Why are HLA alleles associated with drug hypersensitivity?

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Disclosure

In relation to this presentation, I declare that there are no conflicts of interest.

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1. Introduction: HLA associations reveal alternative mechanisms for different pathologies and different drugs

2. Why is abacavir hypersensitivity strongly associated with HLA-B*57:01?
   1. What is the structural composition of complexes recognized by T cells in patients?
   2. X-ray crystallography and peptide binding assays

3. Why does carbamazepine induce severe cutaneous adverse reactions with different HLA associations in different ethnic groups?
   1. HLA-B*15:02, generate complexes +/- drug
   2. HLA-A*31:01, generate complexes +/- drug
   3. Gel filtration chromatography
   4. Drug affinity responsive target stability (DARTS)

4. Why does sulfamethoxazole have multiple HLA associations?
   1. Different mechanisms, class I, class II, hapten, π?
   2. Sulfamethoxazole enhances HLA binding of specific peptides
<table>
<thead>
<tr>
<th>Class I HLA</th>
<th>Class II HLA</th>
</tr>
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<tbody>
<tr>
<td>HLA-B*57:01 Abacavir</td>
<td>HLA DRB1*01:01 Nevirapine</td>
</tr>
<tr>
<td>HLA-B*15:02 Carbamazepine</td>
<td></td>
</tr>
<tr>
<td>HLA-B*58:01 Allopurinol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2002</td>
</tr>
<tr>
<td></td>
<td>2004</td>
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<td></td>
<td>2005</td>
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<tr>
<td></td>
<td>2006</td>
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<tr>
<td></td>
<td>2007</td>
</tr>
<tr>
<td></td>
<td>2008</td>
</tr>
<tr>
<td>HLA-B*57:01 Flucloxacillin</td>
<td>2009</td>
</tr>
<tr>
<td>HLA-B*35:05 Nevirapine</td>
<td></td>
</tr>
<tr>
<td>HLA-A*31:01 Carbamazepine</td>
<td>2011</td>
</tr>
<tr>
<td>HLA-A*02:01 Amox-Clav</td>
<td></td>
</tr>
<tr>
<td>HLA-C*04:01 Nevirapine</td>
<td>2012</td>
</tr>
<tr>
<td>HLA-B*13:01 Dapsone</td>
<td>2013</td>
</tr>
<tr>
<td></td>
<td>HLA-DQA1*02:01 Lapatinib</td>
</tr>
<tr>
<td></td>
<td>HLA-DRB1<em>15:01/DQB1</em>06:02 Amox-Clav</td>
</tr>
<tr>
<td>Drug</td>
<td>HLA Allele</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Abacavir Hypersensitivity Syndrome</td>
<td>B*57:01</td>
</tr>
<tr>
<td>Allopurinol SJS/TEN and DRESS/DIHS</td>
<td>B*58:01</td>
</tr>
<tr>
<td>Carbamazepine SJS/TEN</td>
<td>B*15:02</td>
</tr>
<tr>
<td>Dapsone DRESS/DHIS</td>
<td>B*13:01</td>
</tr>
<tr>
<td>Flucloxacillin DILI</td>
<td>B*57:01</td>
</tr>
</tbody>
</table>
Drug Hypersensitivity: How Drugs Stimulate T Cells via Pharmacological Interaction with Immune Receptors.  

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<th>Hapten concept (immune/allergic stimulation)</th>
<th>p-i concept (pharmacological stimulation)</th>
</tr>
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<tbody>
<tr>
<td>Generation of a complex immune response with activation of the innate immune system, T and B cell reactions</td>
<td>Direct and exclusive T cell stimulation by 'pharmacological' drug-receptor interaction; innate immune system not involved</td>
</tr>
<tr>
<td>Chemical (covalent) stable binding of drug/drug metabolite to proteins or peptides, which act as antigens for B and T cells</td>
<td>Structural binding of drug/drug metabolite to certain HLA or TCR proteins; mostly quite tabic interactions</td>
</tr>
<tr>
<td>Can be dependent on the metabolism of the drug to reactive compound and needs processing of proteins to immunogenic peptides</td>
<td>Metabolism of drugs or processing of proteins are not required to elicit reactivity</td>
</tr>
<tr>
<td>Time for drug metabolism and protein processing within APC is needed (≥4 h)</td>
<td>Mostly immediate (&lt;~10 min) reactivity of T cells</td>
</tr>
<tr>
<td>B and T cells react via specific immune receptors to drug-modified proteins or drug-modified peptides</td>
<td>T cells react via TCR directly to the drug-modified HLA/peptide complex, or the drug has an allogeneic effect on TCR which then reacts to the HLA peptide</td>
</tr>
</tbody>
</table>

