Drug hypersensitivity (DH): classification based on drug-binding & drug-target

Werner J Pichler
BERN,
Switzerland

Conflict of interest statement
ADR-AC GmbH has been involved in Consultation & Funding in 2018/2019 by following companies:
Alnylam, Astellas, Bayer, Becton-Dickinson, Biomarin, Galapagos, Roche, Teleflex
CLASSIFICATION of Drug Hypersensitivity Reactions (DHR)

Timing
<1->6 hr

+ immune mechanism
Gell & Coombs I-IV a,b,c,d

+ mode of action/
drug binding

Does the drug form a new antigen (covalent hapten-carrier conjugate) or does it bind via non-covalent links to receptors, enzymes, ...?
Classification of adverse drug reactions

Type A

Type B = DHR

85%

≈15%

- action of drug (on & off target)
- dose dependent
- predictable

Drug hypersensitivity reactions (DHR)

(= Type B reactions)

Allergic-immune (hapten)

P-i pharmacological interaction with immune receptors

Pseudo-allergic
Classification of DHR based on

a) **how drugs bind to proteins** (covalent vs non covalent bonds)

b) **to which proteins**

- **Covalent bonds** between drug and protein: Formation of a new antigen; Classical, allergic DHR

- **Drugs bind directly to HLA or TCR** (van der Waals, electrostatic, OH bonds)

- **Drugs bind directly to receptors & enzymes of effector cells, no real allergy**

- **T- & B-cells:**
  - IgE: Anaphylaxis, urticaria
  - T cells: MPE
  - IgG: haemolytic anaemia, immune-complex d.

- **Always only T cells:** MPE, DiHS/DRESS, SJS/TEN, AGEP, hepatitis …

- **MRGPRX2/Mast cells**
  - anaphylaxis/urticaria
  - Cyclooxygenase↓/
  - Leukotriens↑, bronchospasm, asthma, urticaria

- **Bradykinin↑:** angioedema
Classification of DHR based on

a) **how drugs bind to proteins** (covalent vs non covalent bonds)

b) **to which proteins**

---

**Immune Mechanism**

Allergic-immune (hapten)

**Pharmacology**

“off target activities” on immune* & inflammatory cells

*) p-i: off target is too simple, as the clinic is always due to T cell reactions, even if drug bound to APC!
amoxicillin

\[
\begin{align*}
&\text{NH}_2 \\
&\text{HO} \\
&\text{OH} \\
&\text{CO}_2\text{H}
\end{align*}
\]


down arrow

hapten/allergic (covalent)

carbamazepin

\[
\begin{align*}
&\text{O} \\
&\text{C} \\
&\text{N} \\
&\text{NH}_2 \\
&\text{CO}_2\text{H}
\end{align*}
\]

down arrow

pharmacologic-immune (p-i)

ciprofloxacin

\[
\begin{align*}
&\text{F} \\
&\text{N} \\
&\text{C} \\
&\text{O} \\
&\text{C} \\
&\text{O} \\
&\text{H}
\end{align*}
\]

down arrow

pseudo-allergic (MasRPGPRX2)
amoxicillin

hapten/allergic (covalent)

pharmacologic-immune (p-i) (TCR, HLA)

pseudo-allergic (MasRPGPRX2)

carbamazepin

ciprofloxacin
Drugs elicit distinct DHR via allergic or p-i mechanism

Flucloxacillin: Exanthem, hepatitis (B*57:01)
Sulfamethoxazole: Exanthem, DiHS/DRESS (p-i TCR)
Piperacillin: Exanthem, DiHS/DRESS

Big difference of the same drug acting via hapten or p-i regarding clinic, HLA-linkage, dose dependence, diagnosis. p-i causes more severe reactions!
How can one differentiate allergic, p-i and pseudoallergic reactions in clinical practice?

1. **experience with drug:**
   - some drugs can elicit only T cell reactions: CBZ, phenytoin, abacavir, allopurinol .... → p-i
   - other drugs are known for pseudoallergy (ACE-inhibitors, NSAID)

2. **Clinical picture/immune reaction:**
   - T cell reactions: allergc (hapten) and/or p-i antibody and T cell types of immunity → hapten
   - Severity of T cell reactions (DiHS/DRESS and SJS/TEN) → p-i, AGEP: p-i > allergic (?), MPE allergic (hapten) and p-i
   - HLA-linked reactions: → p-i HLA oligo/monoclonal T cells: p-i
   - ? Eosinophilia typical for p-i?, abnormal signalling to T cell? LAT bybassed?

