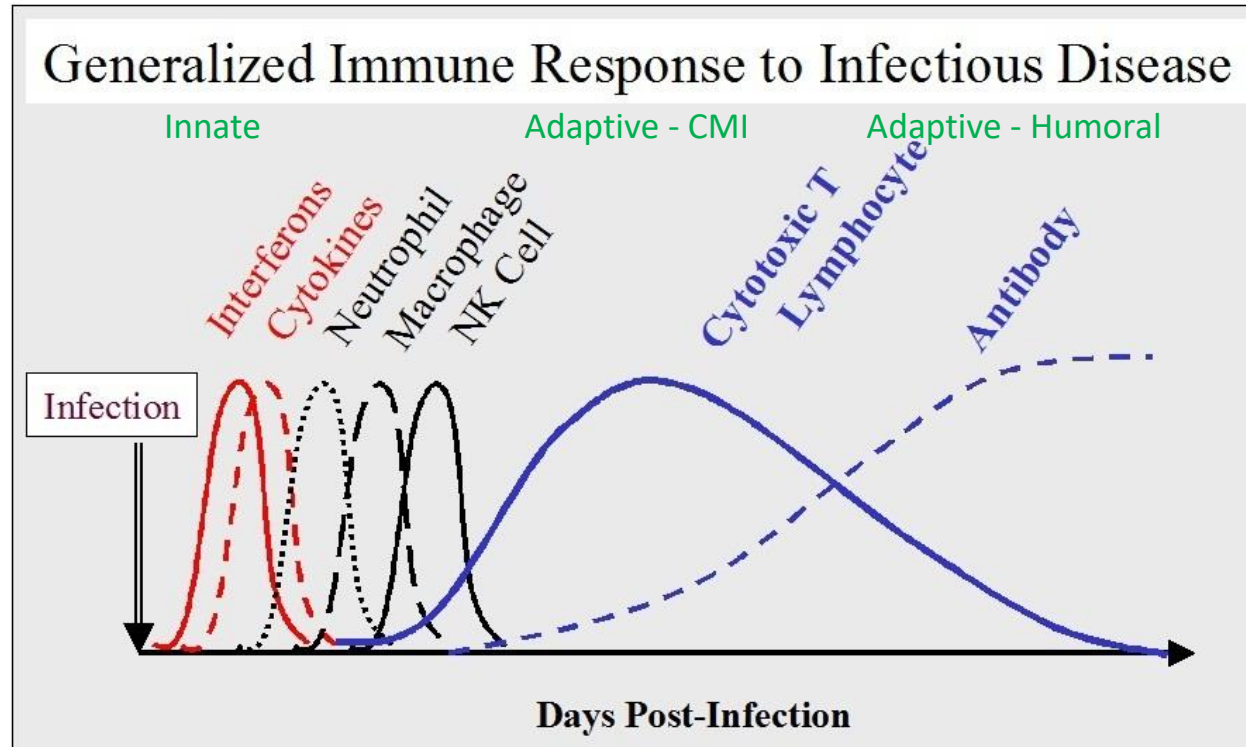
www.ptglab.com



www.classes.midlandstech.edu

Immune System

Response to Infection – An Immune Framework



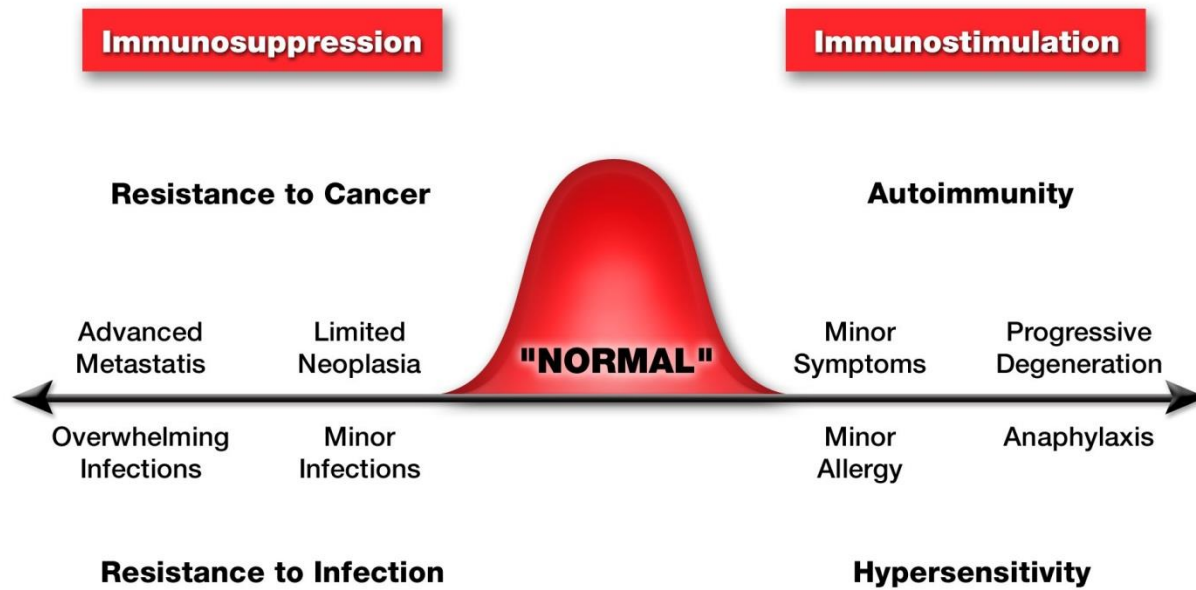
G. Burleson, with permission

Immunotoxicology

DEFINITION

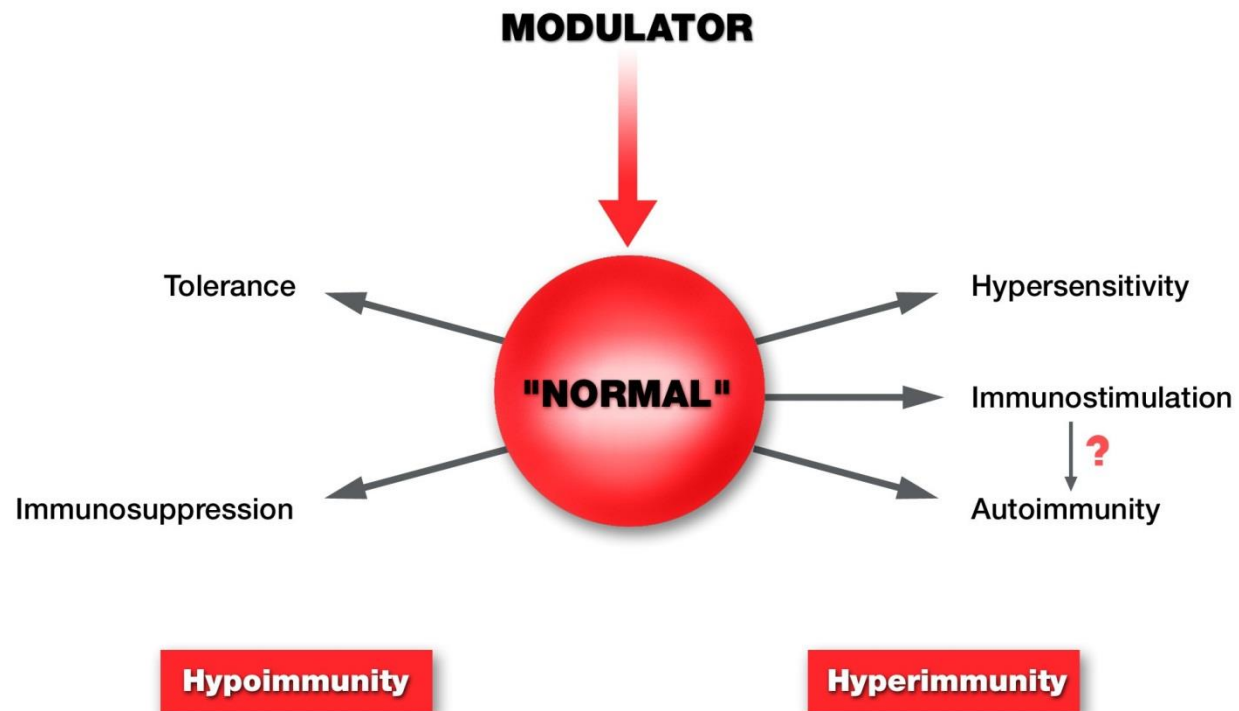
Immunotoxicology is a scientific discipline whose aim is to detect, quantify, and interpret direct or indirect alterations of the immune system that occur as a result of exposure to chemicals, pharmaceuticals, recombinant biologicals, or environmental and occupational pollutants.

Burleson, G.R. and J.H.Dean. Immunotoxicology: Past, Present, and Future. 1995. Methods in Immunotoxicology, Volume 1 pages 3-10.



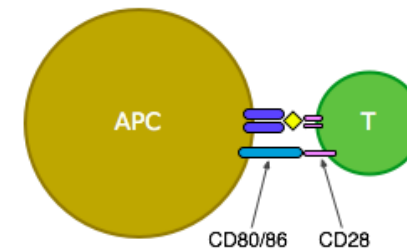
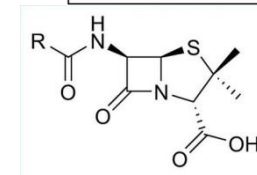
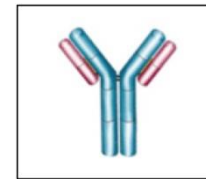
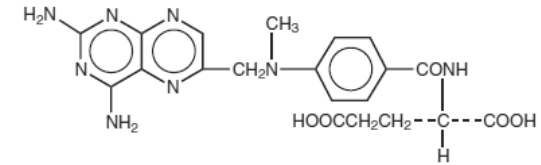
But Is This Correct?

