U.S. Department of Health and Human Services

National Institutes of Health

National Heart, Lung, and Blood Institute

BIOCHEMICAL MECHANISMS OF DRUG TOXICITIES

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TYPES OF ADRs

- Hepatic
- Cardiac
- Skin
- Renal
- Pulmonary
- Neurological
- Lupus
- Anaphylaxis
- Hemolytic anemia
- Granulocytopenia
- Thrombocytopenia
- Aplastic anemia
- Vasculitis

SEVERITY OF ADRs

- **Minor**
- **Severe (SADRs)**
  - **6.2-6.7% hospitalized patients in USA**
  - **over 2 million hospitalized patients**
  - **similar findings in Europe and Australia**
  - **tens of billions of dollars cost burden**

Wilke, et al., Nature Review-Drug Discovery, 904, 2007

LEADING CAUSES OF DEATH IN USA IN 1994

Heart disease	**743,460**
Cancer	**529,904**
Stroke	**150,108**
SADRs	**106,000**
Pulmonary disease	**101,077**
Accidents	**90,523**
Pneumonia	**75,719**
Diabetes	**53,894**

Lazarou et al., JAMA, 279, 1208 (1998)

DRUG WITHDRAWN IN USA

- Azaribine, psoriasis, blood clots, 1976
- Ticrynafen, blood pressure, liver injury, 1980
- Benoxaprofen, NSAID, liver toxicity, 1982
- Zomepirac, NSAID, anaphylaxis, 1983
- Nomifensine, anti-depressant, hemolytic anemia, 1986
- Suprofen, NSAID, kidney failure, 1987
- Temafloxacin, antibiotic, kidney failure, 1992
- Fenfluramine, appetite suppression, heart valve disease, 1997
- Terfenadine, anti-histamine, fatal arrhy-thmia, 1998
- Bromfenac, NSAID, liver injury, 1998
- Mibefradil, blood pressure, muscle damage and fatal arrhythmia, 1998

DRUG WITHDRAWN IN USA

- Grepafloxacin, antibiotic, fatal arrhythmia, 1999
- Astemizole, antihistamine, fatal arrhythmia, 1999
- Cisapride, heartburn, fatal arrhythmia, 2000
- Troglitazone, diabetes, liver toxicity, 2000
- Cerivastatin, cholesterol reduction, muscle damage leading to kidney failure, 2001
- Etretinate, psoriasis, birth defects, 1999
- Levomethadyl, opiate dependence, fatal arrhythmia, 2008
- Rofecoxib, NSAID, heart attack, stroke, 2004
- Valdecoxib, NSAID, skin disease, 2005
- Pemoline, ADHD, liver toxicity, 2005

TYPE A ADRs

- 80% of ADRs
- Relatively frequent and often predictable
- Excessive or diminished pharmacologic effects
- Drug-drug interactions and poly-morphisms
- Mild to severe ADRs
- Often uncovered preclinically

Endres, et al., European Journal of Pharmaceutical Sciences, 27, 501 (2006)

EXAMPLES OF TYPE A ADRS

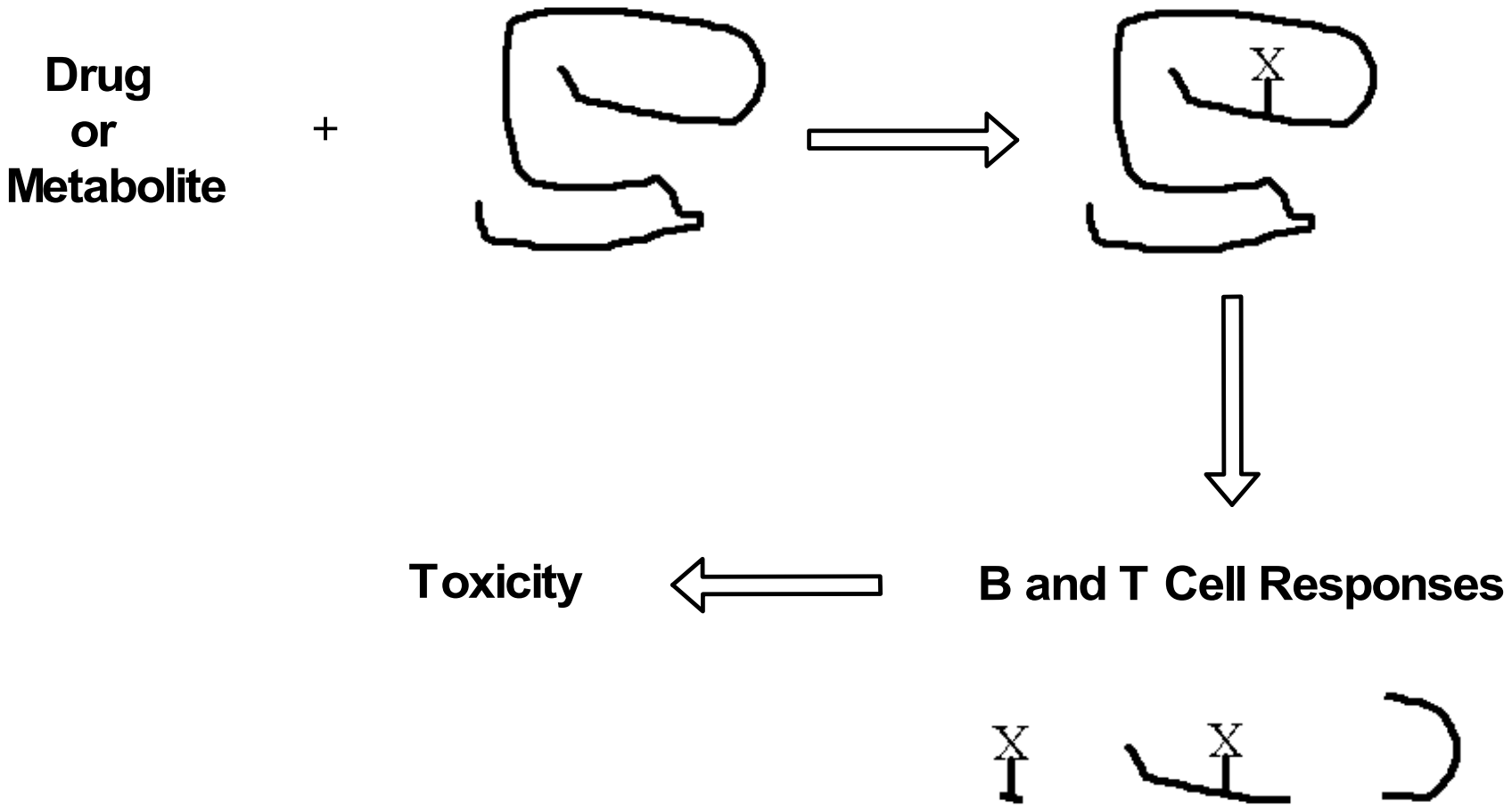
- Drowsiness from antihistamines
- Hypotension from antihypertensive therapy
- Excess bleeding from warfarin
- Prolonged neuromuscular blockade by serum choline esterase deficiency
- Acetaminophen

