Mechanisms of immune mediated drug hepatotoxicity

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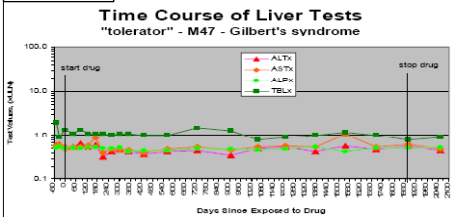
Hepatotoxicity

- Hepatotoxicity is the single most common adverse effect responsible for major drug problems, restrictions on use, drug withdrawals and refusals to approve

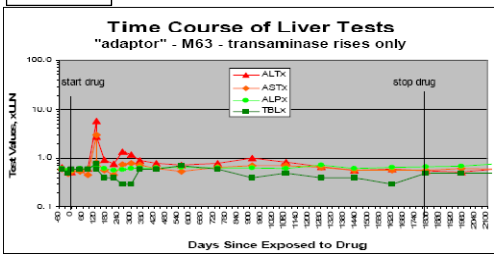
- Idiosyncratic, non-allergic toxicity
- Idiosyncratic, allergic toxicity
- Distinction not clear-cut

Inter-individual variation in response to a drug

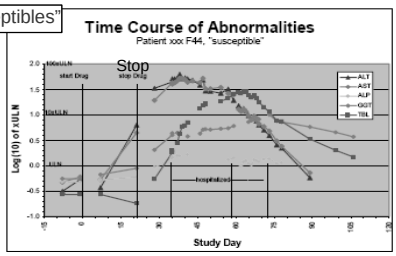
"tolerators"



"adaptors"



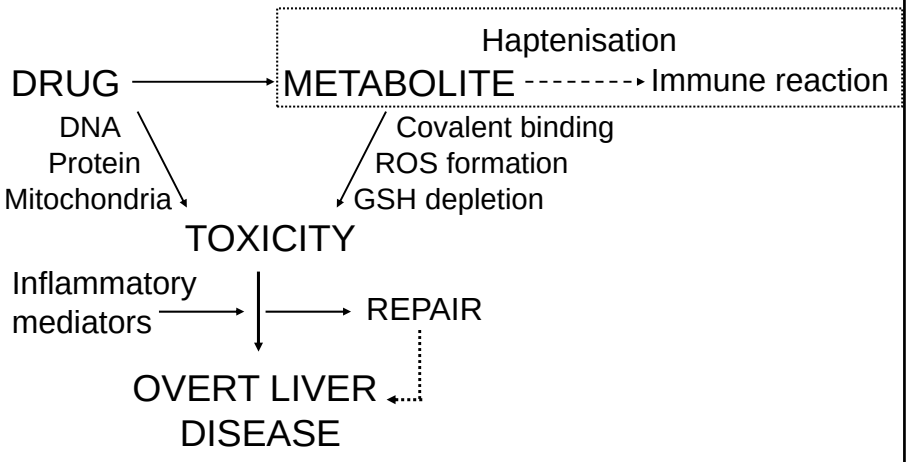
"susceptibles"



ALT
AST
ALP
GGT
TBL

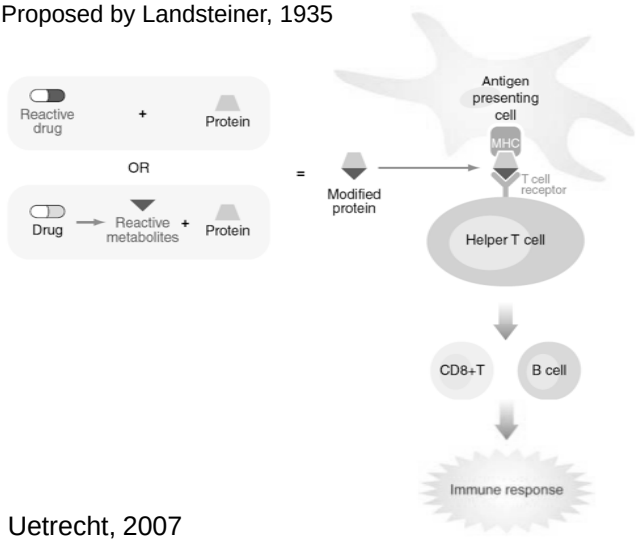
Strand Life Sciences (2008)

