Mechanisms of Drug Hypersensitivity Reactions

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Definitions and Manifestations

**Hypersensitivity**

▶ an inappropriate immune response leading to tissue damage from an otherwise non-toxic agent
Immune-Mediated Adverse Drug Reactions

<table>
<thead>
<tr>
<th>Type</th>
<th>Mediated by</th>
<th>Pathogenesis</th>
<th>Clinical Presentation (Examples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>IgE</td>
<td>Degranulation of basophils and mast cells</td>
<td>Urticaria, anaphylaxis</td>
</tr>
<tr>
<td>II</td>
<td>IgG/M</td>
<td>Cell lysis</td>
<td>Blood dyscrasia</td>
</tr>
<tr>
<td>III</td>
<td>IgG/M</td>
<td>Deposition of immunocomplexes</td>
<td>Vasculitis</td>
</tr>
<tr>
<td>IVa</td>
<td>Th1</td>
<td>IFN-γ activation of monocytes and macrophages</td>
<td>Eczema</td>
</tr>
<tr>
<td>IVb</td>
<td>Th2</td>
<td>IL-4, IL-5 activation of eosinophils</td>
<td>Maculopapular/bullous exanthema</td>
</tr>
<tr>
<td>IVc</td>
<td>CTL</td>
<td>Perforin, granzyme B cytotoxicity</td>
<td>Maculopapular/bullous/pustular exanthema</td>
</tr>
<tr>
<td>IVd</td>
<td>T cells</td>
<td>IL-6 chemotraction and activation of neutrophils</td>
<td>Pustular exanthema</td>
</tr>
</tbody>
</table>

Drug Hypersensitivity

- Off target toxicity
  - Not predictable from the known pharmacology of a drug

- Skin is the organ most commonly involved

- Role of T-cells
  - Proliferation of T-cells from hypersensitive patients but not non-hypersensitive controls
  - Prominent cutaneous infiltration of CD4+ and CD8+ T-cells
Genetic Restriction and Drug Hypersensitivity: Discovery of HLA allele associations

- Discovery of associations between HLA alleles and drug hypersensitivity represents an important advance
- Screening for HLA alleles during clinical practice effectively prevents reactions
  - Abacavir
  - Carbamazepine
- Mechanistic studies using samples from volunteers with HLA risk alleles may shed light on the immune pathogenesis

Pharmacogenetics and Clinical Syndromes

- Abacavir hypersensitivity: HLA-B*5701 OR = 132
- Flucloxacillin DILI: HLA-B*5701 OR = 72
- Carbamazepine SYS/TEN: HLA-B*1502 (Chinese) OR = 1000
- Carbamazepine Hypersensitivity: HLA-A*3101 (Japanese) OR = 11
- Carbamazepine Hypersensitivity: HLA-A*3101 (Caucasians) OR = 30
- Lumiracoxib DILI: HLA-DRB1*1501 OR = 7
- Lumiracoxib DILI: HLA-DQA1*0102 OR = 4.4
- Ximelagatran DILI: HLA-DRB1*0701 OR = 4.4
- Ximelagatran DILI: HLA-BQA1*0201 OR = 9.0

Mallal, 2008; Kindmark et al., 2008; Daly et al., 2009; Chung et al., 2004; McCormack et al., 2011; Singer et al., 2010; Spraggs et al., 2011

Delayed Hypersensitivity Reactions (1)

- Commonest type of cutaneous eruption
- Drug withdrawal
- Symptomatic and supportive treatment

Maculopapular Eruption (MPE)
Delayed Hypersensitivity Reactions (2)

- Systemic manifestations
- Fever, eosinophilia
- Variable extra-cutaneous involvement
- Liver commonest to be affected

Anticonvulsant Hypersensitivity Syndrome: Clinical Characteristics

- Carbamazepine (n=35)
- Lamotrigine (n=34)

- Pneumonitis
- Aplastic anaemia
- Thrombocytopenia
- Lymphadenopathy
- Liver toxicity
- Eosinophilia
- Fever
- Skin rash

% of patients
### Delayed Hypersensitivity Reactions (3)