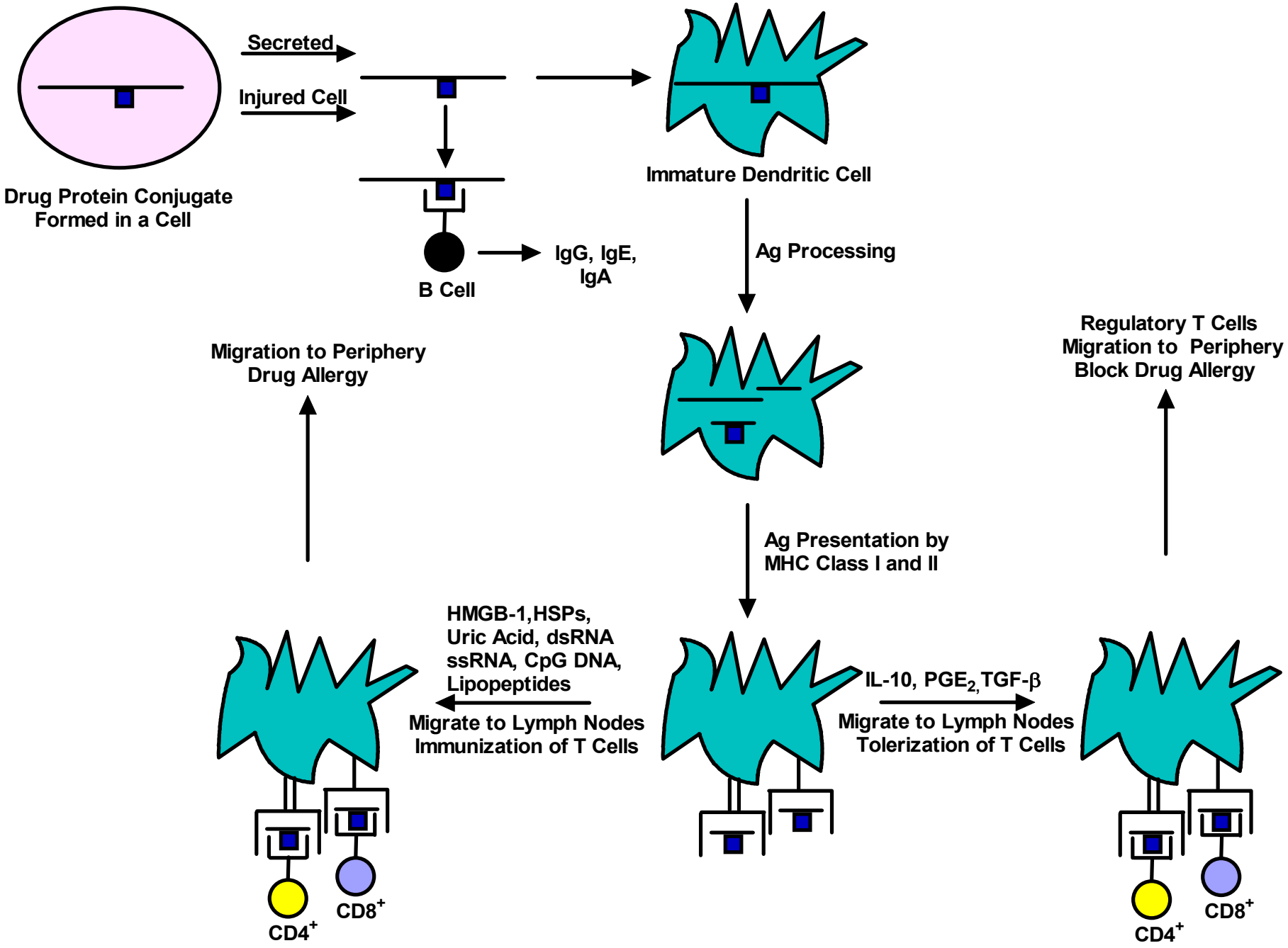
TYPE B ADRs

- 20% of ADRs
- Rare, unpredictable, and highly host-dependent
- Mild to severe ADRs
- Rarely uncovered preclinically in animals or in clinical trials
- Mechanisms often unknown but may be due to:

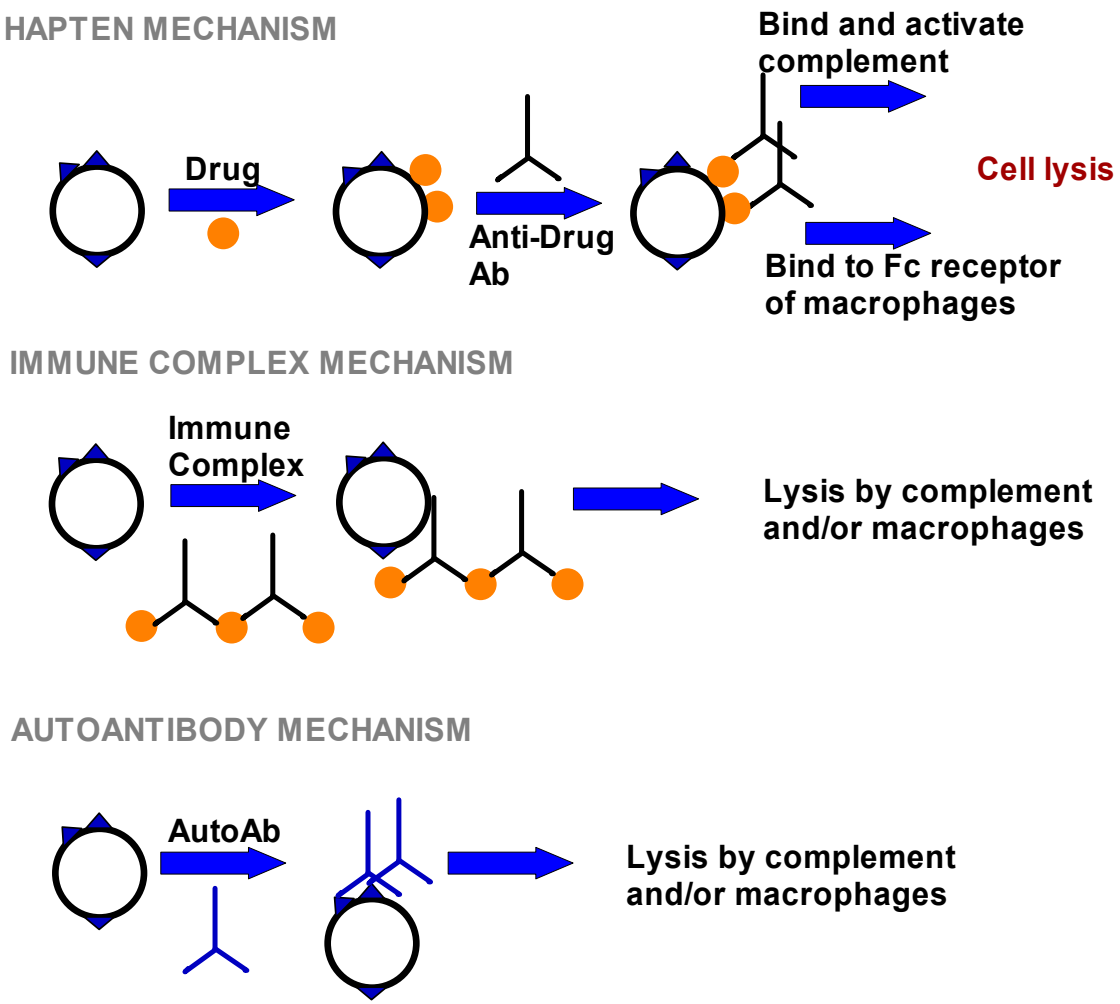
  Allergic Reactions
  Rare Polymorphisms
  Imbalance in Cellular Homeostasis