Pathogenesis of drug-induced liver disease



Hapten hypothesis

Proposed by Landsteiner, 1935

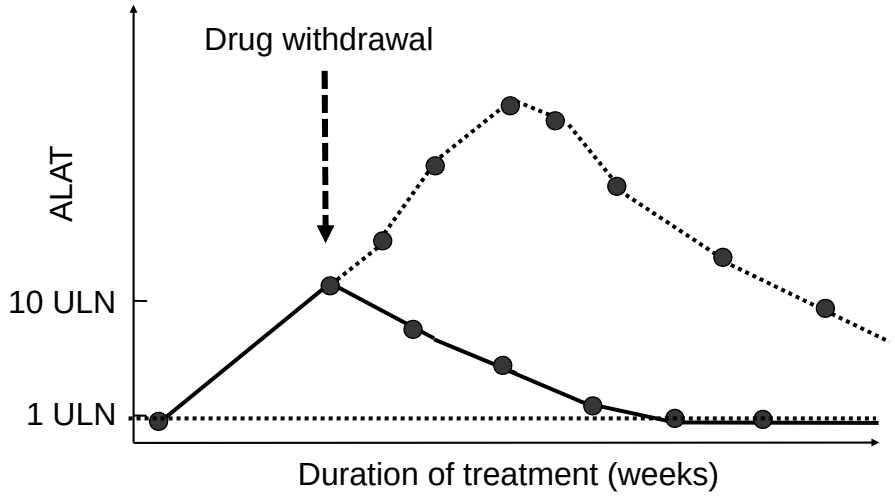


Uetrecht, 2007

Characteristics of immune hepatitis

- Very low frequency of disease, even in the population taking the drug
- Delay between drug administration and onset of disease
- Reduced delay and more severe effect on rechallenge with the drug
- Clinical signs of an immune reaction, such as fever, skin rash and eosinophilia
- Presence of serum autoantibodies, often against native proteins, e.g. P450, UGT or GST form(s)

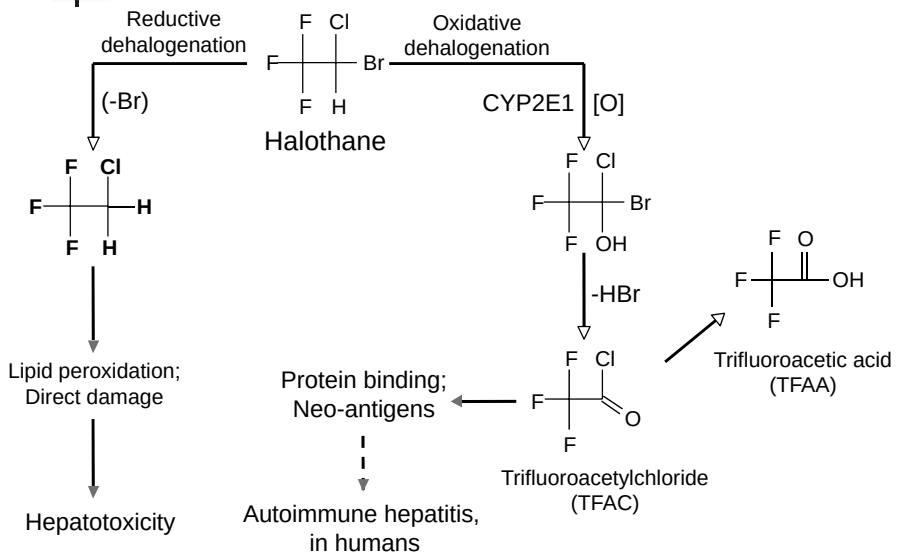
Time-course of immune hepatitis



Halothane

- Once widely used gaseous anaesthetic agent
- Can cause sub-clinical hepatitis in some patients (~20%)
- In rare cases, it can cause severe form of hepatitis (1 in 6,000 - 35,000)
  - Extensive hepatic necrosis
  - Approx. 75% fatality
  - Frequency increased after several doses
- Clinical features of this effect suggest an immune mechanism (fever, rash, eosinophilia)