- Signs of infection – indicating immunosuppression – may be confused with signs of immunostimulation
- This is especially true for inflammation: when observed, is this hypersensitivity/autoimmunity, or the immune system functioning as designed?
- Chronic inflammation can result from persistent viral infection – inability to demonstrate an infectious agent does not mean that what is observed is a form of drug allergy



Examples of Pharmaceutical Immunotoxicity

- Immunosuppression: Infections due to chemotherapy (methotrexate), or direct pharmacological effects (azathioprine)
- Immunogenicity: monoclonal antibodies, other protein drugs
- Hypersensitivity: Penicillin (anaphylaxis)
- Autoimmunity: Pharmaceuticals targeting regulatory proteins in the immune system (anti-TNF)
- Adverse Immunostimulation: Direct or indirect effects (TGN 1412 -- anti-CD 28, oligonucleotides)



Anaphylaxis

- Perhaps first example of experimental immunotoxicology: Richet and Portier (1902) reported an attempt to “immunize” dogs with injected sea anemone material (“prophylaxis”) which produced systemic shock instead (“anaphylaxis”)
- In the same year the US enacted the Biologics Control Act (BCA) after deaths in children due to diphtheria anti-toxin contaminated with tetanus: there was also the evolving understanding that some adverse effects associated with vaccines and anti-toxins could be due to immunogenic contaminants (von Pirquet and Schick, 1906)
- This was supported by studies using rabbits: anaphylaxis could be induced only after a second “challenge” exposure to the causative substance (Auer 1911)

Landsteiner and Draize

- Penicillin: miracle drug that occasionally killed people – often appeared to be “anaphylaxis”
- Landsteiner and Jacobs (1935, 1936) demonstrated that certain chemicals could form “irreversible bonds” with proteins that were immunogenic
- These “haptens” (*partial antigens*) provided an important clue for understanding the basis for immune responses to non-immunogenic molecules
- Penicillin was demonstrated to form biotransformation products *in vivo* which could covalently bind to endogenous proteins and could induce immune responses
- Landsteiner’s work was used to develop what is likely the first immunotoxicology assay: the Draize test for allergic contact dermatitis potential (1944)

Ovary and the PCA Assay

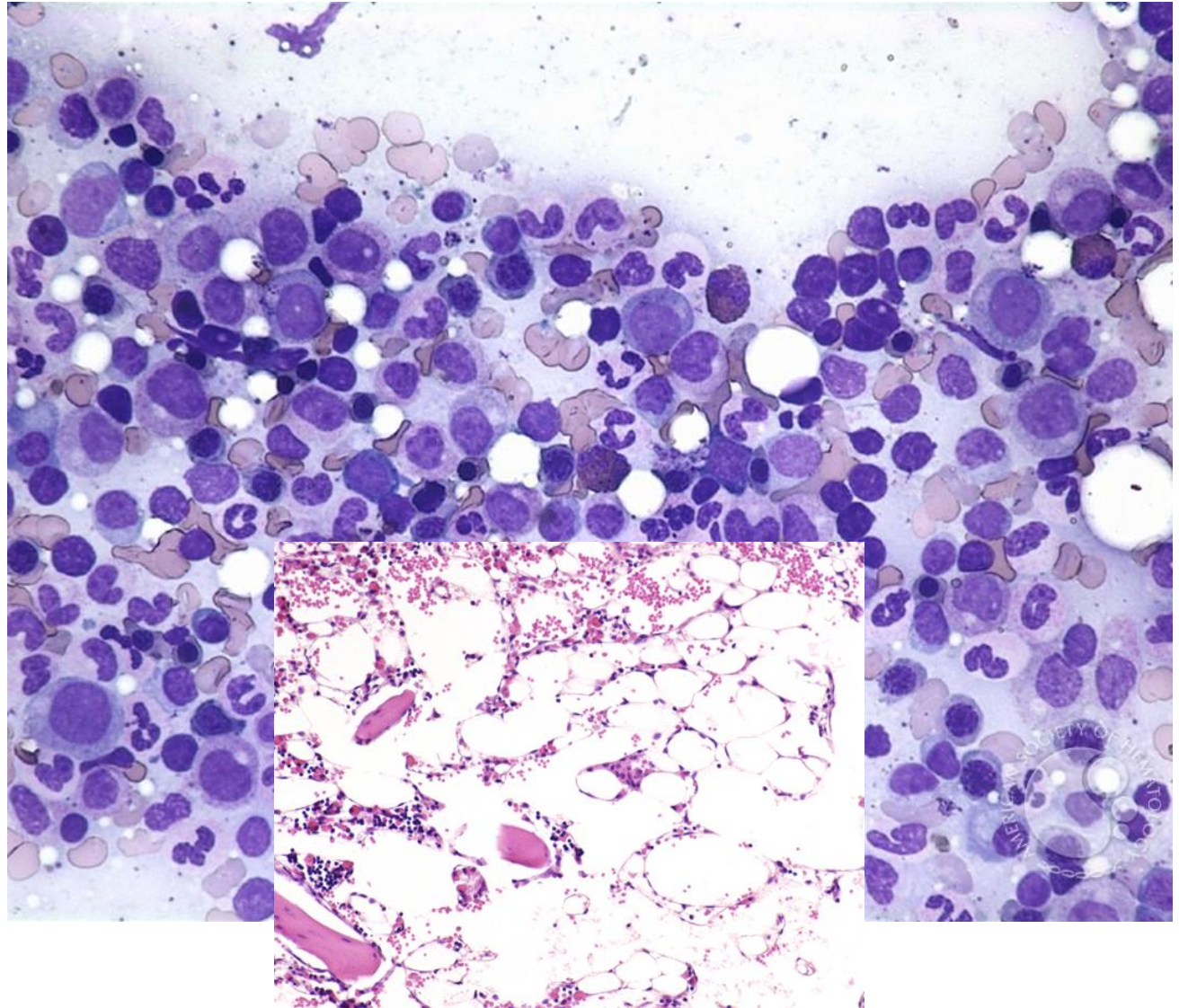
- Ovary and his colleagues demonstrated that the immunoglobulin responsible for anaphylaxis possessed a different molecular weight than those produced in response to infections and that had been shown to be protective (prophylactic, “heterocytotropic”) (Ovary et al, 1963)
- These “homocytotropic” antibodies could be induced by both proteins and haptens – explaining the penicillin reaction
- A relatively simple method for demonstrating anaphylaxis was developed – the passive cutaneous anaphylaxis assay (PCA) (Ovary, 1958)
- Variations on this method have also been developed: active systemic and cutaneous assays

Immunopathology: The Gell & Coombs Categories

- Current classification system was proposed Gell and Coombs (G&C) in the 1960s
- Categories:
 - Type I: immediate hypersensitivity
 - Respiratory
 - Urticaria
 - Systemic
 - Type II: antibody-mediated
 - Antibody-mediated cytotoxicity
 - Antibody-dependent cell-mediated cytotoxicity (ADCC)
 - Type III: immune complex-mediated reactions
 - Type IV: delayed hypersensitivity
 - Dermal
 - Respiratory
 - Systemic

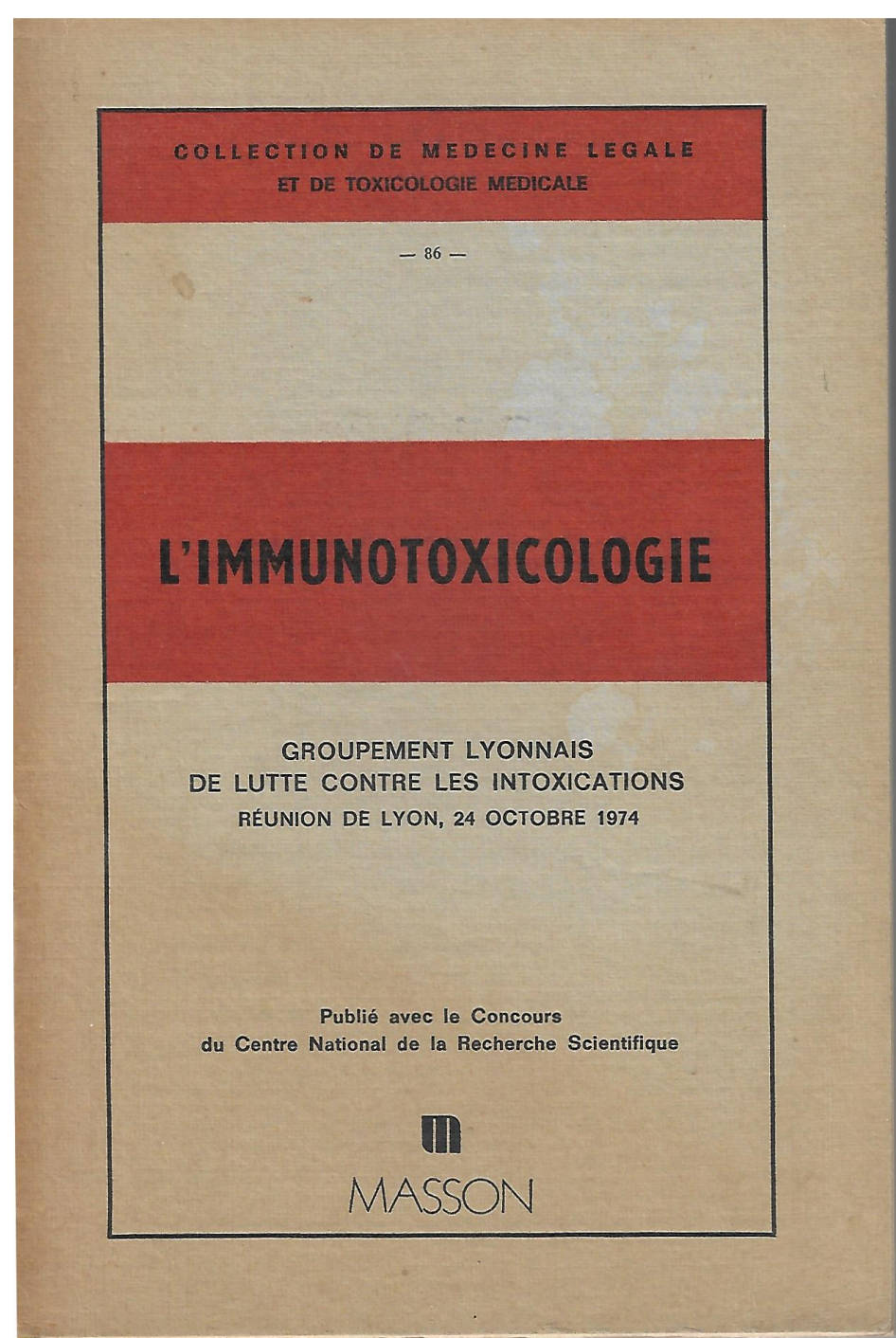
Bone Marrow Toxicity/Myelotoxicity

- Rapidly dividing cells
- Target of ionizing radiation
- Susceptible to cytotoxic anti-proliferative xenobiotics (e.g. alkylating agents)
- Associated with increased susceptibility to infections and leukemia/lymphoma
- Also associated with gastrointestinal and skin toxicities
- Thrombocytopenia



CONTENTS

- Value of radioimmunological techniques in pharmacology and toxicology
- Immunological methods to detect and assay toxicants and medicines in biological fluids (excluding radioimmunological techniques)
- Chemical reactivity - sensitization and poisoning
- Immunological mechanisms of adverse drug events - main aspects of allergic toxidermias
- Drug-induced allergic cytopenias
- Allergic reactions due to pharmaceuticals in nephrology
- Autoimmunization during drug treatments or poisonings