HAPTEN HYPOTHESIS AND DRUG-INDUCED ALLERGIC REACTIONS




Secreted
Injured Cell
Drug Protein Conjugate
Formed in a Cell
B Cell
IgG, IgE,
IgA
Immature Dendritic Cell
Ag Processing
Migration to Periphery
Drug Allergy
Regulatory T Cells
Migration to Periphery
Block Drug Allergy
Ag Presentation by
MHC Class I and II
HMGB-1,HSPs,
Uric Acid, dsRNA
ssRNA, CpG DNA,
Lipopeptides
Migrate to Lymph Nodes
Immunization of T Cells
IL-10, PGE2, TGF-β
Migrate to Lymph Nodes
Tolerization of T Cells
CD4+
CD8+
CD4+
CD8+

MECHANISMS OF DRUG-INDUCED IMMUNE-MEDIATED BLOOD DYSCRASIAS

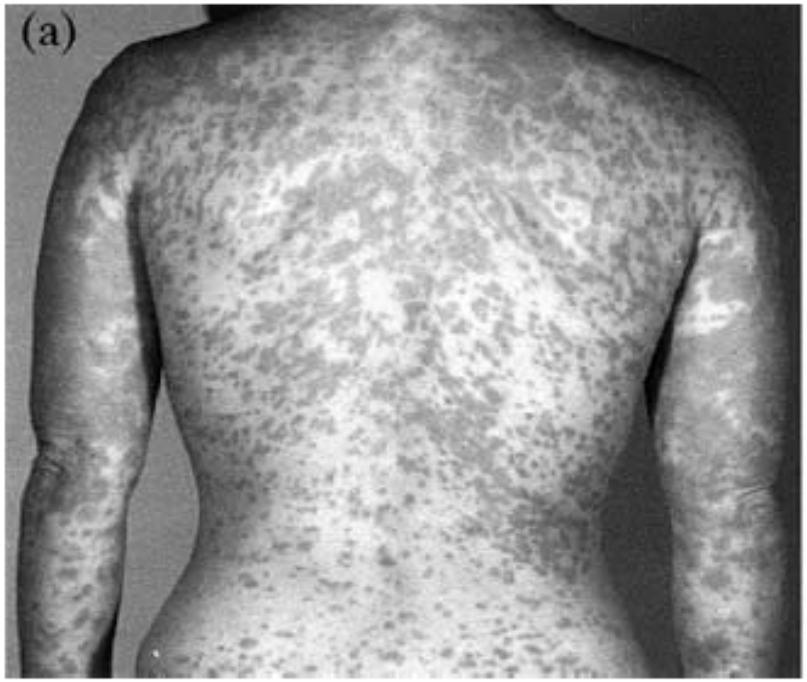


CUTANEOUS DRUG REACTIONS

- **95% are self-limiting rashes**
- **SJS and TEN can be life-threatening with blisters, skin detachment, and mucosa involvement**
- **Most appear to be immune-mediated by drug-specific IgE antibodies while many others by $CD4^+$ and $CD8^+$ T cells**

Roychowdhury and Svensson, AAPS J., 7, E 434 (2005)