Halothane toxicity

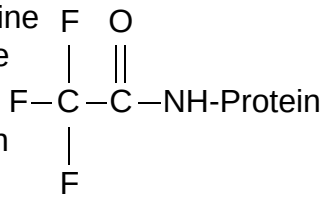


Halothane hepatitis patients: Serum antibodies (% reactivity)

Antigen	TFA-Protein	Native-Protein
CYP2E1		45
PDI (ERp59)	10	5
PDI isoform (ERp57)	55	25
Carboxylesterase	13	5
Calreticulin	5	3
ERp72	30	25
GRP94 (Hsp90b1)	65	28

Halothane-induced hepatitis

- Oxidative pathway is responsible for immune hepatitis
- Oxidative metabolism of halothane leads to formation of trifluoroacetylchloride (CF_3COCl, TFAC)
- Main enzyme responsible is CYP2E1
- TFAC is very reactive and binds to lysine residues in several proteins to produce neoantigens



- CYP2E1 is a major target for alkylation
- Serum from patients (45-70%) contain autoantibodies that recognise CYP2E1

Autoimmune-hepatitis

- Although immune response appears to be provoked by neoantigen, comprising metabolite-modified CYP and other proteins, autoantibodies recognise native (unmodified) proteins

Hypothesis for role of autoantibodies in drug-induced hepatitis

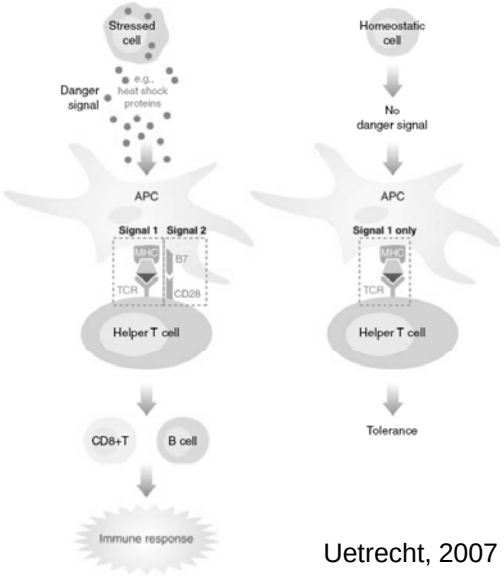
- How do autoantibodies cause hepatotoxicity?
  - Molecular mimicry
  - Activation of cytotoxic T cells and complement system
  - Other

Molecular mimicry

- Minor antigenic differences between neoantigen and host antigen sufficient to lead to induction of immune response by neoantigen?
- Monospecific antibodies against halothane neoantigen, the Nε-trifluoroacetyl-lysine domain, recognise native dihydrolipoamide acetyl transferase (E2) subunit of pyruvate dehydrogenase complex, which contains lipoic acid as prosthetic group
  - Lipoic acid is linked to ε-nitrogen of lysine residue
- Immune response to neoantigen would break tolerance to host epitope
- Cross reactive T or B cell would then induce autoimmune response