3. **research:**
   - In vitro analysis (how drugs stimulate T cell lines, processing dependent or not, .....
<table>
<thead>
<tr>
<th>Hapten</th>
<th>p-i</th>
<th>pseudoallergy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>beta-lactam antibiotics:</strong> Penicillin, amoxicillin, piperacillin, flucloxacillin, cephalosporins, carbapenems, monobactam; <strong>reactive metabolites</strong> like sulfamethoxazole-NO</td>
<td><strong>Anti-epileptics:</strong> carbamazepin lamotrigine, phenytoin <strong>sulfanilamides</strong>; sulfapyrin, sulfamethoxazole, <strong>antibiotics:</strong> Flucloxacillin, amoxicillin, piperacillin, tazobactam, cephalosporin, vancomycin <strong>quinolones</strong> (ciprofloxacin, moxifloxacin, norfloxacin, ..) <strong>radio-contrast media:</strong> iomeprol, iodihexol, ..<strong>local anaesthetics:</strong> lidocain, mepivacain <strong>varia:</strong> allopurinol, oxypurinol metamizole; dapson, ..</td>
<td><strong>NSAID</strong> (acetylsalicylic acide, diclofenac, mefenamic acid, ibuprofen, etc <strong>muscle relaxants:</strong> rocuronium, suxamethylcholin, etc… <strong>quinolones:</strong> ciprofloxacin, moxifloxacin, norfloxacin, .. <strong>radio-contrast media:</strong> iomeprol, iodihexol,… <strong>ACE inhibitors:</strong> Enalapril, lisinopril,…</td>
</tr>
</tbody>
</table>

Only a few drugs are **proven** haptens eliciting an immune response
Summary 1: Classification

- Allergic-immune, p-i, pseudoallergic reactions
  
  All G&C  
  strong T cell  
  typical clinic & drug

- Some drugs can act via allergic or p-i, or allergic or p-i or pseudoallergic mechanism, resulting in distinct clinical manifestations
  
  (e.g. p-i → severe, allergic → mild exanthem)

- Not the drug, but **HOW** the drug binds to proteins and to **WHICH PROTEINS** determines the clinic
Pharmacological interaction with immune receptors

p-i

Drugs binds to immune receptors according to pharmacological rules

The consequence of this drug binding (pharmacology) is an unusual T cell reaction
T cell mediated DHR: interaction between drug, TCR and HLA

Cytotoxic T cell killing keratinocytes

T cell receptor (TCR)

HLA/peptide

HLA/MHC

APC/keratinocyte
Pharmacological interaction with immune receptors (p-i)

a) p-i TCR: the drug binds to the TCR (by non-covalent bonds; not restricted to a HLA-allele)

or

b) p-i HLA: the drug binds to the HLA molecule (NOT to the presented peptide); 
\{HLA+drug+peptide complex\} is recognized by the TCR

TCR >10^{11}/individual

HLA >10.000 population
p-i HLA:

a drug fits into a HLA molecule (e.g. peptide binding groove using van der Waals forces, electrostatic interactions, OH bonds, etc). The drug may fit only in one unique HLA-allele (e.g. abacavir into B*57:01 → HLA-restriction). The \{HLA-drug-peptide\} complex is then recognized by the TCR.

HLA-B*5701:
binding groove for abacavir

Illing et al, Nature 2012
p-i HLA:
The drug binds to the peptide binding groove of HLA

a) It is hidden under the peptide; the T cell reaction is directed against the altered HLA-drug-peptide complex, which looks like an allo-HLA, NOT against the drug.

b) If abacavir binds to HLA inside the cell, novel peptides might be presented («altered peptide repertoire»)

c) The (CBZ) may bind beside the peptide to the HLA and then may directly interact with the TCR as well: this may result in oligoclonal expansions (SJS)
**B*57:01 plus Abacavir ≈ B*58:01**

Abacavir binding to HLA B*57:01 transforms B*57:01 to a molecule looking like HLA-B*58:01