1 Abacavir reactivity may need more time if loading onto HLA-B*57:01 occurs inside the endoplasmic reticulum (see text).
MHC peptide selection and presentation to T cells

Hapten/prohapten model

p-i model

Altered peptide model
Identification of peptides recognized by T cells from abacavir hypersensitivity patients

We identified endogenous peptides that bind HLA-B*57:01 better in the presence of abacavir:

<table>
<thead>
<tr>
<th>Peptide</th>
<th>IC50 (nM) no abacavir</th>
<th>IC50 (nM) + abacavir</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>KVAKVEPAV</td>
<td>40285.6</td>
<td>0.2</td>
<td>ribosome binding protein 1 homolog (RRBP1)</td>
</tr>
<tr>
<td>KSNGTIIHV</td>
<td>10985.6</td>
<td>0.3</td>
<td>CD28</td>
</tr>
<tr>
<td>KIYEGQVEV</td>
<td>9403.3</td>
<td>0.2</td>
<td>60S ribosomal protein L5</td>
</tr>
<tr>
<td>TVAPFNPTV</td>
<td>8992.9</td>
<td>0.1</td>
<td>S100P-binding protein isoform A</td>
</tr>
<tr>
<td>VTTDIQVKV</td>
<td>7308.8</td>
<td>0.2</td>
<td>transcription elongation factor SPT5 isoform A</td>
</tr>
<tr>
<td>RVAGIHKKV</td>
<td>50032.2</td>
<td>171.7</td>
<td>60S ribosomal protein L13 isoform 1</td>
</tr>
<tr>
<td>KAAKIRVSV</td>
<td>4671.1</td>
<td>0.1</td>
<td>GHF-IIH subunit 2-like protein(GTF2H2C)</td>
</tr>
<tr>
<td>RTFHHGVVV</td>
<td>324.7</td>
<td>0.1</td>
<td>nucleoporin 37kDa, isoform CRA_a</td>
</tr>
<tr>
<td>ATIKLQSTV</td>
<td>319.4</td>
<td>0.1</td>
<td>abnormal spindle protein ASP</td>
</tr>
</tbody>
</table>

T cells from patients recognize this peptide better in the presence of the drug.
How does the self-peptide recognized by the TCR?

VTTDIQVKV, corresponding to human transcription elongation factor SPT5 isoform A 976-984, stimulated CD8$^+$ T cells from abacavir hypersensitivity patients.

Self-peptide binds HLA-B*57:01 >30,000 fold better in the presence of abacavir

Crystals of HLA-B*57:01 bound to abacavir and VTTDIQVKV formed by hanging drop
X-ray data of HLA-B*57:01 complexed to abacavir and self peptide
Crystal structure of VTTDIQVKV SPT5a 976-984 bound to abacavir and HLA-B*57:01
Does abacavir bind HLA-B*57:01 with the same orientation with different peptides?
Hydroxyl group of abacavir shows alternate conformations
Hydroxyl group of abacavir shows alternate conformations.
4 crystal structures show H bonds between abacavir hydroxyl group and ordered water molecules.
HLA-B*57:01 Ser70, peptide carbonyl and ordered water molecules form a network H bonded to abacavir hydroxyl group.
Do different peptides adopt alternate conformations when bound to abacavir and HLA-B*57:01?
Crystal structure of VTTDIQVKV SPT5a 976-984 bound to abacavir and HLA-B*57:01

What are the TCR contact residues?

VTTDIQVKV SPT5a 976-984

P4 (Asp) and P8 (Lys)
Are there viral sequences similar to human VTTDIQVKV SPT5a 976-984?

<table>
<thead>
<tr>
<th>Sequence Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>VTQQAQVRL HSV1/2 230–238</td>
</tr>
<tr>
<td>VTTDSVRAL HSV1 12–20</td>
</tr>
<tr>
<td>VTTNIQTKV HIV–1 153–161</td>
</tr>
<tr>
<td>VTTDIQVKV SPT5a 976–984</td>
</tr>
</tbody>
</table>

This HIV-1 sequence was from NCBI, not from an abacavir hypersensitivity patient.
Do viral peptides bound to abacavir and HLA-B*57:01 resemble VTTDIQVKV SPT5a 976-984?