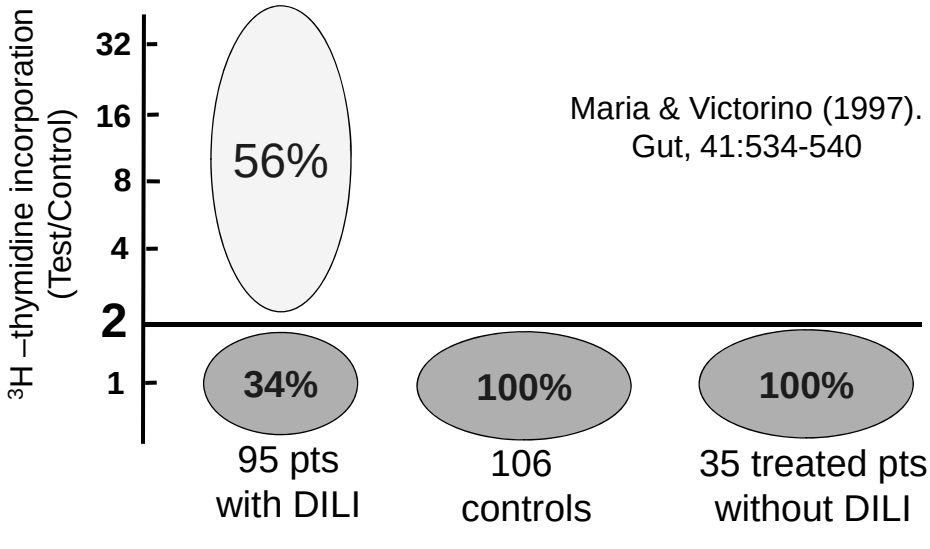
Danger hypothesis

Proposed by Matzinger, 1994



Uetrecht, 2007

Lymphocyte proliferation assay

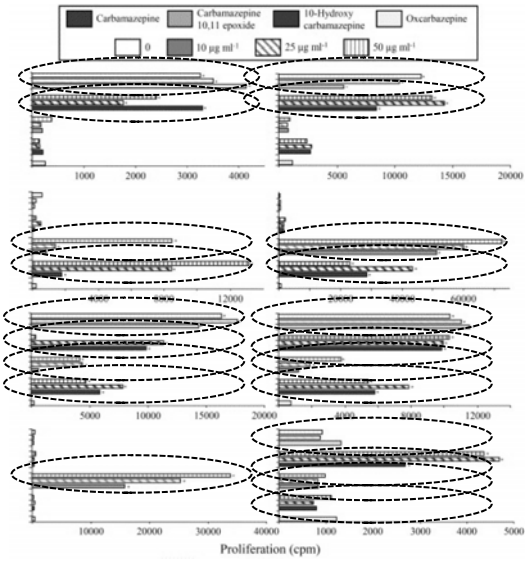


Maria & Victorino (1997). Gut, 41:534-540

Hepatitis in severe drug hypersensitivity syndromes: Immune reactivity elicited by drugs

- Drug specific T-cells can be found in the circulation of patients
- It is possible to generate drug specific T-cell clones (TCC) from the peripheral blood of these patients
- Many T-cells react with parent substance, others also with metabolites or related compounds
- The drug specific T-cells produce high amounts of IFNγ and IL-5
- The drug specific T-cells can kill target cells via a perforin/granzyme B mediated mechanism

T cell clones generated to CBZ

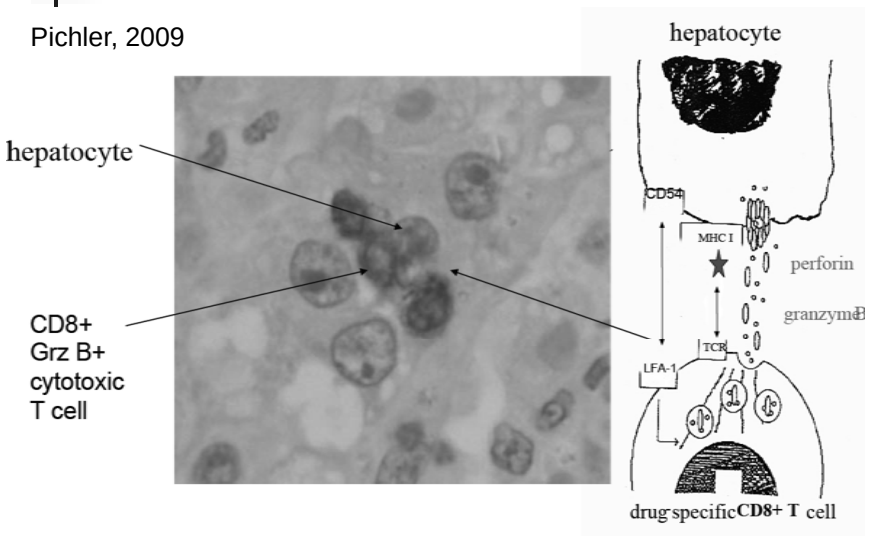


- Often reactivity to CBZ (parent compound)
- Some reactivity to CBZ metabolites
- Cross-reactivity to oxcarbamazepine
- Drug specific T-cells detectable for years after the reaction

- Wu, Naisbitt et al (2006, 2007)