Immunotoxicology Emerges

- Vos, J. G. (1977). Immune suppression as related to toxicology. Reprinted *Journal of Immunotoxicology* 4, 175–200, 2007 5, 67–101
- *Inadvertent Modification of the Immune Response: The Effects of Foods, Drugs, and Environmental Contaminants* (Proceedings of the Fourth FDA Science Symposium, US Naval Academy, Aug. 28-30, 1978)
- “Drug adverse reaction reports related to immunotoxicity” Nelson S. Irey, AFIP
- Of 3900 ADRs, 486 (12%) involved RE system
- Adverse effects reported: tumors and “pseudo-tumors” (transplant patients), opportunistic infections, hypersensitivity reactions (anaphylaxis, vasculitis, other types)

Immunotoxicology: Historical Perspective

- **1979:** The first scientific symposia were the Annals of the New York Academy of Science satellite meeting called "Immune Abnormality", followed by a Gordon Research Conference on drug safety and the effect of environmental chemicals on the immune system (Dean, et al. 1979)
- **1983:** Immunology Today (Trends in Immunology) formally announced the birth of the field of Immunotoxicology resulting from the fusion of immunology and toxicology (Davies, 1983)

Evolution of Guidance on Immunotoxicology Evaluation of Drugs

- *Report to the Drug Safety Subsection Steering Committee of the Pharmaceutical Manufacturers Association on Immunotoxicology and the Pharmaceutical Industry* (Immunotoxicology Task Force; March, 1988)
- No “random screening”: problem-driven approach
- Development of approaches using standard toxicology tests and species
- More research in drug-induced hypersensitivity
- Multidiscipline approach, involve clinicians, don't make genetic toxicology mistakes

ICH S8

- Cause-for-concern approach, with several factors to be considered (findings in nonclinical and clinical studies, patient population, drug classes)
- Recommended assays: TDAR, immunophenotyping, CMI assays, host resistance – all from original NTP tier design
- Adverse immunostimulation included as important issue, but no real guidance on studies
- Problem: no recommendations on bridging to clinical trials (biomarkers, timing, PK, etc.)

Immunosuppression: Examples of Indicators

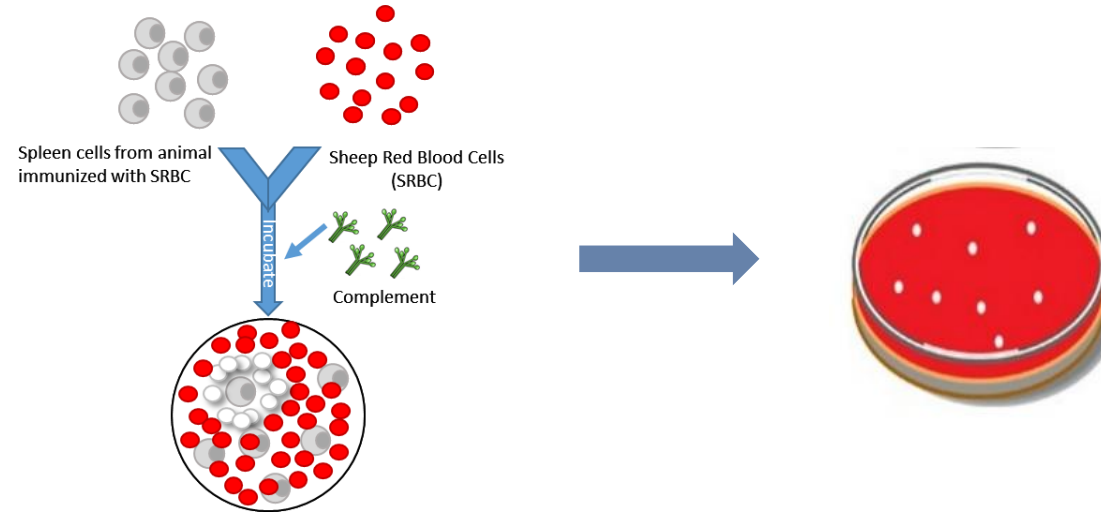
- **Evidence of myelosuppression: e.g. leukopenia, pancytopenia, lymphopenia**
- **Gross pathology findings: involution of thymus**
- **Alterations in immune system organ weights and/or histology: hypocellularity of immune system tissues**
- **Increased incidence of infections: UTIs in rodents**
- **Increased incidence of tumors**
- **Decreased serum immunoglobulin levels**
- **Distinction between unintended (adverse) and intended (pharmacodynamic) immunosuppressive effects**

When Signs of Immunosuppression Observed

- Usual recommendation: test for T-cell dependent antibody response (TDAR) e.g. sheep red blood cell primary (IgM) antibody response (plaque assay)
- TDAR with alternative immunogen (KLH, TT, phage)
- Can be adapted for IgG response
- ELISA/ELISPOT to quantitate antibody response
- Dose, duration and route should be consistent with standard nonclinical studies in which signs of immunosuppression were observed
- Identify causative organism for increased infections
- If considered due to stress, MTD may not be useful: also consider biomarker (e.g. urine corticosterone in rodents)

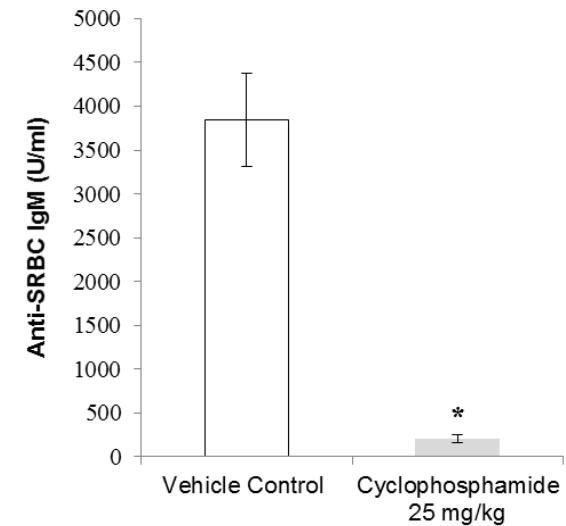
Functional Tests - TDAR

- PFC Assay



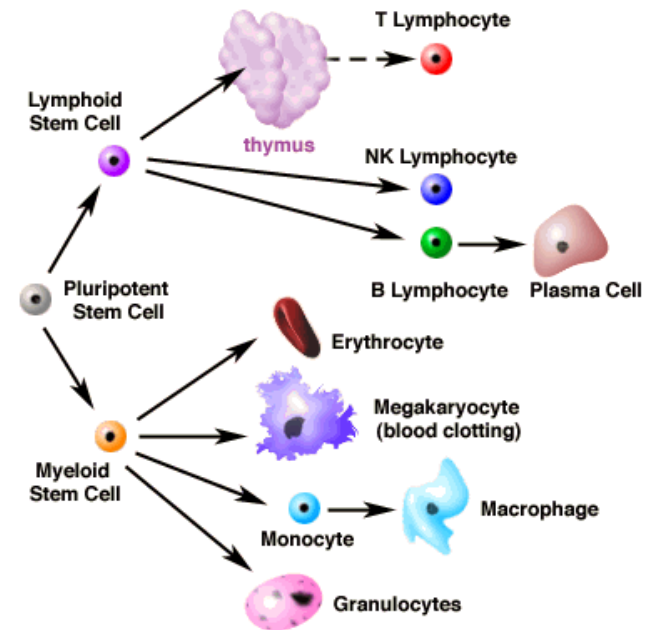
- ELISA

Treatment Group	Anti-SRBC IgM (U/ml)	SE
Vehicle Control	3849	530
Cyclophosphamide 25 mg/kg	210	46



Additional Determinations: Effect of Xenobiotic Exposure

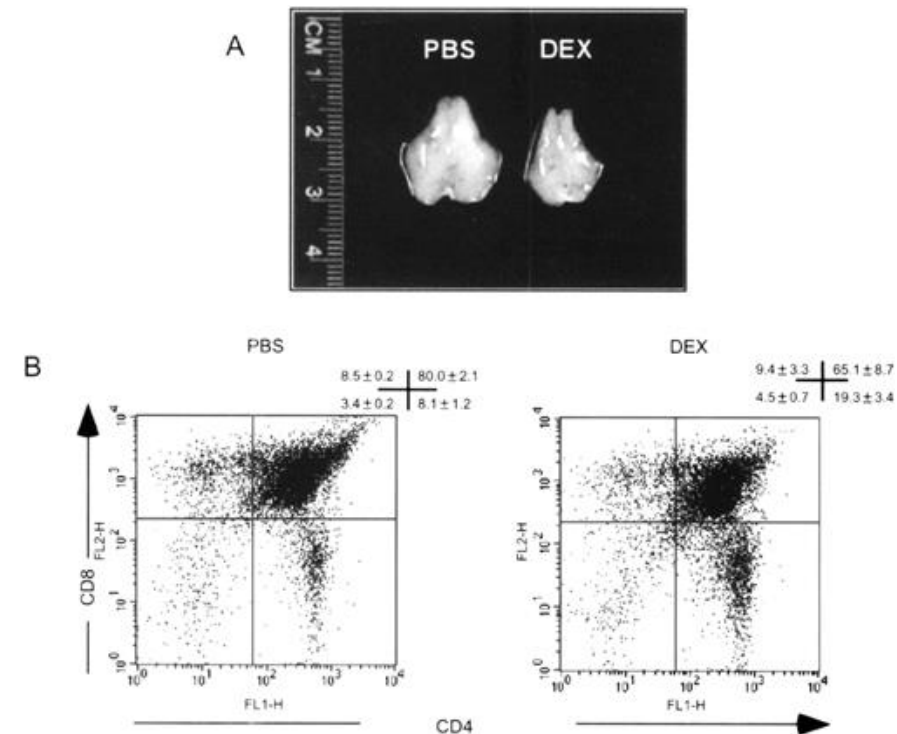
- T-cell independent antibody assay
- Natural killer cell function
- Cytotoxic T cell function
- Cytokine production
- Delayed-type hypersensitivity response
- Host resistance assays
- Complement activation
- Colony-forming unit assays
- Macrophage or neutrophil function
- Immune cell phenotypes



Immunophenotyping

Flow cytometry

- Additional non-functional evaluation
- May be used as an adjunct to hematology in STS or as part of follow-up studies
- Provides information on alterations in leukocyte populations in blood as well as other organs (e.g. spleen, thymus)



Host Resistance Models

- Influenza
- *Herpes simplex 1*
- *Gammaherpesvirus 68*
- Murine cytomegalovirus (primary or latent)
- *Streptococcus pneumoniae*
- *Staphylococcus aureus*
- *Listeria monocytogenes*
- *Pseudomonas aeruginosa*
- *Candida albicans*
- *Plasmodium berghei*
- EL4 mouse lymphoma
- B16F10 mouse melanoma

Immunotoxicity Testing

Immune Assays to Establish Concordance

Tests were used to establish predictability using immune assays with relevance in the risk assessment process for resistance to infection or neoplasia.

Concordance was established on the basis that (a) the test material resulted in a dose-response response or altered two or more immune assays, or (b) compared to host resistance assays since the primary responsibility of the immune system is to protect against infectious or neoplastic disease.