Colored by B factor

Colored sequence similarity between self and viral peptides

Blosum62 similarity values are: blue, 40–50, cyan, 50–60, green, 60–70, yellow, 70–90, orange and 90–100, red.
What are most the solvent exposed peptide side chains available for TCR recognition?

P4 (Asp) and P8 (Lys) in VTT\textit{DIQKV} SPT5a 976-984
P4 (Asp) and P8 (Lys) in VTT DIQVKV SPT5a 976-984
Sharing of TCR contact residues between self and viral epitopes may influence hypersensitivity.

In summary:

1) Viruses are capable of generating sequences with the potential to form stable complexes with drug and HLA.

2) Similarity in self/virus peptide TCR contact residues may influence crossreactive mechanisms driving T cell responsiveness.
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Why does carbamazepine induce severe cutaneous adverse reactions with different HLA associations?

**HLA-B*15:02 Asian**

**HLA-B*31:01 Caucasian**

Peptide elution studies show no strong alteration of peptide repertoire.

Canonical anchors retained at P2 and P9 in the presence of drug.

Drug does not interact structural pockets that accommodate anchor residues.

Different from abacavir.

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**Supplementary Table 5b. Summary of CBZ-induced repertoire shift**

Position specific changes in the amino acid usage of HLA-B*15:02 ligands after treatment with abacavir. Noteworthy changes in prevalence of these amino acids are shown. No significant change was observed for the anchor residues at P2 Leu and P9 Tyr/Phe.

<table>
<thead>
<tr>
<th></th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
<th>P7</th>
<th>P8</th>
<th>P9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp ↓</td>
<td>Leu remains dominant anchor residue</td>
<td>Ala ↑</td>
<td>Gly ↑</td>
<td>Ala ↑</td>
<td>Gly ↑</td>
<td>His ↑</td>
<td>Ser ↑</td>
<td>Tyr/Phe remain dominant anchor residues</td>
<td></td>
</tr>
<tr>
<td>Glu ↓</td>
<td>Asp ↓</td>
<td>Val ↓</td>
<td>Pro ↑</td>
<td>Asp ↓</td>
<td>Thr ↓</td>
<td>Lys ↓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln ↑</td>
<td>Cys ↓</td>
<td>Phe ↓</td>
<td>Val ↓</td>
<td>Ile ↓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Can structural interactions between drugs and the associated HLA molecules be defined with purified components?

1. Generate HLA complexes in the presence and absence of drugs.
2. Characterize HLA complexes in solution (gel filtration, SDS-PAGE, mass spectrometry, peptide sequencing).
3. Characterize drug binding sites with drug affinity responsive target stability (DARTS) assay.
4. Structure determination by X-ray crystallography.

Codon optimized pET plasmids
Transform BL21(DE3)pLysS
Grow 6-12 liters *E. coli*
Isolate inclusion bodies
SDS-PAGE
Refold + peptides
Peptides for refolding with HLA-B*15:02, HLA-A*31:01, HLA-B*58:01

Since the peptide sequence does appear to influence drug/HLA interactions, we selected peptides likely to bind each HLA molecule.

**HLA-B*15:02**
QLAGAGHSY Illing motif, not natural sequence
HLASSGHSY irf7 like 195-203, natural sequence
ELRAREESY HSV, T cell epitope
ELRAREEAY VZV, T cell epitope

**HLA-A*31:01**
RLRDLLLIAAR Env769-779, T cell epitope
RLAAAAAAAAAR poly-alanine with anchors for binding

**HLA-B*58:01**
KTINALVYF camelpox 11-19, T cell epitope
KTAAAAAAFF poly-alanine with anchors for binding
HLA-A*31:01 can be refolded with peptides +/- carbamazepine

Superdex 75

12 kD + 33 kD = 45 kD

RLRDLLLIAAR Env769-779, T cell epitope
RLAAAAAAAAR poly-alanine with anchors for binding
HLA-B*15:02 forms multimeric complexes when refolded with peptides +/- carbamazepine by non-denaturing PAGE.

Fractions from gel filtration of HLA complexes run on non-denaturing gel.

Higher molecular weight complexes with no drug, consistent with FPLC.