Cytotoxic killing of hepatocytes in DRESS*

Pichler, 2009

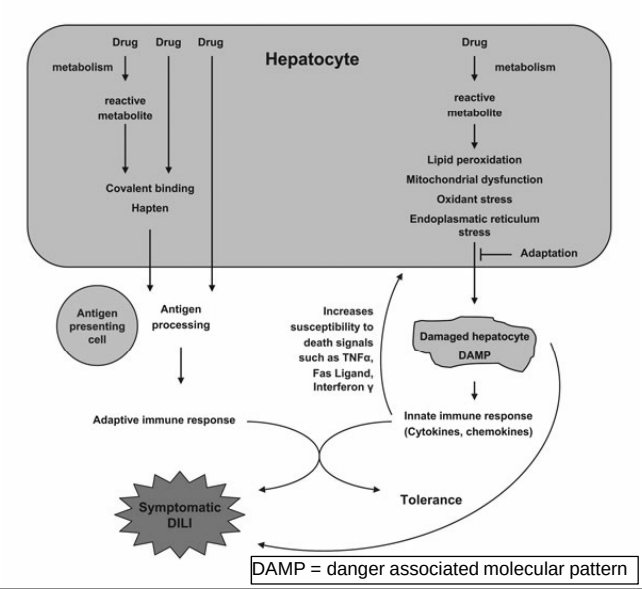


*Drug Reaction with Eosinophilia and Systemic Symptoms

Immunology of DRESS

- Drug specific CD8+ (>CD4+) T cells infiltrate the liver
- They have cytotoxic potential
- They kill (activated?) hepatocytes, which undergo apoptosis
- The exact mechanism of T-cell activation and the role of the drug (parent compound or of metabolites) is unclear

Idiosyncratic DILI

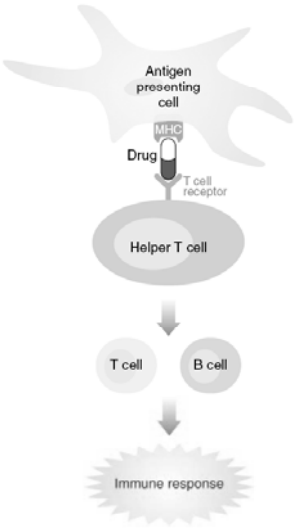


Stirnimann, et al (2010)

DAMP = danger associated molecular pattern

Pharmacological interaction (PI) hypothesis

Proposed by Pichler, 2002

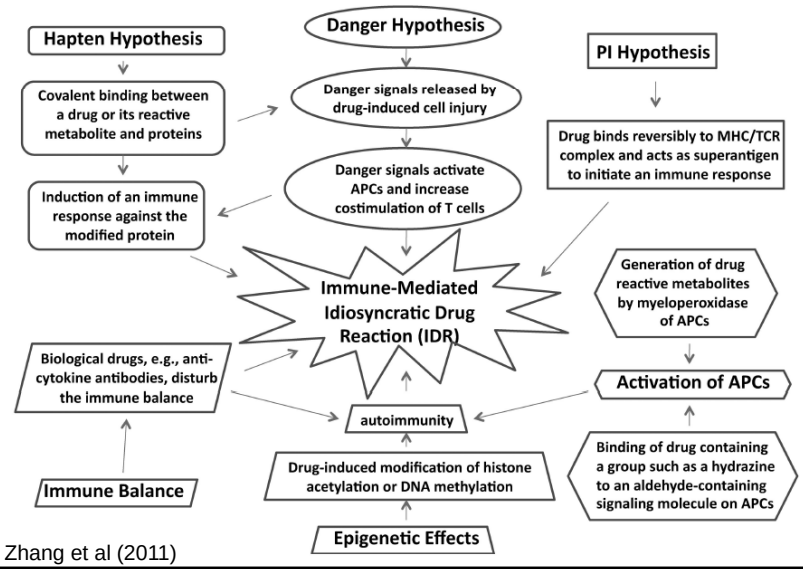


Pharmacologic interaction with immune receptors: the 'p–i concept'