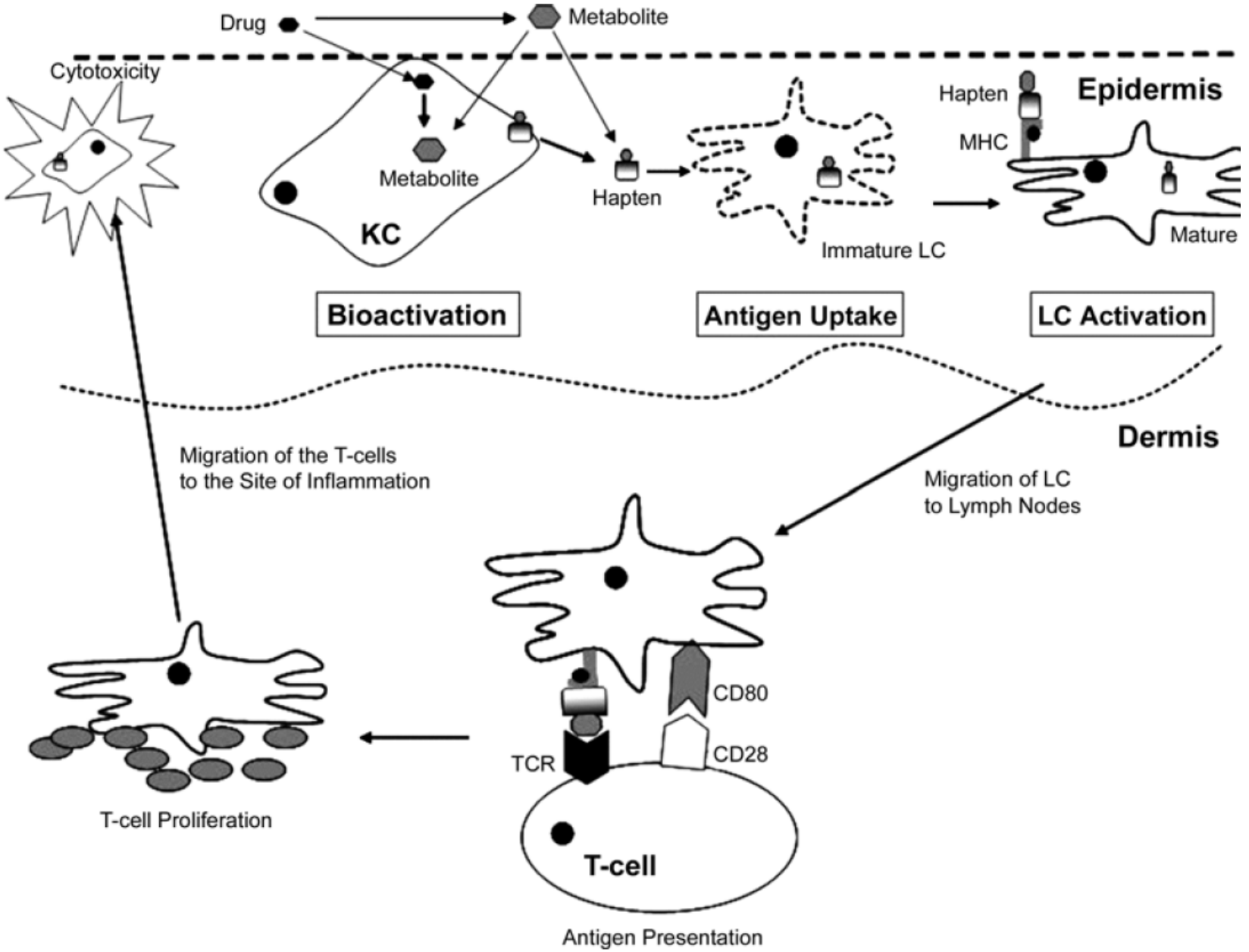
MACULO-PAPULAR EXANTHEM AND TOXIC EPIDERMAL NECROLYSIS



T CELL REACTIVITY TO DRUGS CAUSING CUTANEOUS ADRS

- **Lidocaine**
- **Sulfonamides**
- **β-Lactam antibiotics**
- **Phenytoin**
- **Carbamazepine**

Lebrec et al., Cell Biology and Toxicology, 15, 57 (1999); Naisbitt, et al., Expert Opin. Drug Saf., 6, 109 (2007); Posadas and Pichler, Clin. Experimental Allergy, 37, 989 (2007)


Drug
Metabolite
Cytotoxicity
Metabolite
KC
Hapten
Immature LC
Hapten
MHC
Epidermis
Mature
Bioactivation
Antigen Uptake
LC Activation
Dermis
Migration of the T-cells
to the Site of Inflammation
Migration of LC
to Lymph Nodes
CD80
CD28
TCR
T-cell
T-cell Proliferation
Antigen Presentation

HLA-B*1502 ASSOCIATED WITH CBZ-INDUCED SJS/TEN

- **Seen in south-east Asians but not in Caucasians**
- **98.3% (59/60) CBZ-SJS/TEN positive**
- **4.2% (6/144) CBZ-tolerant positive**
- **High sensitivity/specificity of this test can be used to screen patients receiving CBZ**

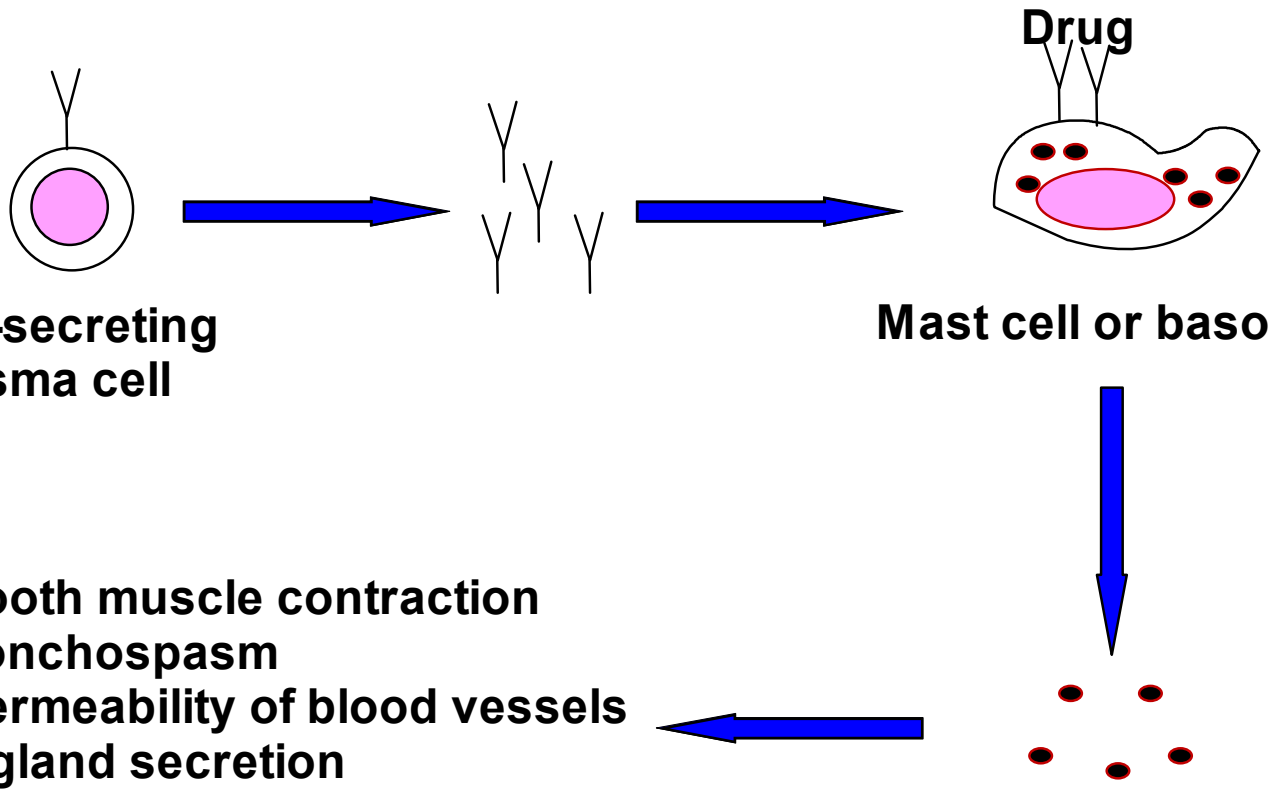
Chung, et al., Curr. Opin. Allergy Clin. Immunol., 7, 317 (2007)