**Stevens-Johnson Syndrome**
- 1-10% of skin blistered
- Two mucous membranes involved
- 10% mortality

### Delayed Hypersensitivity Reactions (4)

**Toxic Epidermal Necrolysis**
- >30% of skin blistered
- Two mucous membranes involved
- 30% mortality
Delayed Hypersensitivity Cutaneous Reactions

- Maculopapular exanthem
- Hypersensitivity syndrome
- Stevens-Johnson Syndrome
- Toxic Epidermal Necrolysis

Severity → Mortality → Rarity

Phenotypic Heterogeneity

- Skin
- Liver
- Kidney

- Phenotypic heterogeneity common in drug allergies
  - Same drug can cause different manifestations in different patients
  - Within the same organ, clinical picture can vary
  - Within different patients, a number of organs can be affected

- Important to have clear phenotypic criteria to allow for phenotype standardisation
Phenotype is Crucial

The Phenotype Standardization Project: Improving Pharmacogenetic Studies of Serious Adverse Drug Reactions

M Firmehamed, GP Aithal, E Behr, A Daly and D Roden

Phenotype Standardization for Immune-Mediated Drug-Induced Skin Injury

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Case Definition and Phenotype Standardization in Drug-Induced Liver Injury

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Cellular pathophysiology of drug hypersensitivity reactions in skin: characterization of T-cell clones

Tissue damage in skin

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The Hapten Hypothesis

1. Small compounds (<1000Da) – incomplete antigen

Pathways of T-cell activation

Nature of the binding interaction

Non-covalent

Covalent
Abacavir Hypersensitivity: Translational Paradigm

- Discovery of HLA-B*5701
- Replication
- Demonstration of cost effectiveness
- Randomised controlled trial (PREDICT-1)
- Implementation (UK from 2006)

Change in Peptide Repertoire

Immune self-reactivity triggered by drug-modified HLA-peptide repertoire

- Patricia T. Illeg 1, Julian P. Vivian 1, Nadine L. Duijs 1, Lydia M. Koster 1, Zhou Jian Chen 1, Mandvi Bhuradn 1, John J. Mills 1, Lars Jør 1, Michael 1, Stephanie Grant 1, Nicholas A. Williamson 1, Scott R. Brown 1, Anthony W. Parec 1, Jamie Roush 1, and James McCluskey 1

Drug hypersensitivity caused by alteration of the MHC-presented self-peptide repertoire

- David A. Ostrov 2, Barry J. Grant 2, Yuri A. Pompe 2, John Sidney 2, Mikkel Harndahl 2, Scott Southwood 2, Carla osteoff 2, Shun Lu 2, Jean Jakonce 2, Cesar Augusto F. de Oliveira 2, Lui Yang 2, Hu Mei 2, Eming Shi 2, Jeffrey Shabana 2, A. Michelle English 2, Amanda Writson 2, Andrew Lucas 2, Elizabeth Phillips 2, Simon Mellis 2, Howard M. Grey 2, Alessandro Sette 3, Donald F. Hunt 3, Soren Buus 3, and Bjoern Peters 4

Abacavir induces loading of novel self-peptides into HLA-B*57:01: an autoimmune model for HLA-associated drug hypersensitivity

- Michael A. Nocness 4, Shen Luo 4, Li Lu 4, Michael T. Boyne 4, Mary Gomartell 4, Aaron D. Renneil 4, Janet Woodcock 4, David H. Margulies 4, Curtis McMurtry 4, Stephen Vernon 4, William H. Hildebrand 4, and Rico Buchli 4
HLA-B*57:01 and Abacavir Hypersensitivity

- Peptides from untreated cells show standard peptide profile
- ABC treated cells show novel self-peptides (20-25%) with Ile/Leu occupying C-terminal anchor protein
- No change in peptide profile with closely related allotypes

Illing et al, 2013, Curr Opin Immunol

Immunogenic HLA-B*57:01-peptide complexes (after Illing et al, 2013)
**Drug Sensitivity**

**Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS): A Multiorgan Antiviral T Cell Response**

- Patients with DRESS due to carbamazepine, allopurinol, sulfamethoxazole
- EBV, HHV-6 and HHV-7 reactivation in 76%
- Increased secretion of cutaneous homing markers, TNF-α, IFN-gamma
- CD8+ T cells recognised several EBV epitopes
- Culprit drugs triggered production of EBV from patient B cells
- Link between altered peptide repertoire and viral reactivation

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**HLA-B*5701** genotype is a major determinant of drug-induced liver injury due to flucloxacinil

- 52 cases
  - NNT for abacavir 12
  - NNT for flucloxacinil 13,819
  - Illumina 1 million SNP array
  - Strong (P=10^-30) association with SNP in LD with HLA-B*57:01

**Collaboration between UK DILIGEN and SAEC**
**Why HLA-B*57:01?**

Abacavir

Flucloxacillin

1. 8.5 in 100000 patients
2. Elderly and prolonged course

Chance finding or is there a common mechanism?