Luster et al. 1992. Risk assessment in immunotoxicology: I. Sensitivity and predictability of immune tests. *Fund Appl Toxicol* 18:200-210

Luster et al. 1993. Risk assessment in immunotoxicology: II. Relationships between immune and host resistance tests. *Fund Appl Toxicol* 21:71-82

Luster et al. 1995. Immunotoxicology and risk assessment. In: *Methods in Immunotoxicology, Volume 1*, pp. 51-68, Chapter 5, (Eds: G.R. Burleson, J.H. Dean and A.E. Munson), Wiley-Liss, Inc., New York, N.Y.

Immunotoxicity Testing

Immune Assays to Establish Concordance

While the predictive values of individual immune assays for host resistance tests range from relatively good (AFC assay: 73%; NK activity: 73%; DTH: 82%) to poor (lymphoproliferative response to LPS < 50%, combinations of multiple immune tests can, and should, increase concordance to as high as 100%.

- There are 8 three-way combinations of immune parameters that achieve 100 % concordance
- Of these 8 combinations :
 - Acquired cell-mediated immune functional measures were included in 7 of the 8 combinations
 - Humoral immune functions (TDAR) was included in 4 of the successful combinations.

Immunosuppression: Considerations for Additional Studies

- Intended patient population: If drug will be administered to women of child bearing age, evaluate F1 generation hematology and lymphoid histology in reproductive toxicity studies.
- Known drug class effects
- Pharmacokinetics (concentration of drug and/or metabolites in immune tissues)
- Immunosuppression in clinical trials
- Stress related immunosuppression should be supported with adequate data, e.g. measure cortisol levels or compare treatment groups

Lymphoid organs to be examined for immunotoxic effects (ICH S8)

Lymphoid organ	Tissue Compartment		Distribution
Thymus	Cortex	Subcapsular region	Immature T cells (CD4CD8 duple negative cells)
		Deep cortex	Immature T cells (CD4CD8 double positive cells)
	Medulla		Mature T cells (CD4 single positive cells, CD8 single positive cells)
Spleen	White pulp	Lymphoid follicle	B cells
		Marginal zone	B cells
		Periarteriolar lymphoid sheath (PALS)	T cells
	Red pulp		Erythrocytes and macrophages
Lymph node	Cortex		B cells
	Paracortex		T cells
	Medulla	Medullary cord	B cells and plasma cells
Peyer's patch	Follicle		B cells
	Interfollicular area		T cells
Bone marrow	Erythroid cells		
	Myeloid cells	Proliferation phase cells	
		Mature granulocytes	
	Megakaryocytic cells		
Adipose tissue			

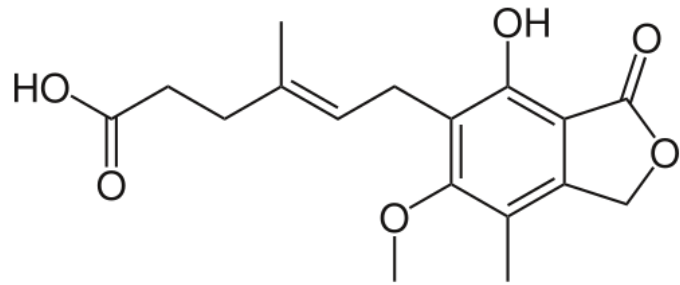
Weight of Evidence Review

- A decision is made after the WoE (weight of evidence) review
- From ICH S8: “A finding of sufficient magnitude in a single area should trigger additional immunotoxicity studies. Findings from two or more factors, each one of which would not be sufficient on its own, could trigger additional studies. If additional immunotoxicity studies are not performed, the sponsor should provide justification.”
- Timing of assessment: ICH S8 suggests prior to Phase III. However, talk to the respective Division for specific drug development programs.

Immunosuppressant Drugs

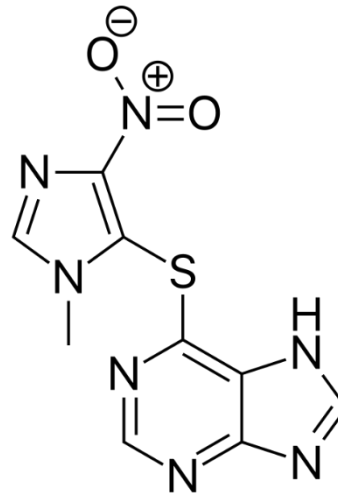
- Cytotoxic cancer chemotherapeutics (e.g. cyclophosphamide, 5-fluorouracil, vincristine, rituximab)
- Transplant drugs (e.g. azathioprine, cyclosporine, tacrolimus, rapamycin, mycophenolate mofetil)
- Anti-inflammatory drugs (prednisone, methotrexate, natalizumab, anti-TNF α mAbs)

Cytotoxic Anti-proliferative Immunosuppressants



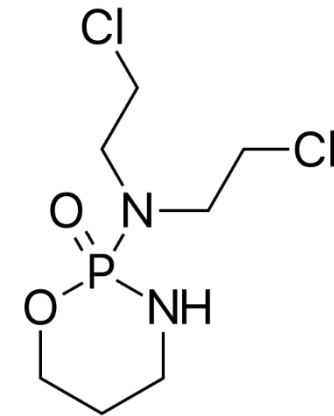
Mycophenolic Acid

Inhibits inosine monophosphate dehydrogenase needed for *de novo* T- and B-cell purine synthesis



Azathioprine

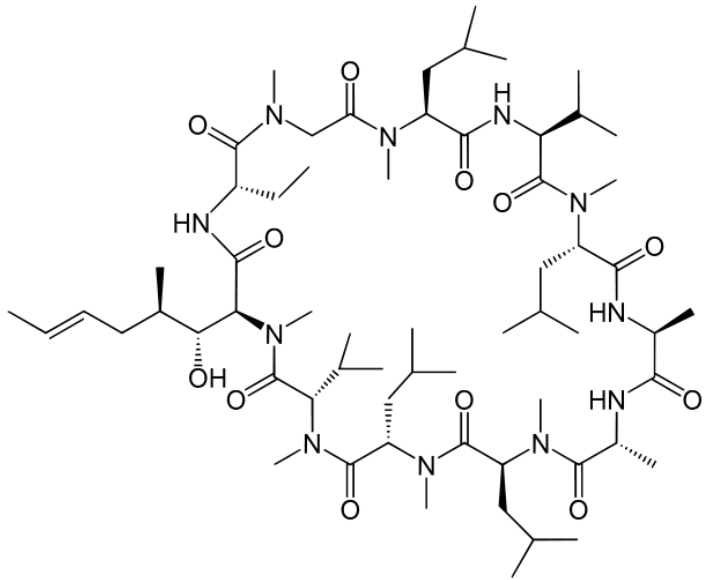
Anti-metabolite → inhibits purine synthesis



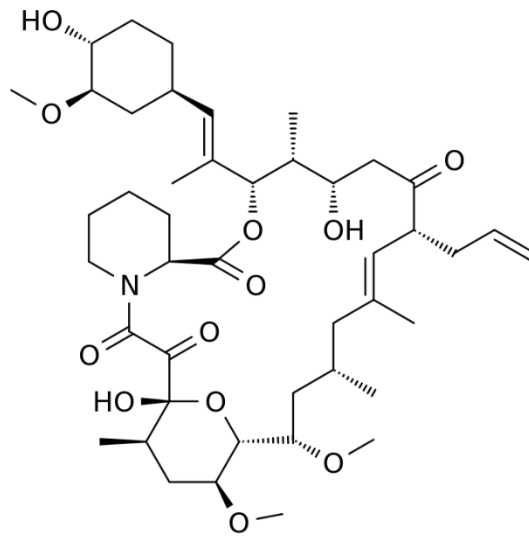
Cyclophosphamide

Metabolite phosphoramidate cross-links DNA

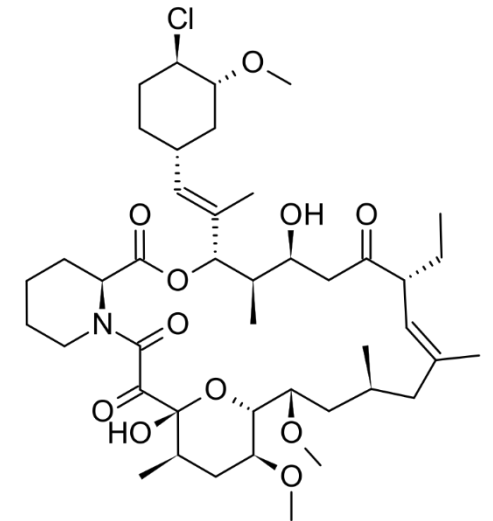
Calcineurin Inhibitors



Cyclosporine A



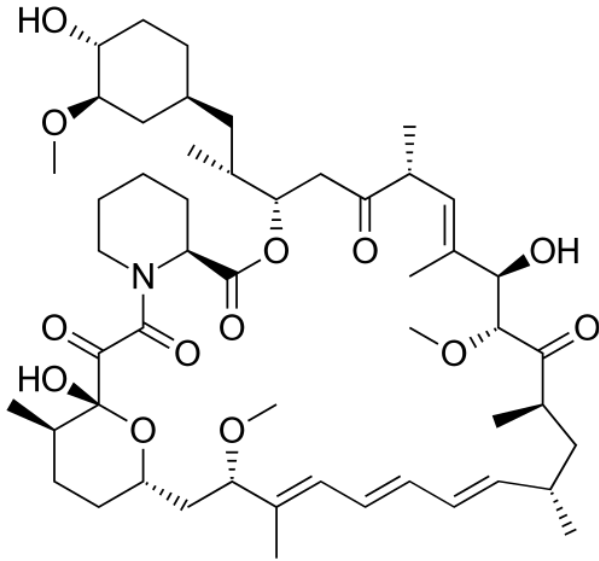
Tacrolimus



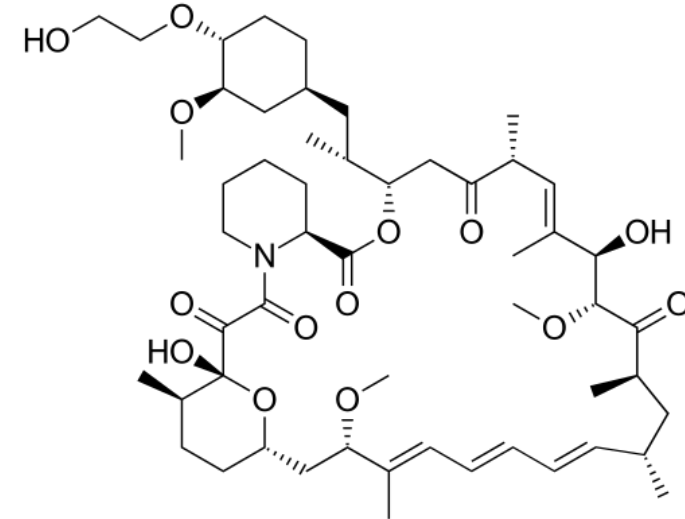
Pimicrolimus

Inhibit IL-2 transcription

mTOR Inhibitors



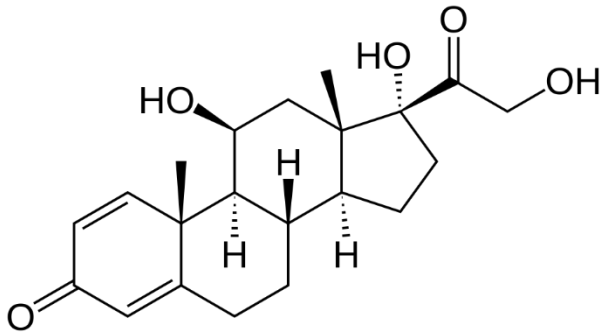
Rapamycin



Everolimus

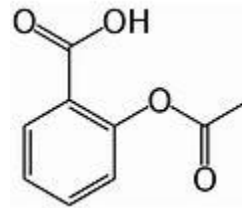
Inhibit T- and B-cell activation

Anti-inflammatory Drugs



Prednisolone

Binds glucocorticoid receptor → inhibits inflammatory and induces anti-inflammatory gene products