IgE-MEDIATED ANAPHYLACTIC DRUG REACTIONS

Alcuronium
Cephalosporins
Penicillins
Protamine
Streptokinase
Sulfamethoxazole
Suxamethonium
Thiopentone
Trimethoprine
Tubocurarine

Park et al., Chem. Res. Toxicol., 11, 969 (1998);Thong and Chan, Ann. Allergy Asthma Immunol., 92, 619 (2004)

MECHANISM OF DRUG-INDUCED ANAPHYLAXIS



1. Airway smooth muscle contraction leading to bronchospasm
2. Increase permeability of blood vessels and mucous gland secretion
3. Inflammation (eosinophils and neutrophils)
4. Respiratory, gastrointestinal, cutaneous, and cardiovascular systems can be involved

DRUG-INDUCED LIVER DISEASE IS A MAJOR HEALTH PROBLEM

It is a major cause of acute liver failure and a major safety reason for:

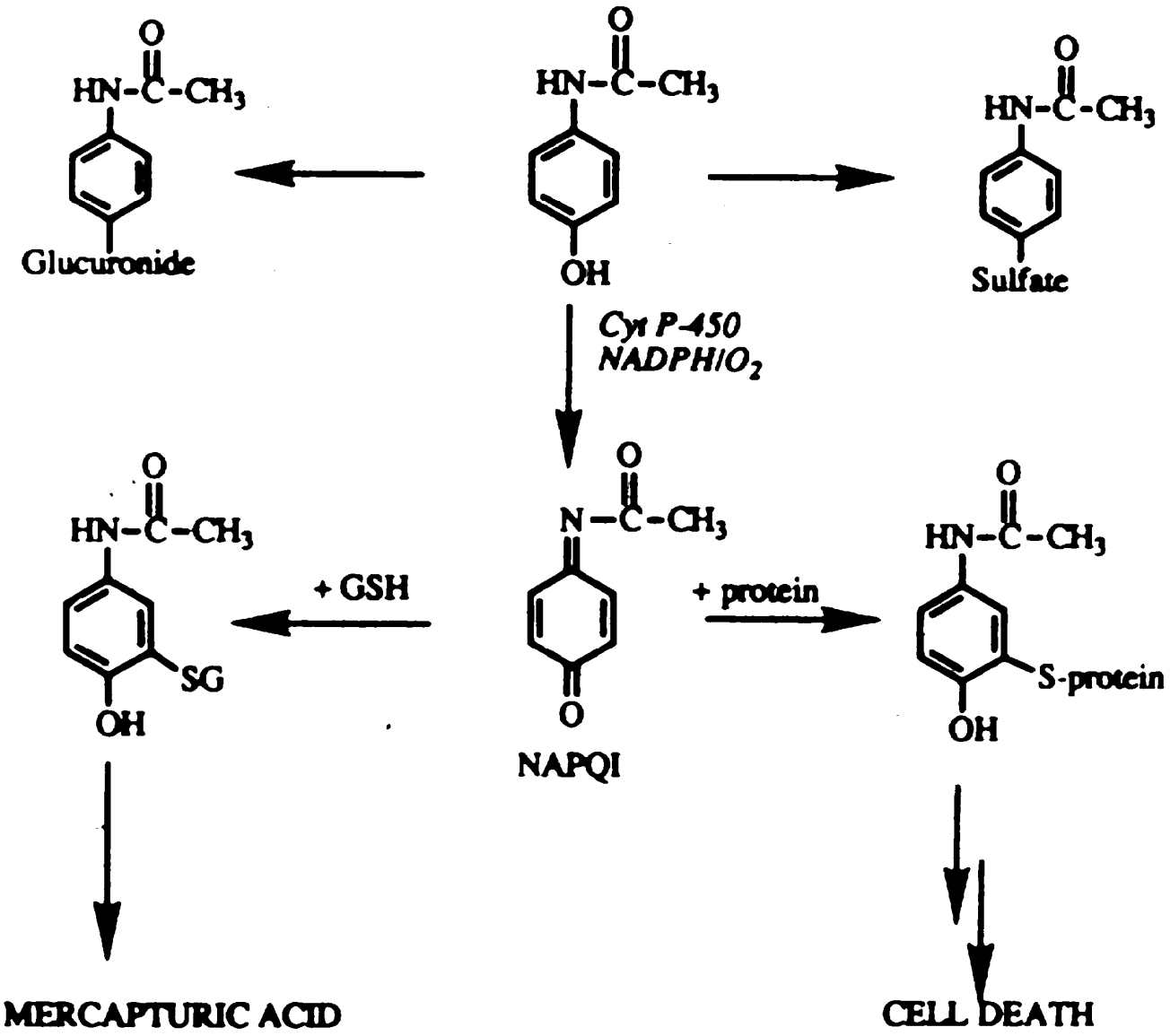
- **Stopping preclinical development of drugs**
- **Terminating clinical trials of drugs**
- **Withdrawing drugs postmarketing**