1. Reversible binding of drug to TCR
2. Stabilised by MHC/peptide interaction

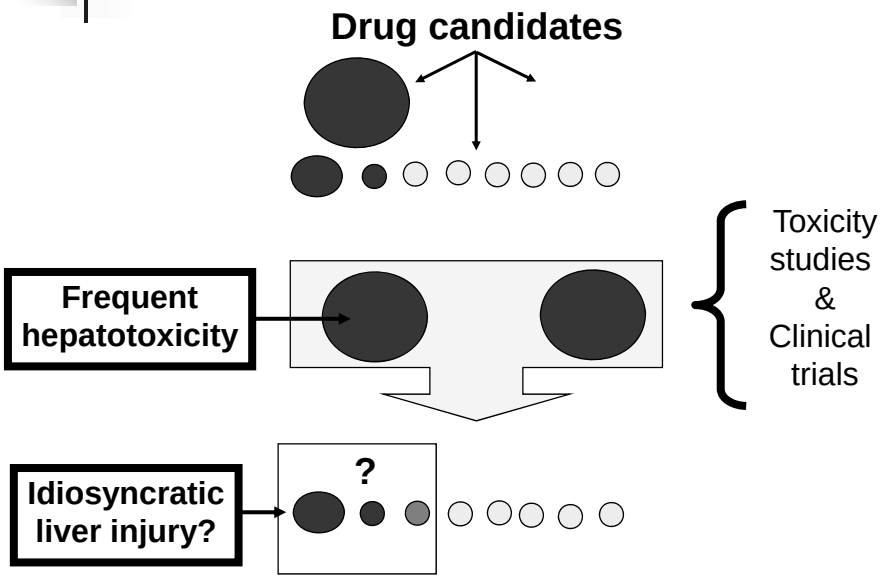
Uetrecht, 2007

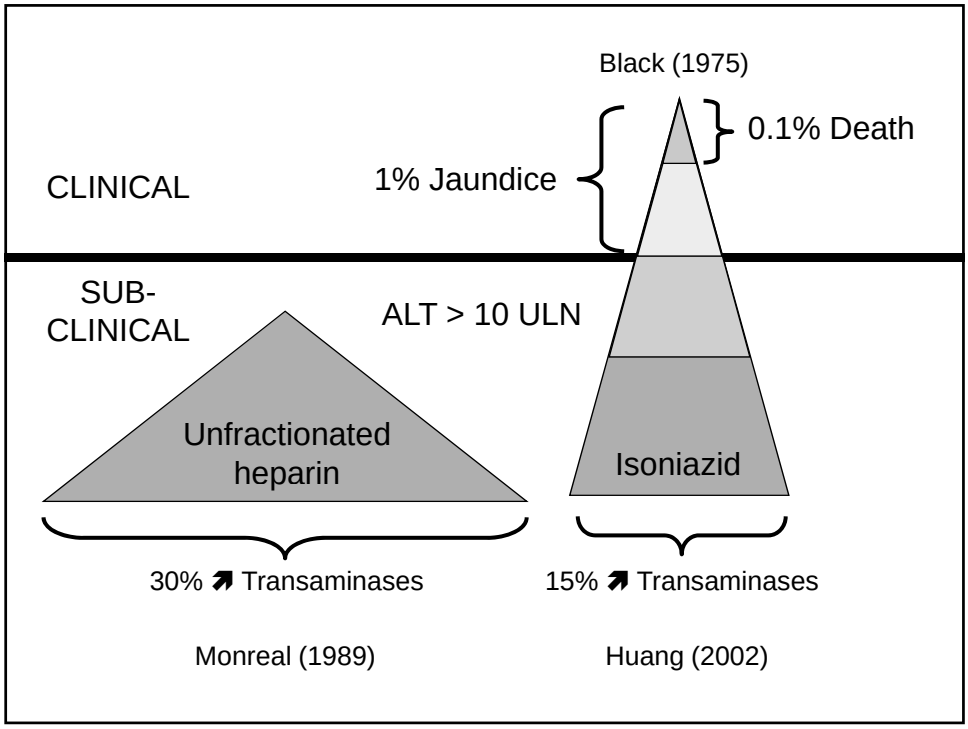
Hypothesised mechanisms for DILI



Zhang et al (2011)

Idiosyncratic liver injury is not easy to predict





Predicting sensitisation in DILI

- It is possible to detect sensitisation in sensitized individuals
- Examples for isolated hepatitis with demonstration of sensitisation (lymphocyte transformation test, patch test):
  - Omeprazole
  - Aminopterin (methotrexate)
  - Abacavir
  - Clarithromycin; and others
- It is possible to detect drug specific T-cells in non-exposed individuals at risk of abacavir hypersensitivity (HLA-B*5701); IFNγ production by $CD8^+$ T cells; not detectable in HLA-B*5701 negative individuals
- *In vitro* generation of hapten and non-hapten (p-i mechanism) specific T cell clones possible from non-sensitised individuals

