Genetic in Severe Drug Allergy Reactions

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Severe Drug Hypersensitivity Reaction

DRESS  Drug Rash with Eosinophilia and Systemic Symptoms

- Other Names: DHS, DIHS
- Fever
- Massive eosinophilia (> 1,5G/Lt), lymphoadenopathy)
- Internal organ: liver, kidney, lungs
- Late onset of the symptoms (> 2-10 Weeks treatment)

Severe Drug Hypersensitivity Reaction

SJS / TEN

- Stevens Johnson Syndrome (SJS, 10 % Body surface)
- Toxic Epidermo Necrolysis (TEN, > 30 % Body surface)
- Caused by drug hypersensitivity >95%
- Mortality: 10-35%
- Massive keratinocyte apoptosis
  - Epidermal detachment / formation of bullae
  - Involvement of mucosa
Pathogenesis Present Concept

- Immune mediated
  - Adaptive immunity
  - Activation and clonal expansion of T cells
  - MHC-restricted drug presentation → HLA Class I association
  - Initiation by cytotoxic T lymphocytes (CTL)

- Immune mediated Cytotoxicity:

![Diagram](image)

Genome wide association studies

- Rapid scanning of markers across the genome of many people to find genetic variations associated with a particular disease.

- Patients cohorts
  - Patients with SJS/TEN or DRESS due to a define drug
  - Patients tolerating the drug
  - Healthy individuals (not exposed to the drug)

Genetic association with DHR

<table>
<thead>
<tr>
<th>Drug</th>
<th>HLA-Patient</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepin</td>
<td>B*1502 in Han-chinese 100% association (SJS) 3% positive predictive value</td>
<td>Chung et al., Nature 2004</td>
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<tr>
<td></td>
<td>A*3101 in Japanese population 60% association (SJS) OR 10.8</td>
<td>Ozekii et al., Hum Mol Genet 2011</td>
</tr>
<tr>
<td></td>
<td>A*3101 in European population OR 12.4 (DRESS) 8.6 (MPE), 25.9 (SJS)</td>
<td>Mc Cormick et al., NEMM 2011</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>B*5801 in Han-chinese 100% association (SJS &amp; TEN)</td>
<td>Hung et al., PNAS 2005</td>
</tr>
<tr>
<td></td>
<td>B*5801 in Caucasian 55% association (SJS) OR 80 Undefined association for allopurinol-induced DRESS</td>
<td>Lejeune et al., Pharmacogen. 2008</td>
</tr>
</tbody>
</table>
**Genetic association with DHR**

<table>
<thead>
<tr>
<th>Drug</th>
<th>HLA-B*5701</th>
<th>100% association of confirmed cases</th>
<th>55% positive predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abacavir</td>
<td></td>
<td></td>
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<tr>
<td>Flucloxacillin</td>
<td></td>
<td>Drug induced liver disease</td>
<td>84.3% association in a DILI-cohort OR 80 Prevalence: 1 in 500-1000 HLA-B*5701+</td>
</tr>
</tbody>
</table>

**Genetic associations and functional involvement**

Absolute 100% association:

- Carbamazepine: HLA-B*1502: 100% (Han Chinese)
- HLA-A*3101: >60% (Caucasian)
- Allopurinol: HLA-B*5801: 100% (Han Chinese)
- HLA-B*5801: >50% (Caucasian)
- Abacavir: HLA-B*5701: 100%

Seeing these extremely high proportions of identified HLA allele in cohorts, **HLA molecules must be functionnally involved** in the pathogenesis of drug hypersensitivity disorders!

**What are HLA molecules?**

Membrane surface proteins presenting peptide antigens to T cells:
- MHC class I, HLA-A, - B and - C → CD8+ T cells
- MHC class II, HLA-DP, -DQ, DR → CD4+ T cells
Involvement of T cells in severe hypersensitivity reaction

- HLA associations preferentially with HLA class I
- CD8 activation
- Cytotoxic mechanism (Granzyme B, Granulysin)
- Severe reactions (SJS, hepatitis)

CD8 Staining

Perform staining

Top view of the HLA-molecule

Empty HLA molecule

Peptide containing HLA molecules

Extremely polymorphic

> 1500 alleles identified

Side view of the HLA-molecule

Anchored peptide: multitudes of possible peptides

Positions 2 and 9 are called “anchor residues” because they fix the presented peptide by tight interaction with the HLA molecule
Possible mechanisms involving HLA association

Hapten theory

Interaction within the Peptide binding groove

Hapten mechanism

Cova lent binding of the drug on proteins

How could be the presentation of haptenized peptides HLA allele restricted

Drug X
Hapten binds to lysins

Peptide 23-29
Peptide 34-40
Peptide 45-51

Processing

Binding of modified peptides to certain/fitting HLA-alleles

HLA- A*0201
HLA- B*5701
HLA- B*2703
How could be the presentation of haptenized peptides HLA allele restricted

Drug X Hapten binds to lysins

Processing

The hapten-modification of a protein generates many different peptides, which bind to various “fitting” HLA-alleles;

Thus, hapten induced T cell reactions are not HLA-restricted

HLA- A*0201  HLA- B*5701  HLA- B*2703

*pi-concept: Pharmacological Interaction with Immune Receptors

Labile, non-covalent interaction

The Abacavir model

T cells isolated peripheral blood mononuclear cells from HLA B*5701/healthy donors were stimulated for 15 days with ABC or with culture medium alone (negative control).

Without ABC  With ABC

0.06  0.87
Small polymorphism between within the B*57/58 haplotype

114 Asp → Asn
116 Ser → Tyr
156 Leu → Arg

B*5701
B*5702
B*5801

Immediate activation of specific CD8+ T cells by Abacavir

Intracellular Calcium influx measurement

→ 200 seconds are too short to allow processing of Abacavir modified peptide
Carbamazepin model: Absence of modified peptides

HLA-B*1502 eluted peptide did **NOT** carry the drug (CBZ).

Yang CW, JACI, 2007

Why Carbamazepine induces SJS/DRESS in less than 5% of HLA-B*1502+ individuals?

**HLA-B*1502**
- 100% association in the cohort
- But <5% of HLA-B*1502 will develop SJS to CBZ
- Specific Clonotype in the population

Ko et al, JACI, 2011

Summary

- GWAS identified HLA-allele association with severe drug hypersensitivity
  - Carbamazepin: HLA-B*1502/A*3101
  - Allopurinol: HLA-B*5801
  - Abacavir, Flucloxacillin HLA-B*5701
- Genetic associations were discovered with very high proportions (100%) in DHR cohorts.
- Abacavir and HLA-B*5701 typing: first example of successful personalized medicine
Summary

• HLA molecules are crucial protein involved in peptide antigen presentation
• Drug can interact with HLA at very precise locations and produces / modifies antigenic determinants
• Phenotype of main in-vitro generated drug reacting T cells is inflammatory (IFNγ production) and cytotoxic.

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