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**Flucloxacillin Binding to HSA**

Flucloxacillin

Protein

Protein conjugate

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![Image](thiazolinedine_ring.png)

**19FPKLWAVAVAR**

Unmodified parent ion mass = 1038.6
Modified parent ion mass = 1473.5

Lys190

Lys212
Flucloxacillin-Induced Liver Injury

- **Flucloxacillin forms an antigen in patients**
  - Jenkins et al., 2009

  Albumin conjugates in 8/8 patients (Lys 190, 212)

- **Association between the expression of HLA-B*5701 and liver injury**
  - 4/64 controls
  - 63/74 cases

**HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin**


**MISSING LINK:**

Evidence to show that reactions to flucloxacillin are driven by drug-specific activation of cytotoxic CD8+ T lymphocytes

**Patient cohort:** Six patients with clinically well-defined flucloxacillin-mediated liver injury

**Phase 1 study:**
- LTT
- *negative in all patients*
- IFN-gamma ELISPOT
- *positive in 5/6 patients*

**HLA-B*5701 restricted activation of flucloxacillin responsive T-cells: The immunogenetic basis for DILI**
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- **Phase 2 study:**
  - Characterization of drug-specific cytotoxic T-cells
  - Generation and characterization of T-cell clones
  - Role of the HLA risk allele in the immune response

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**HLA-B*5701 Restricted Activation of Flucloxacillin Responsive T-cells: The Immunogenetic Basis for DILI**

- **Patient cohort:** Six patients with clinically well-defined flucloxacillin-mediated liver injury
- **Pilot study:**
  - LTT
  - *negative in all patients*
  - IFN-gamma ELIspot
  - *positive in 5/6 patients*
- **Phase 2 study: on-going**
  - Characterization of drug-specific cytotoxic T-cells
  - Generation and characterization of T-cell clones
  - Role of the HLA risk allele in the immune response
Detection of Drug-Specific T-cell Responses in HLA-Typed Drug-Naive Volunteers

**READOUTS**
- proliferation
- cytokine secretion
- cell surface markers

**Provision of co-stimulatory signals through endogenous activators**

**Proliferation assay against suspect chemicals**

**Cell bank containing functional lymphocytes**

**Dissemination of results**

**Volunteer Demographics (N=400)**

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>White</th>
<th>Indian</th>
<th>Other</th>
<th>Chinese</th>
<th>Black</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>Female</td>
<td>64%</td>
<td>36%</td>
<td></td>
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</tr>
<tr>
<td>29 ±10 years</td>
<td>Range</td>
<td>18-60 years</td>
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</table>
**HLA-B*57:01 Restricted Activation of Flucloxacillin Responsive T-cells: The Immunogenetic Basis for DILI**

**Volunteer cohort:** Frozen PBMC from 26 drug-naive individuals expressing HLA-B*5701

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**Flucloxacillin: 3 patients**

**Patient 1**
- Cholestatic hepatitis after 2 weeks of flucloxacillin
- Confirmed on biopsy

**T-cell**
- HLA-B*57:01

**Patient 2**
- Acute generalised exanthematous pustulosis
- Rechallenge with recurrence of reaction

**T-cell**
- HLA-B*57:01

**Patient 3**
- Anaphylaxis
- Positive skin and intradermal testing

**IgE**
- HLA-B*57:01

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**What factor(s) determine phenotype specificity?**
Key knowledge gaps

Frequency / Severity of Drug Hypersensitivity

1. Relationship between drug protein binding and immunogenicity is not defined

2. The immunological processes responsible for sensitization and tolerance are not defined

3. The importance of genetic (HLA) predisposing factors (do they relate to the T-cell response?)

4. How T-cells cause different forms of severe skin injury is not known

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