Aspirin

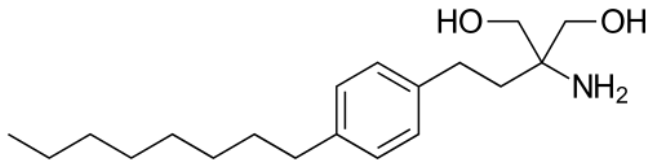
Inhibits cyclooxygenase



Methotrexate

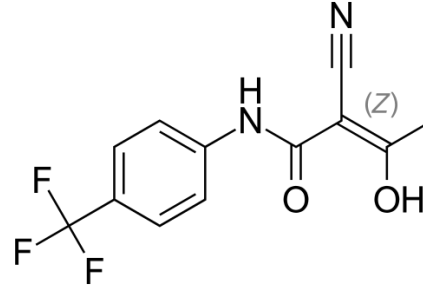
Inhibits dihydrofolate reductase

Anti-inflammatory Drugs (2)



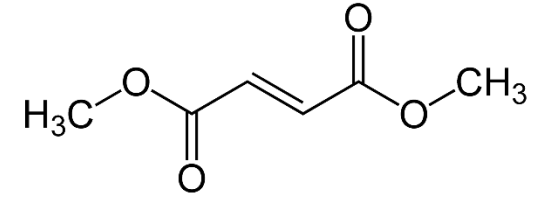
Fingolimod

Sphingosine-1-phosphate receptor modulator – traps lymphocytes in lymph nodes



Teriflunomide

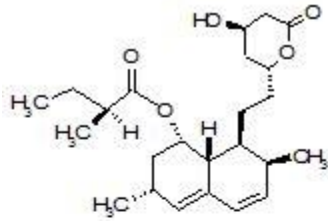
Dihydroorotate dehydrogenase inhibitor → blocks *de novo* pyrimidine synthesis



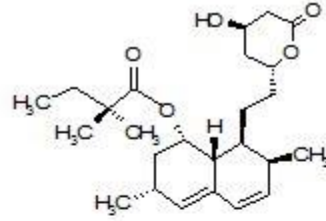
Dimethyl fumarate

Activates Nrf2 pathway?

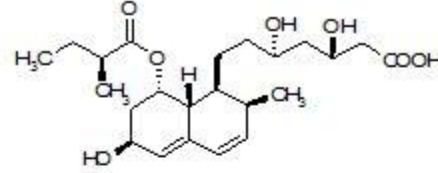
Statins



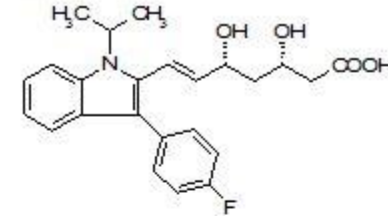
LOVASTATIN



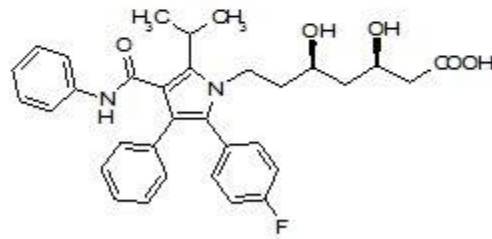
SIMVASTATIN



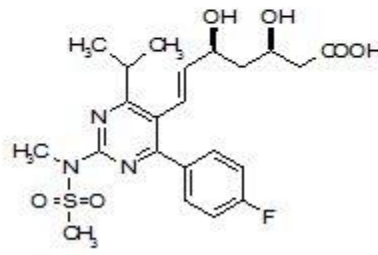
PRAVASTATIN



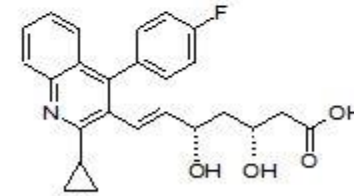
FLUVASTATIN



ATORVASTATIN



ROSUVASTATIN



PITAVASTATIN

3-hydroxy-3-methyl-glutaryl coenzyme A reductase inhibition

Suppression of IFN γ -induced MHC II expression on vascular endothelial cells

Immunomodulatory mAbs

- Natalizumab → α 4-subunit of integrins
- Basiliximab → IL-2 receptor
- Daclizumab → CD25 (IL-2 receptor)
- Rituximab → CD20
- Infliximab, etanercept, adalimumab → TNF α
- Omalizumab → IgE
- Ipilimumab → CTLA-4
- Nivolumab → PD-1
- Avelumab → PD-L1

Cancer in Transplant Patients

- Most experience with renal transplants
- Two important databases: Cincinnati Transplant Tumor Registry and Australian/New Zealand Combined Dialysis and Transplant Registry
- Lifetime risk of cancer in transplant patients approaches 70%; risk of cancer (excluding skin malignancies) might be as high as 100 X general population
- Skin (esp. lip and squamous cell carcinoma) and non-Hodgkin's lymphoma most prevalent
- Rare tumors also more common (e.g. Kaposi's sarcoma)
- Cancers tend to be more serious
- Some (esp. lymphomas) may regress with cessation of immunosuppressant therapy

Immunogenicity

- Can be adverse effect associated with biologic drugs
- Proteins, polypeptides: inherently immunogenic
- Threshold: > 5,000
- SMW: covalent binding to protein
- Minor alterations in protein structure can enhance immunogenicity
- Immune responses (antibodies, T cells) can alter PK and PD: esp. important in toxicology studies (false negatives for adverse reactions)
- Anti-drug antibodies (ADA) associated with hypersensitivity, other types of immunopathies
- Induction of human ADA (HADA) may not be predicted by nonclinical studies
- Can be basis for autoimmune reactions (esp. if intended as replacement for endogenous protein)

Unintended Immunogenicity

- Drug allergy → immune response resulting in variety of immunopathies (e.g. anaphylaxis, organ-specific immunopathology, autoimmune reactions, systemic hypersensitivity)
- Deleterious effects on drug pharmacodynamics (e.g. neutralizing antibodies, alterations in pharmacokinetics)
- Product quality → immune responses to altered proteins, process contaminants

What is Drug Allergy?

- *Drug allergy* is an adverse drug reaction (ADR) that *seems* to have an immunological basis
- Immune-Mediated Drug Hypersensitivity Reaction (IDHR)
- Some ADRs *appear* to be drug allergy, are not immune-mediated: pseudo-allergy
- Spectrum of pathologies is daunting
- Allergic contact dermatitis
- Maculopapular (morbilliform) skin reactions following non-dermal exposure
- Anaphylaxis
- Toxic epidermal necrolysis/Stevens-Johnson syndrome

Adverse Drug Reactions

- Type A: dose-related, relatively predictable, and likely related to pharmacological action of the drug;
- Type B: not related to dose, not generally predictable, likely to have an immunological basis or related to a metabolic idiosyncrasy
- Type C: cumulative-dose related, likely associated with defect in clearance or other pharmacokinetic parameter
- Type D: time-related, such as chronic exposure resulting in drug-induced tumors
- Type E: withdrawal of drug resulting in adverse effects
- Type F: failure of efficacy.

Edwards and Aronson: *Lancet*, 356, 1255, 2000.

Allergy

Sensitization

Induction Phase

Challenge

Effector Phase



Drug Allergens

- β -lactam antibiotics
- Sulfonamides
- Local anesthetics
- Radiocontrast media
- Anticonvulsants
- ACE inhibitors
- NSAIDS
- Biologics

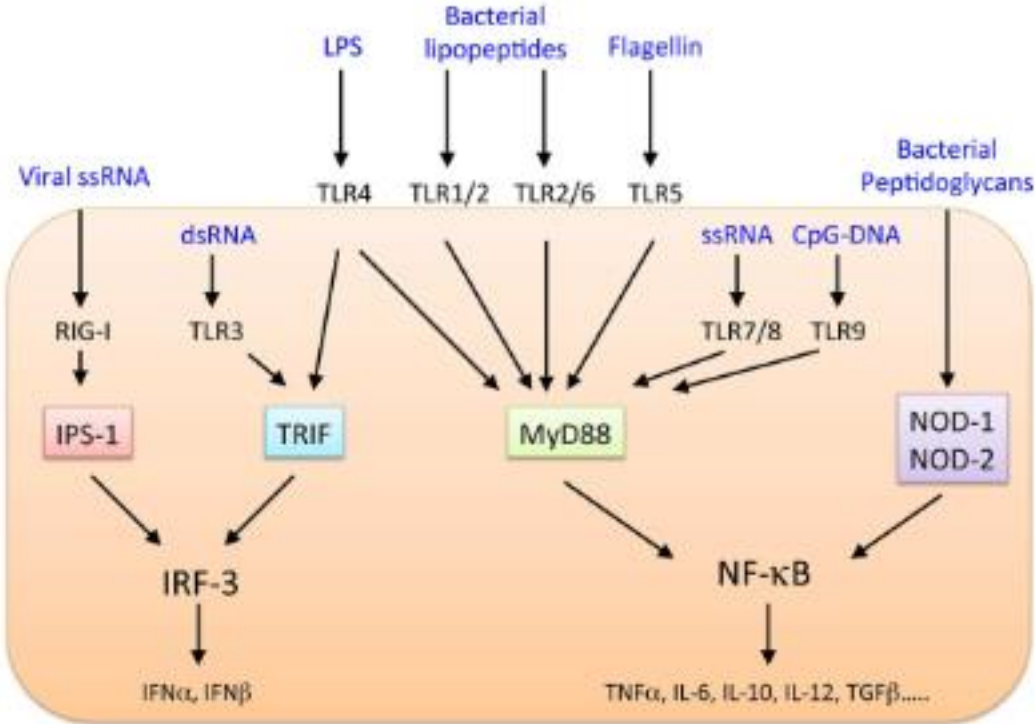
Hapten Formation

- Biotransformation \neq metabolism (e.g. penicillin)
- Chemotherapeutics that covalently bind to nucleic acids or other potential tumor targets and form potential haptens
- Can be important therapy-limiting ADR
- Photoactivation – can result in hapten formation and photoallergy
- Metabolism is a complex factor: enzyme polymorphisms, xenobiotic-induced metabolism, and even body weight