MHC alleles and DILI susceptibility

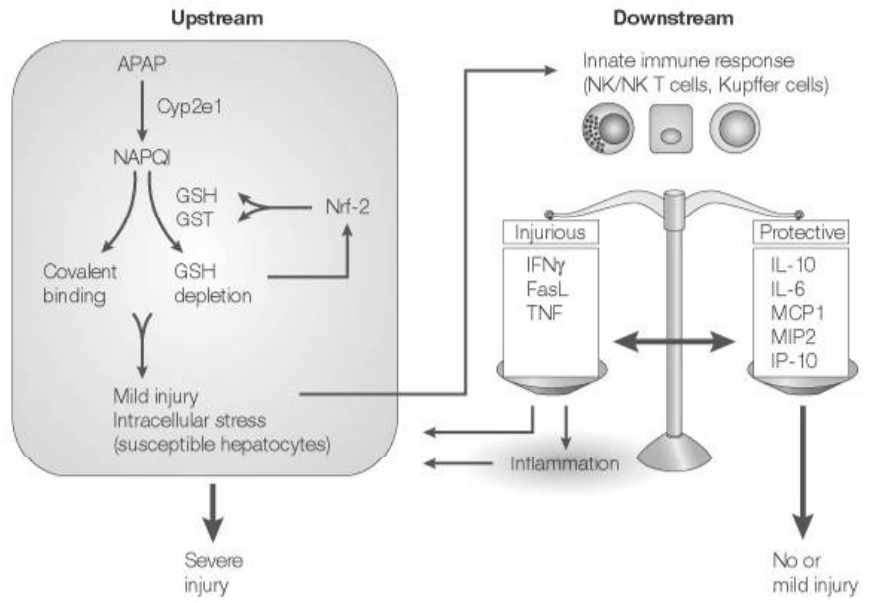
Drug	HLA	Odds Ratio* (Case/Control)
Amox/Clavulanate[1]	DRB1*1501	10X
Ximelegatran[2]	DRB1*07	4X
Lumaricoxib[3]	DRB1*1501	6X
Flucloxacillin[4]	B*5701	80X
Ticlodipine[5]	A*3303	13X

* Odds Ratio does not reflect predictive value or absolute risk measures

1. Hautekeete et al. Gastro. 1999; O'Donohue et al., Gut, 2000
2. Kindmark et al., Pharmacogenomics J., 2007
3. www.aasld.org/conferences/educationtraining/Documents/Hepatoxicit/Wright
4. Daly et al., Nature Genetics, 2009
5. Hirata et al., Pharmacogenomics J., 2008

Predicting immune-mediated DILI

- Escape of local (hepatic) immune tolerance
- Consider (human) genetic background:
  - HLA-B*5701, *1502, *5801, *07. Consequently, animal models of limited value
  - Other genes (drug transporters, P450s, ...)
- Biochemistry/metabolism of compound: Generation of hapten-like compounds intrahepatically
  - Relevant mitochondrial damage?
  - Danger signals?
  - Individually different?
- Demonstration of *in vitro* sensitisation in non-sensitised subjects



Role of innate immune system From Kaplowitz (2005)

Summary

- CYPs are major autoantigens associated with DIAH
- Other antigens and neoantigens may be important in DIAH, depending on chemistry of reactive metabolite(s)
- Evidence for T cells associated with drug-induced autoimmune hepatitis (DIAH)
- Importance of genetic background of individual
- Increasing evidence for importance of innate immune system in all drug-induced liver injury

Further information

- Kaplowitz N (2005). Idiosyncratic drug hepatotoxicity. *Nature Rev Drug Discov* **4**, 489-499
- Uetrecth J (2009). Immune-mediated adverse drug reactions. *Chem Res Toxicol* **22**, 24-34
- Adams DH *et al* (2010). Mechanisms of immune-mediated liver injury. *Tox Sci* **115**, 307–321
- Cjaga AJ (2011). Drug-induced autoimmune-like hepatitis. *Dig Dis Sci* **56**, 958-976