Next, mass spec and peptide sequence of multimers.

ELRAREESY HSV, T cell epitope
Can structural interactions between drugs and the associated HLA molecules be defined with purified components?

**Target identification using drug affinity responsive target stability (DARTS).**
*Proc Natl Acad Sci U S A. 2009 Dec 22;106(51):21984-9*

Takes advantage of a reduction in the protease susceptibility of the target protein upon drug binding

Universally applicable because it requires no modification of the drug and is independent of the mechanism of drug action

We are attempting the DARTS method to define the carbamazepine binding site on HLA-A*31:01.
Carbamazepine protects fragments of HLA-A*31:01 complexes from proteolysis

1 mg/ml complex
Add drug or vehicle control
Pronase at different concentrations
15 minutes RT
Stop reaction
SDS-PAGE

HLA-A*31:01

β₂-microglobulin

At 1:300 pronase with no drug, complete destruction of HLA
At 1:300 pronase with carbamazepine, fragments are protected

Next, DART with HLA-B*58:01 and oxypurinol

+ carbamazepine - carbamazepine
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Why does sulfamethoxazole have multiple HLA associations?

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<th>HLA</th>
<th>Reference</th>
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<tbody>
<tr>
<td>SJS/TEN</td>
<td>HLA-A29, -B12, -DR7</td>
<td>Roujeau 1986</td>
</tr>
<tr>
<td></td>
<td>HLA-B38</td>
<td>Lonjou, 2008</td>
</tr>
<tr>
<td>Fixed drug eruption</td>
<td>HLA-A30, -B13, -Cw6</td>
<td>Ozkaya-Bayazip, 2001</td>
</tr>
</tbody>
</table>
Does sulfamethoxazole have multiple HLA associations because different mechanisms are involved?

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1 Abacavir reactivity may need more time if loading onto HLA-B*57:01 occurs inside the endoplasmic reticulum (see text).
Can effects of sulfamethoxazole on peptide/HLA binding affinity be measured?

Mechanistic insights in T cell recognition of sulfamethoxazole may be learned from structures of drug-specific TCRs complexed to drug/peptide/HLA complexes.

Few drug specific TCR transfectants have been characterized, but there are several sulfamethoxazole specific TCRs restricted to HLA-DR1.

Can effects of sulfamethoxazole on peptide/HLA-DR1 binding affinity be measured?

“The B-LCL used as APC could be either autologous or MHC-matched: concerning the TCR transfectants analyzed in this study, HLA-DR1-matched B-LCL were sufficient to activate the transfectant cells and induce IL-2 production (data not shown). However, if B-LCL with unmatched HLA-DR (e.g., HLA-DR7/13) were used, no IL-2 production was seen. ”

Is sulfamethoxazole buried in the antigen binding cleft or bound to the solvent accessible surface?

Model of sulfamethoxazole docked to NGTLNGLDYDDYVYP and NGTLNGLDADDYVYP complexed to HLA-DR1
Is sulfamethoxazole buried in the antigen binding cleft or bound to the solvent accessible surface?

Model of sulfamethoxazole docked to NGTLNGLDYDDYVYP and NGTLNGLDADDYVYP complexed to HLA-DR1.
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Is sulfamethoxazole buried in the antigen binding cleft or bound to the solvent accessible surface?

Model of sulfamethoxazole docked to NGTLNGLDYDDYVYP and NGTLNGLDAADDYVYP complexed to HLA-DR1
Can crystallizable complexes of sulfamethoxazole bound to peptide/HLA-DR1 be generated?

TCR αβ from transfectants + HLA-DR1 complexed to FVNPVEAFQFKFELL and sulfamethoxazole (Kd=79 nM)


Schmid DA¹, Depta JP, Lüthi M, Pichler WJ.
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Summary

1. Abacavir binds in the cleft of HLA-B*57:01 in 4 crystal structures. Peptides can be identified from self and viral peptides that bind HLA with higher affinity in the presence of the drug.

2. HLA-A*31:01 and HLA-B*58:01 form standard HLA monomers +/- carbamazepine or oxypurinol.

3. Carbamazepine appears to alter HLA-B*15:02 complex formation.

4. Sulfamethoxazole enhances the affinity of specific peptides for HLA-DR1.
Thank you organizers of this meeting!
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The Structural and Functional basis of HLA-Associated Drug Hypersensitivity

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