Necessary Second Signal

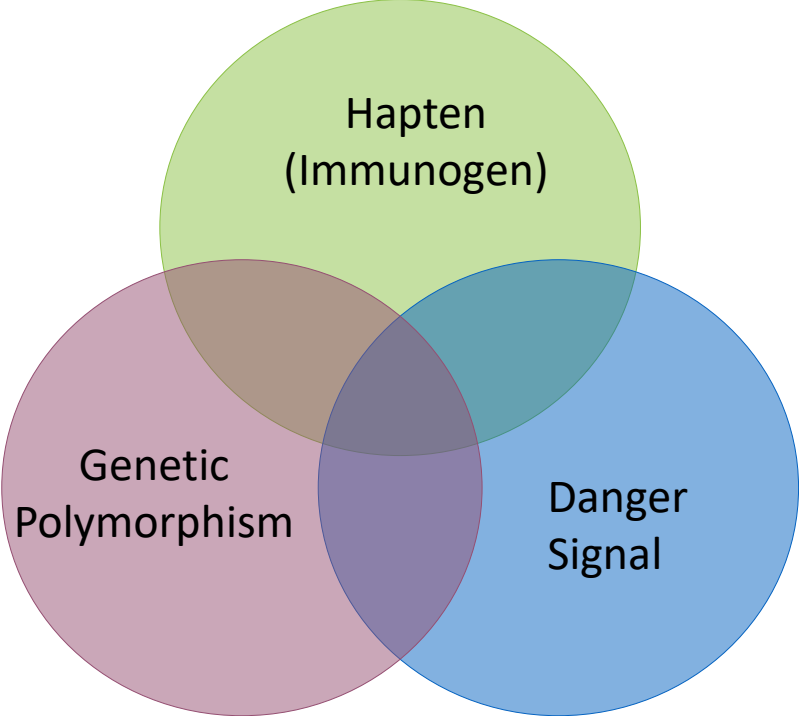
- “Danger signal”
- T helper, Innate Immunity
- Cytotoxicity and reactive metabolite(s)
- Basophil activation, mast cell tryptase
- Toll-like Receptors (esp. TLR-4, TLR-9)
- NOD-like Receptors
- Cytosolic DNA Sensors
- α -defensins 1–3
- Damage-associated molecular patterns (DAMPs) and the alarmin system
- Pathogen-associated molecular patterns (PAMPs)

Toll-like Receptors



Genetic Susceptibility for Drug Allergy

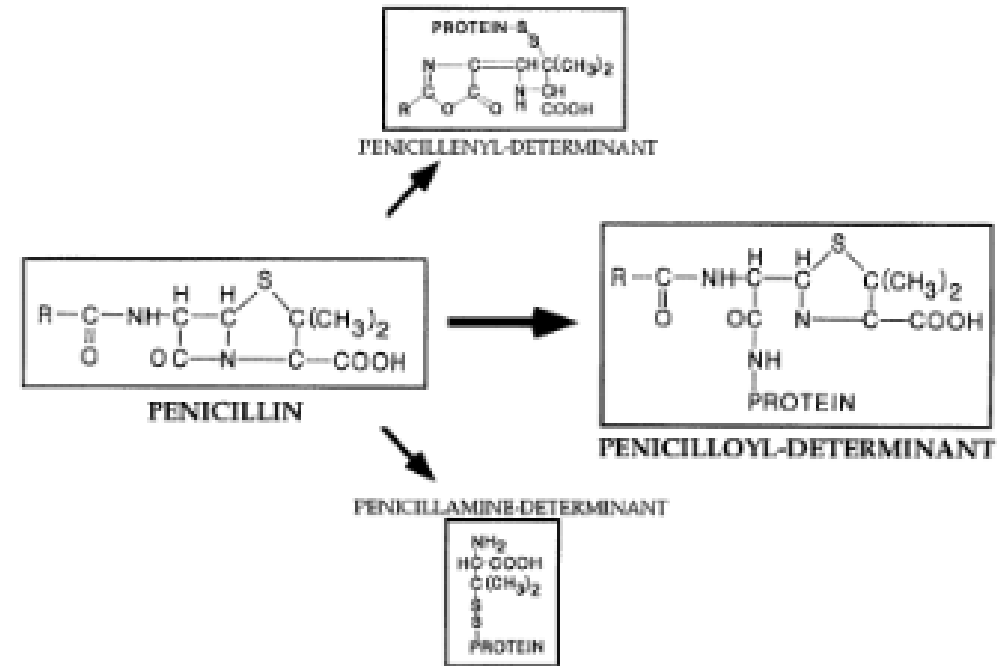
- Cytokine polymorphisms (e.g. IL-4/IL-4RA)
- NLRP3 polymorphisms
- CRTH2 polymorphisms and eotaxin-2
- Flucloxacillin (Hepatotoxicity) *HLA-B*570*
- Allopurinol (SCAR) *HLA-B*5801*
- Abacavir (Hypersensitivity rxn) *HLA-B*570*
- Carbamazepine (SJS) *HLA-B*1502*
- Penicillin (Anaphylaxis) *E237G variant of FcεR1β*



Penicillin Allergy

- Haptens form in vivo in the context of an alerted immune system
- In a patient with the predisposing haplotype, this would constitute the triad of events that may be necessary to induce an allergic reaction

Penicillin in fact represents an excellent example of context determining effect: it is not a particularly toxic drug, but under the appropriate conditions it can induce a life-threatening immune reaction



Immunopathy: Beyond Gell & Coombs

- With a few exceptions (anaphylaxis, urticaria, ACD), drug allergies may not be of a single type
- *Toxic epidermal necrolysis/Stevens-Johnson syndrome*: appears to be type IV, but may also have type III features
- *Drug reaction with eosinophilia and system symptoms (DRESS)*: associated with anticonvulsant therapy, characterized by fever, rash, lymphadenopathy, eosinophilia, interstitial pneumonitis, myocarditis, and in most cases hepatitis
- *Hemophagocytic lymphohistiocytosis (HLH)*: systemic inflammatory reaction also associated with anticonvulsants, esp. lamotrigine, characterized by fever, rash, splenomegaly, cytopenias, elevated serum triglycerides and ferritin, decreased blood fibrinogen, decreased/absent NK cells, elevated CD25-positive white cells, and hemophagocytosis (demonstrated by bone marrow, spleen, or lymph node biopsy)

Xenobiotic-induced Immunopathy: Other Forms

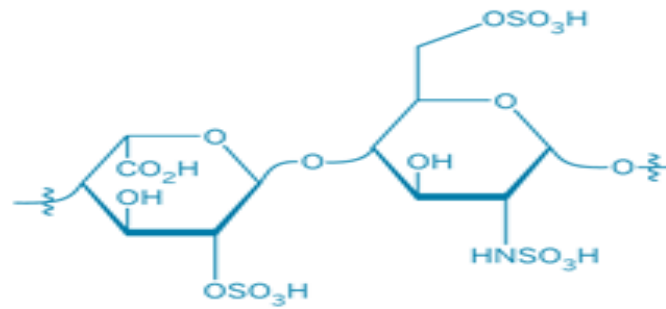
- Drugs such as sulfonamides and β -lactams can cause any of the Gell and Coombs' immunopathies, and occasionally signs of more than one form are observed concurrently (especially true for sulfa drugs)
- Idiopathic drug-induced liver injury (iDILI): many forms appear to be immune-mediated, "Hy's Law" – 3X ALT, 2X bilirubin ULN
- Antibody-dependent enhancement (ADE): 5th type of immunopathology?
- Pharmacological interaction: non-covalent MHC II binding
- Viral activation
- Pseudo-allergy

Anaphylaxis or Anaphylactoid?

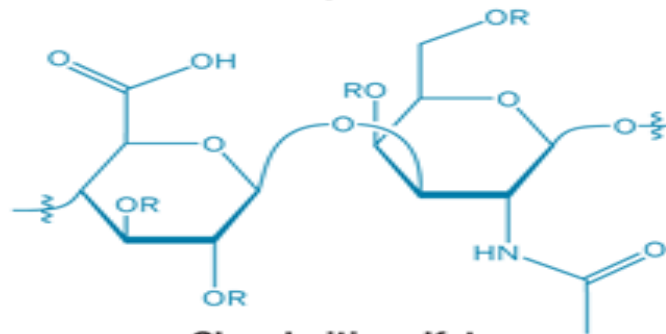
- IgE-mediated
- Previous exposure to antigen
- Rarely modeled in animals
- Old tests: Passive Cutaneous Anaphylaxis (PCA), Active Systemic Anaphylaxis (ASA), Active Cutaneous Anaphylaxis (ACA) assays
- *May* be able to diagnose with skin tests, RAST, or ELISA
- Direct effect on mast cells/basophils, with release of vasoactive amines
- Activation of classic or alternate complement pathway
- Alterations in arachidonic acid metabolism
- *May* be modeled in animals

Contaminated Heparin Associated with Adverse Clinical Events and Activation of the Contact System

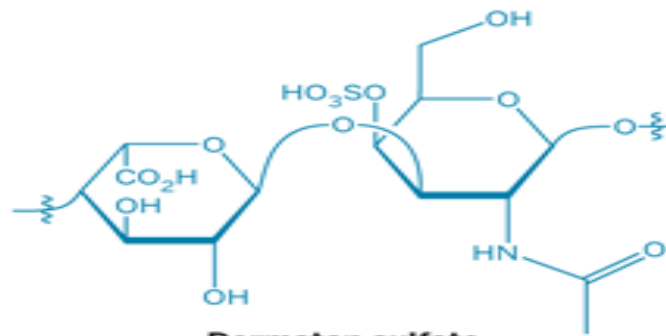
Takashi Kei Kishimoto, Ph.D., Karthik Viswanathan, Ph.D., Tanmoy Ganguly, Ph.D.,
Subbiah Elankumaran, Ph.D., Sean Smith, B.S., Kevin Pelzer, Ph.D.,
Jonathan C. Lansing, Ph.D., Nammalwar Sriranganathan, Ph.D., Ganlin Zhao, M.D.,
Zoya Galcheva-Gargova, Ph.D., Ali Al-Hakim, Ph.D., Gregory Scott Bailey, B.S.,
Blair Fraser, Ph.D., Sucharita Roy, Ph.D., Thomas Rogers-Cotrone, M.S.,
Lucinda Buhse, Ph.D., Mark Whary, Ph.D., James Fox, Ph.D., Moheb Nasr, Ph.D.,
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and Ram Sasisekharan, Ph.D.