F. Ballet, J. Hepatol., 26 (Suppl. 2), 26 (1997)

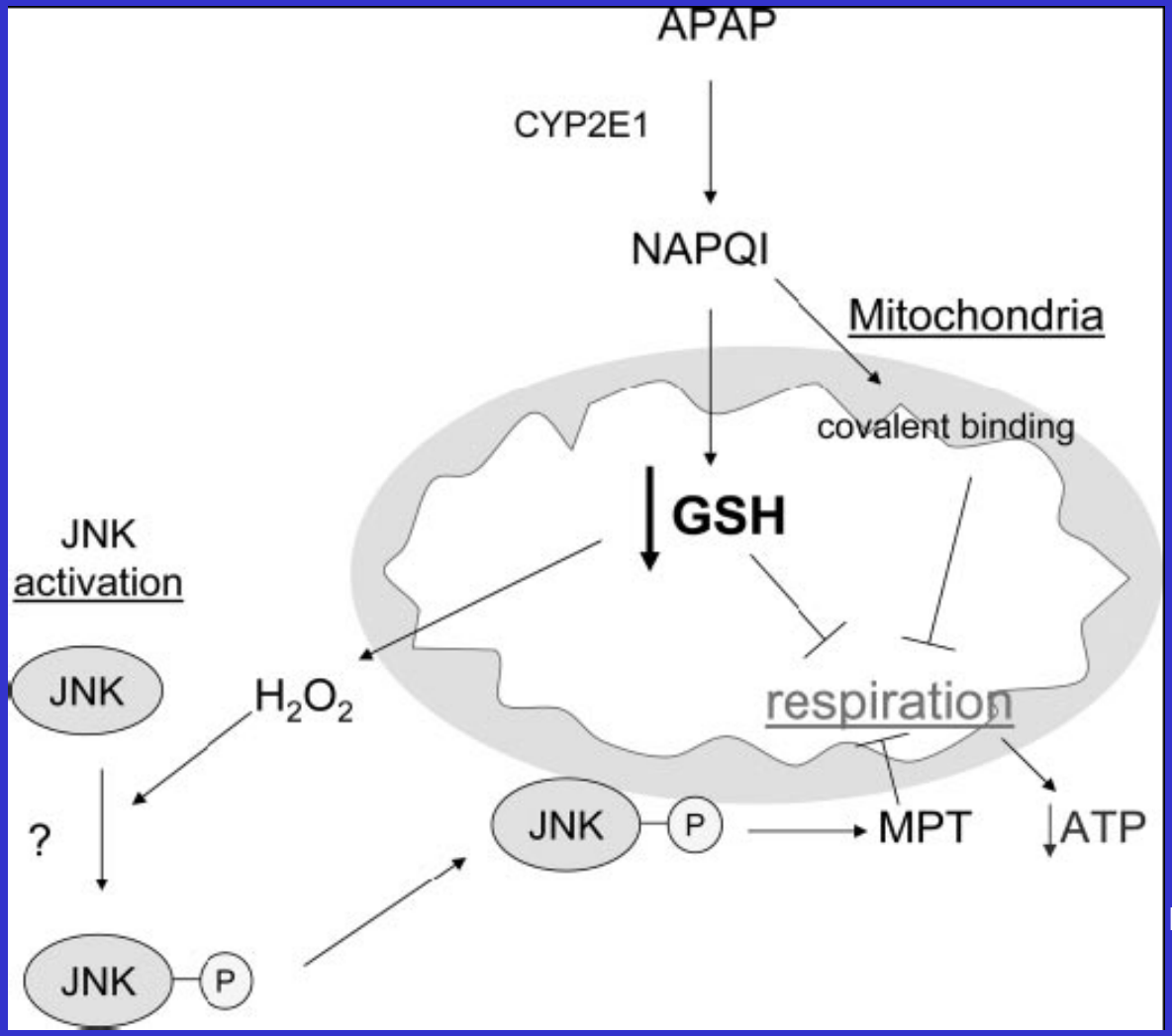
DRUGS WITHDRAWN / NOT APPROVED DUE TO LIVER DISEASE

Iproniazid	1956
Ibufenac (Europe)	1975
Ticrynafen	1980
Benoxaprofen	1982
Perhexilene (France)	1985
Dilevalol (Portugal and Ireland)	1990
Bromfenac	1998
Troglitazone	2000
Nefazodone (Serzone)	2003
Ximelagatran (Exanta)	2004

ACETAMINOPHEN LIVER INJURY



MITOCHONDRIAL DAMAGE IN AILI



Hanna et al., JBC, 283, 13565 (2008)

DRUGS CAUSING DILI ASSOCIATED WITH MITOCHONDRIAL INJURY

- Troglitazone
- Diclofenac
- Nimesulide
- Mefenamic acid
- Tolcapone
- Valproic acid
- Leflunomide
- Amiodarone
- Trovafloxacin
- Simvastatin
- Perhexiline
- Isoniazid
- Dantrolene
- Sulindac
- Lamivudine
- Stavudine
- Fialuridine

U.A Boelsterli and P.L.K. Lim., Toxicol. Appl. Pharmacol., 220, 92 (2007)

FIALURIDINE-INDUCED MITOCHONDRIAL INJURY IN PATIENTS

- **FIAU is a uridine analog developed for hepatitis B treatment**
- **Administration to 15 patients resulted in 7 developing severe mitochondrial liver damage with 5 dying and 2 receiving liver transplant**
- **Toxicity was not predicted from rodent studies**

MECHANISM OF FIAU LIVER INJURY

- **Toxicity of FIAU is apparently due to FIAU-TP which inhibits mitochondrial DNA polymerase-γ and DNA synthesis**
- **Humans and not rodents have human nucleoside transporter 1 (hENT1) in the mitochondrial membrane**

E.W. Lee, et al., J.Biol.Chem., 281, 16700 (2006)

INNATE IMMUNE CELL INJURY CAN FOLLOW INITIAL INTRINSIC DILI

DAMPs: HMGB-1, MIF, HSPs

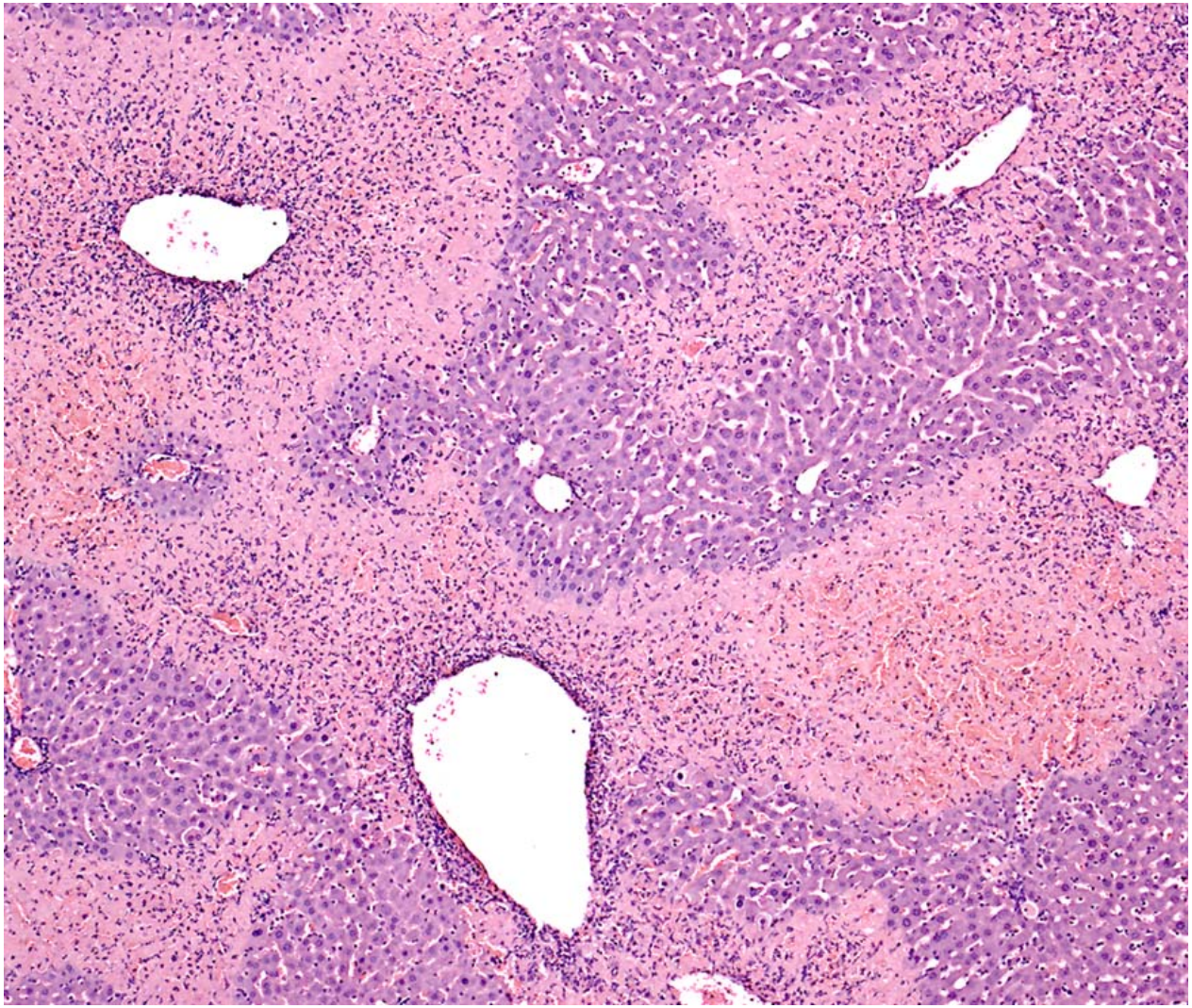
Protoxicant Factors: IFN-γ, osteopontin, IL-6, ROI, RNI