Heparin



Chondroitin sulfate



Dermatan sulfate

$\text{R} = \text{H}$ or SO_3H

Animal Model of Anaphylactoid Reaction

- Patients demonstrated signs consistent with anaphylaxis, but unusual for heparin
- IgE-mediated reactions difficult to model in animals (PCA, ASA works with some allergens, but not predictive or useful in forensic setting)
- Anaphylactoid reactions can be modeled, however
- Contaminated heparin, OSCS direct activation of kinin-kallikrein system with generation of vasoactive bradykinin in human plasma
- Concurrent generation of C3a and C5a, potent anaphylatoxins
- Both effects linked to activation of factor XII
- Effect modeled in swine

Hypersensitivity Cases Associated With Drug-Eluting Coronary Stents

A Review of Available Cases From the Research on
Adverse Drug Events and Reports (RADAR) Project

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Jennifer M. Hoffman, PHARM.D,*|| Matthew H. Samore, MD,*|| Jorge Alvarez, MD,‡#
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Brian K. Whisenant, MD,*|| Paul R. Yarnold, PhD,¶ Steven M. Belknap, MD,¶ Dennis P. West, PhD,¶
Jonathan E. Gage, MD,‡‡ Richard E. Morse, MA,†¶ Gordana Gligoric, MD, PhD,‡#
Laura Davidson,†¶ Marc D. Feldman, MD, FACC‡#

*Salt Lake City, Utah; Chicago, Illinois; San Antonio, Texas; Albuquerque, New Mexico; Bethesda, Maryland;
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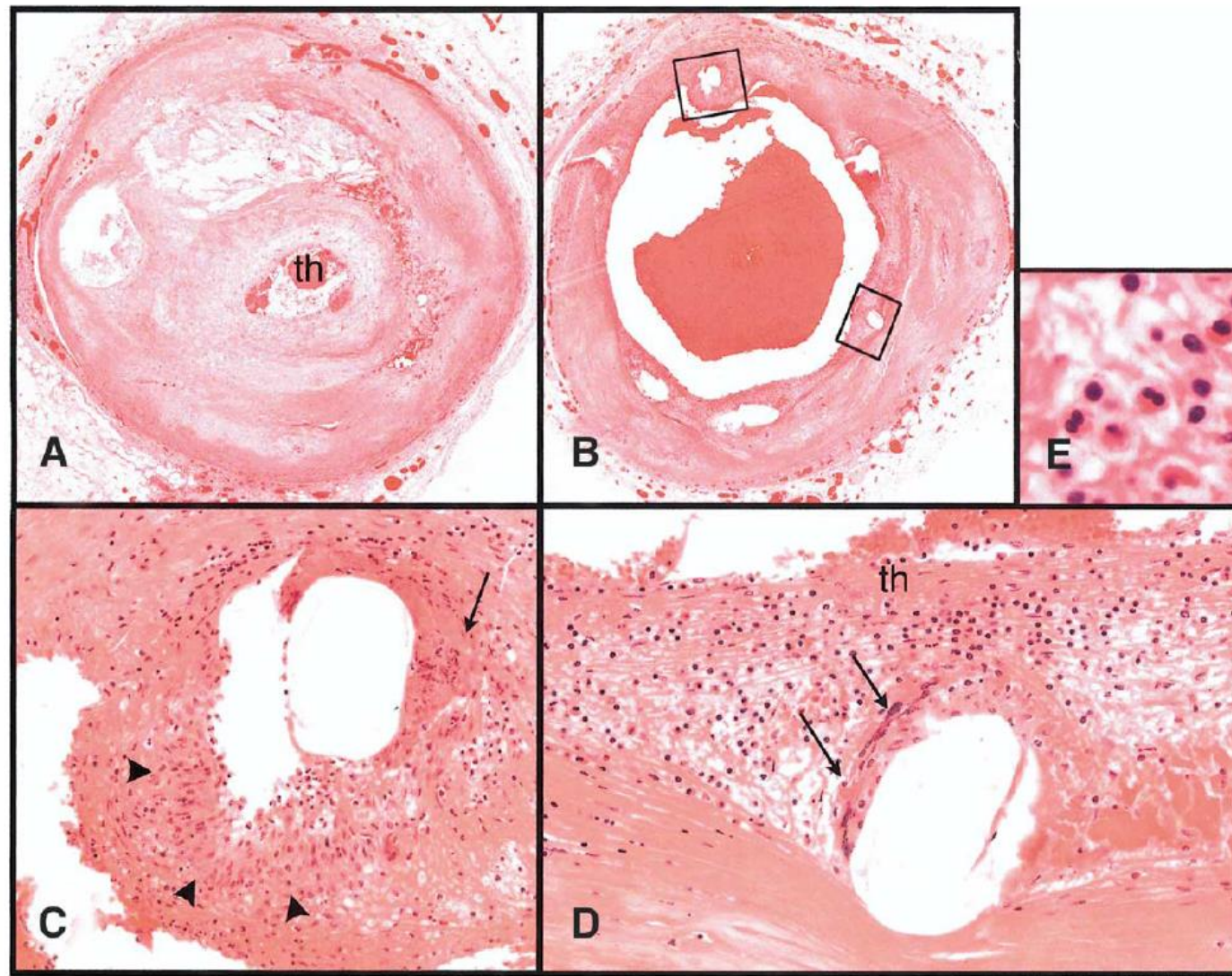
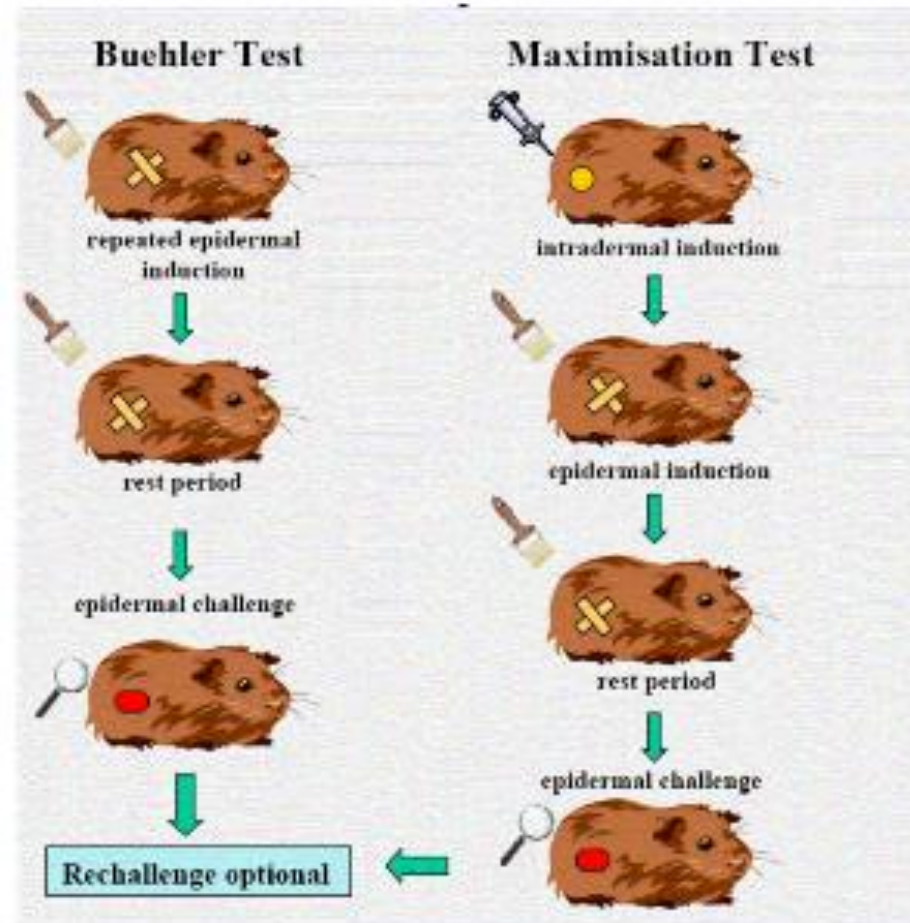
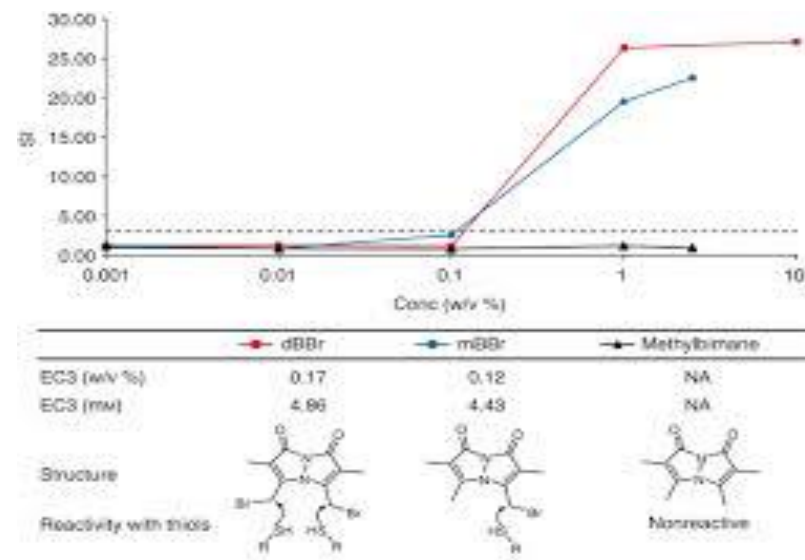
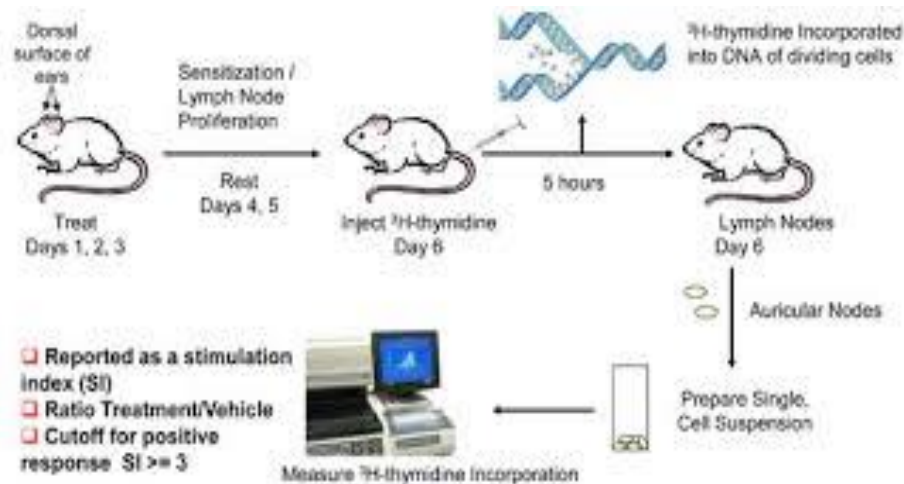


Figure 2. Photomicrograph of the non-stented coronary artery of Patient #2 just proximal to the stent showing severe stenosis and non-occlusive luminal thrombus (th) (A). In B is shown the proximal stented artery with marked inflammatory reaction around stent struts; high-power magnification of the boxed areas in B is shown in C and D, note that there is severe granulomatous reaction consisting of macrophages (arrowheads) and giant cells (arrows). In between the stent struts, there is severe eosinophilic and T-cell infiltration (high-power E) with only rare spindle-shaped cells seen close to the lumen. There is absence of endothelium in D; instead there is a surface thrombus.

Nonclinical Tests for Allergic Contact Dermatitis



Murine Local Lymph Node Assay



Modifications not using radioactive markers:

LLNA:DA (daicel adenosine triphosphate)

LLNA:BrdU-FC (bromodeoxyuridine detected using flow cytometry)

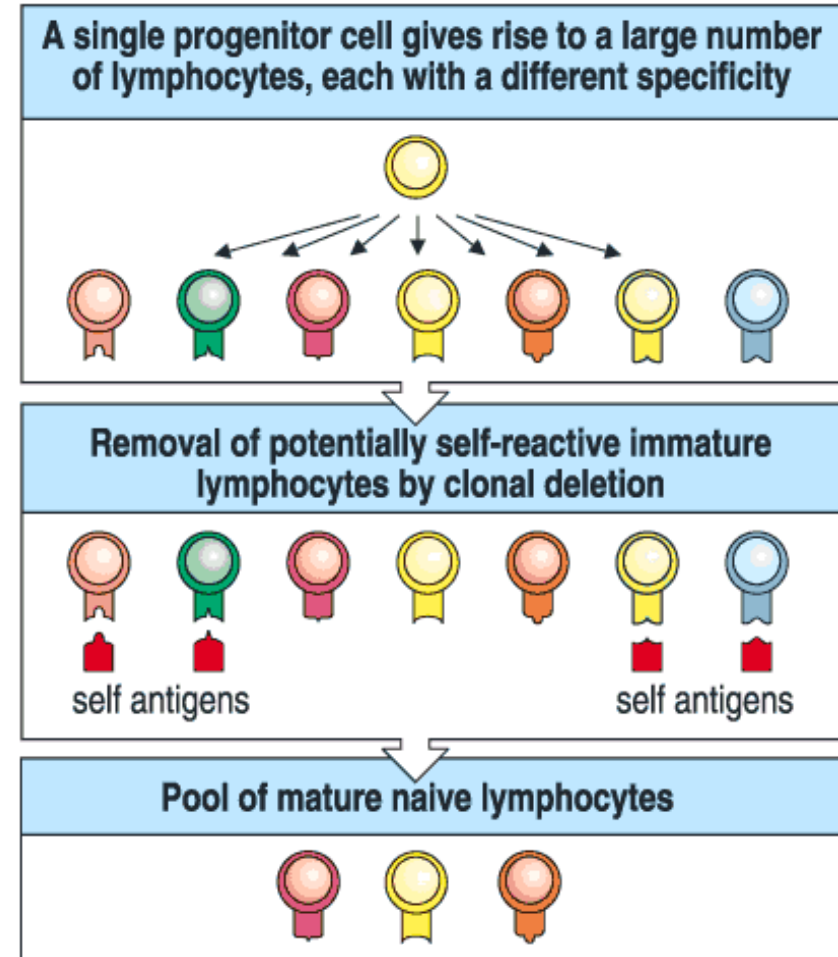
LLNA:BrdU-ELISA (bromodeoxyuridine detected by ELISA)

LLNA for Respiratory Sensitization

- T_H1 pattern: IL-2, IFN γ
- T_H2 pattern: IL-4, IL-10, IL-13
- If LLNA +, examine cytokine pattern
- Respiratory allergens are subset of ACD, produce T_H2 pattern
- Important co-factor: irritation

Central Tolerance

- Mechanism by which lymphocytes are rendered non-reactive to self
- Occurs in primary lymphoid organs
 - T cells = Thymus
 - B cells = Bone marrow
- Lymphocytes that recognize self antigens are neutralized (negative selection)
 - Clonal deletion
 - Anergy
- T cells must also recognize MHC (positive selection)
- Not 100% effective



Peripheral Tolerance

- Mechanisms by which self-reactive lymphocytes that escape central tolerance are neutralized
- Occurs in the immune periphery
- T cell peripheral tolerance
 - Ignorance (no access to “immunopriviliged” organs)
 - Lack of costimulatory signals
 - Lack of antigen presentation
 - Suppression by other immune cells (T_{regs})
- B cell peripheral tolerance mechanisms are less well understood

Xenobiotic-induced Autoimmunity

- Impairment of immune tolerance → xenobiotic damage to T_{REGs}
- No standard methods for evaluating autoimmunity
- Glomerulonephritis, lupus-like syndrome, hemolytic anemia, vasculitis, loss of tissue architecture with lymphocytic infiltrates
- Popliteal lymph node assay (PLNA) has been proposed as a possible predictive tool
- Also: predisposed animal models (e.g. BN rat)
- Screening for autoantibodies has been used with limited success
- Replication-impaired virus → expression of viral antigen
- IL-17/IL-17r, IL-22, IL-23 polymorphisms

Drug-associated Autoimmune Reactions

- Hydralazine
- Procainamide
- Isoniazid
- Methyldopa
- Quinidine
- Minocycline
- Chlorpromazine

Immune-mediated Blood Dyscrasias

- Autoimmune hemolytic anemia (type II): associated with penicillin, other hapten-forming xenobiotics
- Antineutrophil antibody associated neutropenia (type II)
- Heparin-induced thrombocytopenia (HIT): immune response to heparin-platelet factor 4 complex resulting in type III immunopathy
- Thrombotic thrombocytopenic purpura (TTP): thrombocytopenia, microangiopathic hemolytic anemia, multiorgan system failure, neurologic signs – associated with xenobiotic-induced antibody response to a protease (ADAMTS13) involved in coagulation
- Hemolytic uremic syndrome (HUS): similar to TTP but no anti-protease antibodies

ORIGINAL ARTICLE

Pure Red-Cell Aplasia and Epoetin Therapy

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and Nicole Casadevall, M.D.

Eprex and Pure Red-Cell Aplasia

- 1988 – 2004: 175 cases of PRCA reported in Europe and Canada associated with Eprex (~ 500 cases world-wide)
- 18/100,000 patient/years with Eprex without human albumin excipient
- Human albumin removed due to concern for transmission of Creutzfeldt-Jacob disease
- Organic compounds from rubber plungers and silicone may have acted as adjuvants
- Antibodies inhibited bone marrow erythroid-colony formation
- Changes in manufacturing led to 80% decrease in incidence

Adverse Immunostimulation

- Antigen-nonspecific, inappropriate, or unintended activation of some component of the immune system
- Chronic inflammation (probably more of an issue with vaccines, medical devices)
- In some cases, overlaps with pseudo-allergy
- Cytokine release syndrome (CRS)
- Systemic inflammatory response syndrome (SIRS)
- Complement activation-related pseudo-allergy (CARPA)
- Sterile sepsis (“cytokine storm”)
- Tegnenero (agonist anti-CD28 IgG₄ mAb)

Tegenero

- Humanized agonistic anti-CD28 IgG4 mAb
- Initial indication: rheumatoid arthritis
- Therapeutic rationale: anti-inflammation by induction of regulatory cytokines/chemokines
- Induce cytokine storm, massive release of pro-inflammatory mediators
- New concept: “Sterile Sepsis”
- Continuum of effects: activation of immune effector function without specific immunogen/antigen
- mAb infusion and “anaphylaxis”

mAb Adverse Effects

- Infusion site reactions
- Rash
- Immunogenicity/hypersensitivity/
autoimmunity
- Types II and III immunopathies
(e.g. serum sickness)
- Anaphylaxis/pseudo-allergic reactions
- Cytopenias/ intravascular hemolysis
- Exaggerated pharmacodynamics (e.g.
tumor lysis syndrome)
- Immunosuppression/infections/
malignancies
- Lymphoproliferative disorders
- Organ toxicities (liver, heart, lung,
kidney)
- Capillary leak syndrome
- Complement activation-related
pseudo-allergy (CARPA)
- Cytokine release syndrome
- Sterile sepsis

MABEL

- Minimal Anticipated Biological Effect Level
- *In vitro* assay(s) using human cells (usually PBMCs), either plate bound or soluble (flow cytometry)
- Assessment of immune activation, cytokine release, ligand/receptor blockade
- Hazard identification for cytokine release syndrome → can be used to determine safe starting dose in clinical trials (risk assessment)

Nonclinical Safety Evaluation of the Immunotoxic Potential of Drugs and Biologics Guidance for Industry

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to <http://www.regulations.gov>. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document, contact (CDER) David McMillan, 240-402-1009, or (CBER) Office of Communication, Outreach and Development, 800-835-4709 or 240-402-8010.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

February 2020
Pharmacology/Toxicology

New Guidance

- This guidance replaces the withdrawn guidance for industry *Immunotoxicology Evaluation of Investigational New Drugs* (October 2002).
- This guidance covers the evaluation of functional, histomorphologic, and cellular aspects of the immune system in nonclinical studies for new drugs, therapeutic proteins, and recombinant/plasma-derived blood proteins regulated by the Center for Drug Evaluation and Research (CDER) and the Center for Biologics Evaluation and Research (CBER)
- Cell and gene therapies, adjuvanted vaccines, and other biologics are not within the scope of this guidance, although some of the principles in this guidance may also be applicable to these product types; for these products, direct consultation with the review division is strongly recommended.

New Guidance

- For the purposes of this document, the term immunomodulator could be any therapeutic that modifies the immune response, including those that act in a manner that is not overtly immunosuppressive or immunostimulatory and may have subtle or even mixed effects
- Includes better discussion of immunosuppression-associated carcinogenicity: A WOE-based risk assessment is particularly relevant for drugs and biologic products that lack the intended pharmacological activity in rodents and for biologics for which significant formation of anti-product antibodies diminishes interpretability of rodent studies

New Guidance

- For therapeutic biologic products intended to stimulate an immune response either directly or indirectly, a starting dose based on a minimal anticipated biologic effect level (MABEL) or a pharmacological effect level (PEL) may be more appropriate than a starting dose based on toxicology endpoints such as the no observed adverse effect level (NOAEL)
- Emphasis on use of pharmacology assays for specific end-points, such as cytokine release
- The murine local lymph node assay no longer recommended, should use guinea pig assays
- Specific mention of innate immunity, developmental and juvenile animal studies
- Discussion of ePPND studies in nonhuman primates