Protective Factors: IL-4, IL-6, IL-10, IL-13, COX-2

Cells: Kupffer cells, PMNs, NK, NKT cells and hepatocytes

M.E. Bianchi, J. Leukoc. Biol., 81, 1 (2007); D.J. Antoine et al., Expert Opin. Drug Metab.Toxicol, 4, 1415 (2008)

INFLAMMATORY CELL INVOLVEMENT IN AILI IN A IL-10$^{-/-}$ MOUSE



PAMPS CAN ACTIVATE THE INNATE IMMUNE SYSTEM

- **TLR1/2 and TLR2/6 activated by bacterial triacylated and diacylated lipopeptides, respectively**
- **TLR4 activated by LPS, several HSPs, heparan sulfate products, hyaluronic acid fragments**
- **TLR5 activated by bacterial flagellin**
- **TLR 3 activated by viral dsRNA**
- **TLR7 and 8 activated by viral ssRNA**
- **TLR9 activated by bacterial unmethylated CpG DNA**

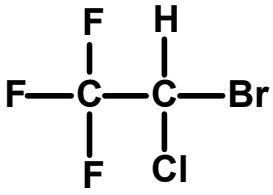
E. Seki and D.A. Brenner, Hepatology, 48, 322 (2008)

POTENTIAL ROLE OF GUT-DERIVED LPS ENDOTOXIN IN DILD

- **Rat and mouse models of DILD have been produced by LPS + drug treatments**
- **Diclofenac, chlorpromazine, trovafloxacin, and ranitidine**
- **LPS activates TLR4 which can lead to activation of monocytes, macrophages, dendritic cells, mast cells and other cells.**

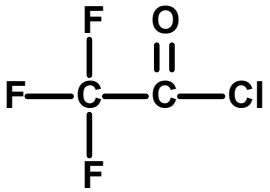
P. J. Shaw et al., Toxicol. Sci. 107, 270 (2009)

HALOTHANE-INDUCED ALLERGIC HEPATITIS

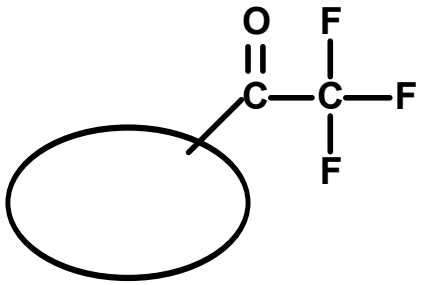


Halothane

P450



Trifluoroacetyl Chloride



Hepatocyte

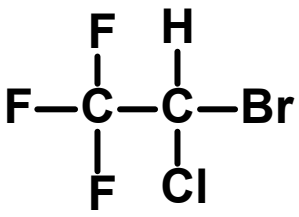
Humoral and Cellular Immune Responses

Toxicity

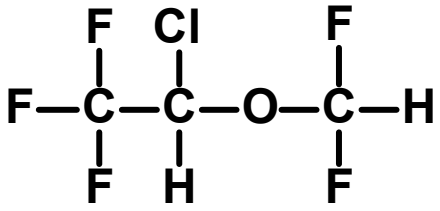
HALOTHANE HEPATITIS PATIENTS' SERUM ANTIBODIES (% REACTIVITY)

Antigen	TFA-Protein	Native-Protein
PDI	10	5
PDI isoform	55	25
Carboxylesterase	13	5
Calreticulin	5	3
ERP72	30	25
GRP94	65	28
CYP2E1		45

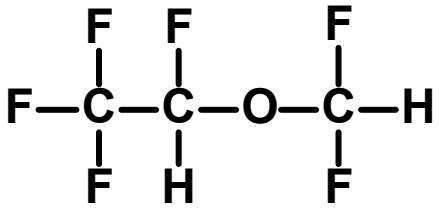
OTHER HALOTHANE DERIVATIVES



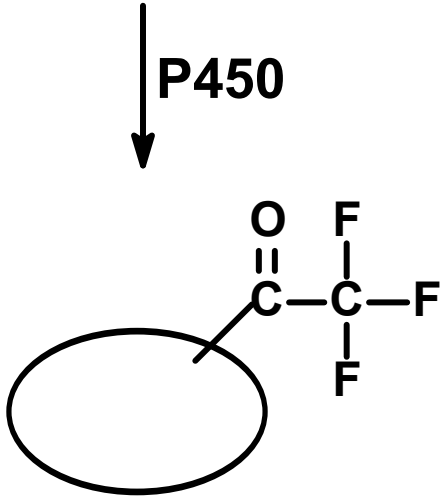
Halothane



Isoflurane



Desflurane



Hepatocyte

ANTIBODIES ASSOCIATED WITH OTHER DRUGS CAUSING HEPATITIS

Drug	Antigen
Tienilic acid	CYP2C9
Dihydralazine	CYP1A2
Ethanol	CYP2E1, CYP3A4, CYP2E1-hydroxy-ethyl radical

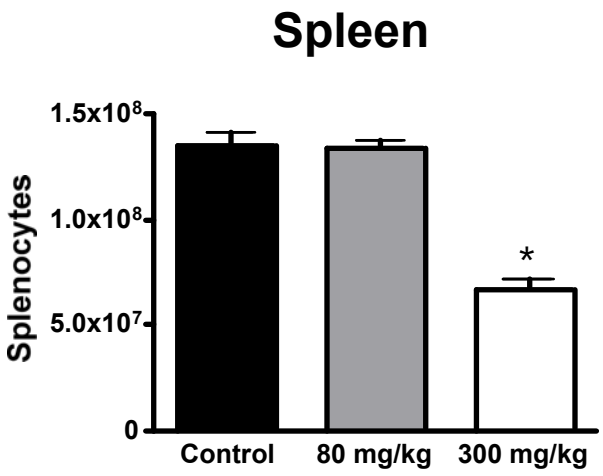
T CELL REACTIVITY ASSOCIATED WITH DRUGS CAUSING ALLERGIC HEPATITIS

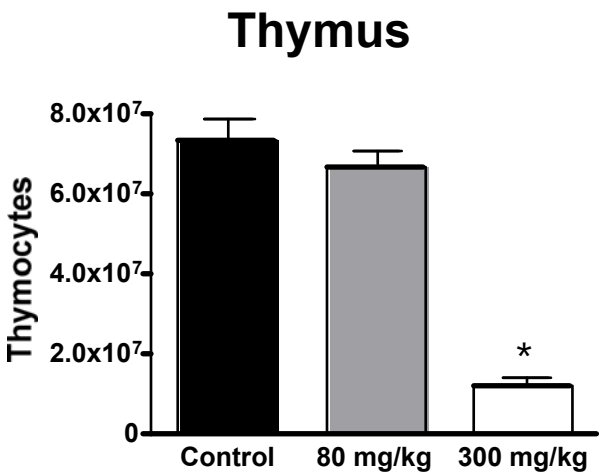
Cotrimoxazole
Erythromycin
Ketoconazole
Ampicillin
Allopurinol
Ibuprofen
Captopril
α-Methyldopa
Enalapril

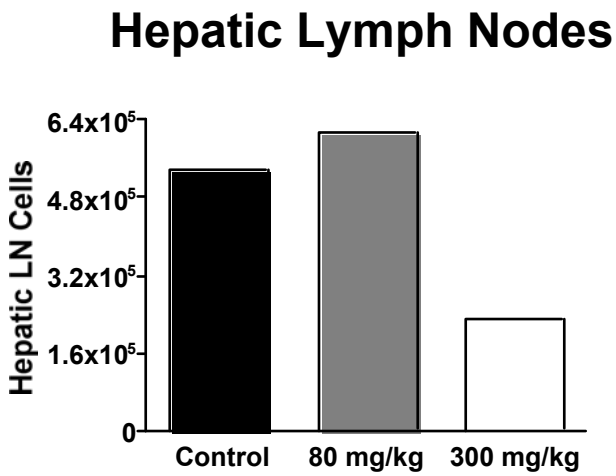
Chlorpromazine
Amineptine
Dothiepine
Phenytoin
Carbamazepine
Tamoxifen
Glibenclamide
Lovastatin
Propylthiouracil

Gut, 41, 534 (1997)

HEPATOTOXIC DOSE OF APAP DEPLETES LYMPHOCYTES WITHIN 24 HOURS

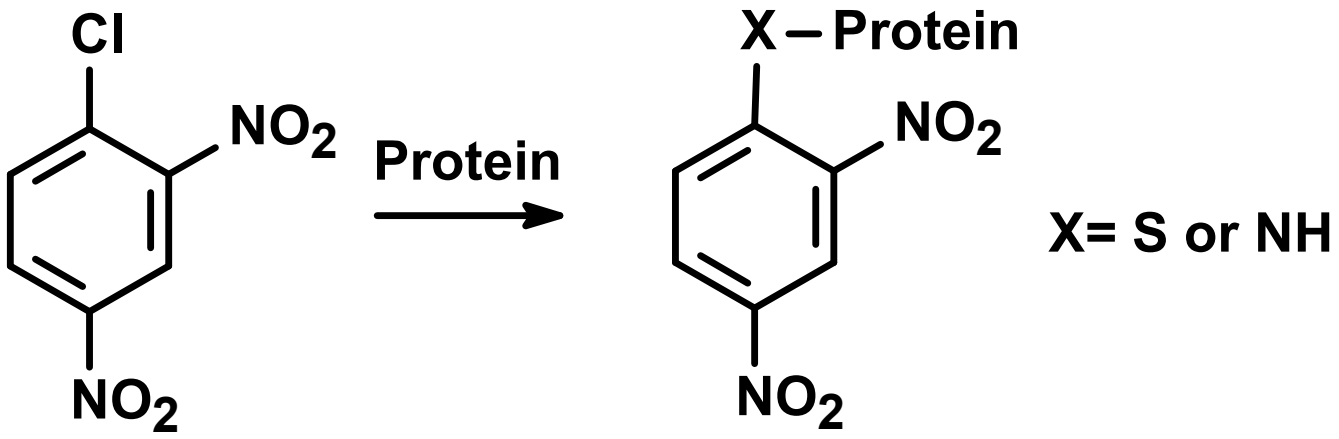






M.J. Masson, et al., Chem. Res. Toxicol., 20, 20 (2007)

DNCB AS A MODEL DRUG ALLERGEN



DNCB

DNP — Protein Adduct

PAINTING DNCB ON SKIN CAUSES DTH IMMUNE REACTION

TOLERANCE PROTOCOL

Sensitization
with 5% DNCB

Determine DTH
response

0 7 12 13 **Day**

i.v. DNP-BSA
or BSA

Challenge with
2.5% DNCB

C. Ju, et al., Chem. Res. Toxicol., 16, 1514 (2003).

Summary

- Drug-drug interactions are the major cause of ADRs, but are often predictable. Polymorphisms can also play a role.
- Many SADRs are rare, highly host-dependent and difficult to predict. Multiple genetic and environmental factors may have a role as well as the innate and adaptive immune systems.
- Designing drugs that will not be metabolized to reactive metabolites may eliminate many SADRs
- Newer preclinical screening tests may also prevent many SADRs