T cells react to abacavir-HLA-peptide complex as to an allo-allele (B*58:01)

4.7-5.2% of abacavir reactive T cell clones of various B*57:01+ donors react with B*58:01

p-i HLA in DHR ≈ allostimulation severe symptoms because drugs can change your identity

• The T cell repertoire contains ca 20% of allo-reactive T-cells able to react DIRECTLY with allo-alleles (e.g. HLA-B*58:01 in a HLA-B*57:01 + individual); in clinic, an acute graft vs host disease, e.g. bone marrow transplantation, may occur

• The HLA-molecule is your identity card! Binding of drugs (abacavir, oxypurinol, dapsone, flucloxacillin, …..) to the HLA-peptide complex changes your identity and make the HLA-drug-peptide complex look like an allo-allele: Symptoms like in acute graft versus host disease appear: massive MPE, SJS/TEN, DiHS/DRESS
Model for p-i TCR: Sulfamethoxazole & other sulfanilamides

Generation of sulfamethoxazole specific T cell clones (H13, 1.3, ..... ) & TCR hybridomas
**p-i TCR:** drug interacts with T cell receptor of Sulfamethoxazole stimulated T cell clones (TCC, «H13» & «1.3»)

SMX-specific Clone 1.3:

SMX binds to a unique site on the **CDR3-α** loop of the SMX specific **TCR 1.3**
Orientation of sulfanilamide in TCR interaction: stimulatory (1/12) or blocking (11/12)

11/12 sulfanilamides (SDZ, SMP, SPD,...) were binding, but not stimulatory
TCC stimulation by SMX – it could be blocked by the 11 other sulfanilamides, (proliferation and/or Ca++ influx).
These 11 other sulfanilamides bound to the same site, but were not activating the TCR/TCC (their NH2 ending was directed to TCR)
p-i TCR: drug interacts with T cell receptor of sulfamethoxazole stimulated T cell clones (TCC, «H13» & «1.3»)

Drug binds to CDR2: allosteric effect (H13)

St. Watkins & WJ Pichler, OJI, 2013
Drug (SMX) binding to the TCR-Vβ CDR2 loop of TCC H13

6 of 12 analyzed sulfanilamides (SMX and 5 other) fit into the pocket formed by the CDR2 region of TCR H13. The same 6 SA were stimulatory (Ca influx, proliferation, cytokine secretion)

S Watkins & W J. Pichler: Activating Interactions of Sulfanilamides with T Cell Receptors, Open J Immunology, 2013
Visualizing the Binding of SMX to TCR H13

Molecular dynamic simulations of TCR H13 and HLA-DR*10:01 with SMX binding
Allosteric effect of SMX binding to CDR2-Vβ pocket of TCR H13

TCR H13 binds with 7 fold higher affinity to HLA-DR B1*10:01 and laminin peptide than without drug
The interaction of sulfanilamides with the «TCR» shows that the drug interaction with immune receptors (p-i TCR) follows the rules of «normal» drug interactions with receptors:

- drug binds to these immune-receptors
- It can or cannot result in signalling
- Binding by the drug can even inhibit the signal transmission induced
  - by other drugs
  - or other ligands (e.g. allostimulation)
- The drug binding to TCR can have an allosteric effect on the TCR, which enhances interaction with «normal» peptide/HLA

Drugs are potential tools to interfer with the specific immune system
the SMX binding site is on the constant part of TCR, namely on Vβ20.1, which is used in ca. 1-3% of all TCR

Why is not every human reacting to SMX?

T cell reactivity is an important cofactor
Some drug hypersensitivity reactions depend on **cofactors** like strong T cell activation during generalized herpes-virus or HIV infections.

**Sulfamethoxazole**

HIV positive

~ 3% exanthem

All carry TCR-Vβ20.1

~ 50% «exanthem»

*Cofactor: viral activation*
Important cofactors for clinical manifestation of p-i reactions (DH):
the threshold of T cell activation (e.g. p-i TCR) is reduced by
prior T cell activation: risk for DH ↑

- **Viral infections in childhood:**
exanthem after penicillins

- **Generalized virus infections (EBV, CMV, HIV,...):** exanthem after SMX,
aminopenicillins,…

- **Some severe autoimmune diseases: active SLE, Mb Still:**
exanthems after drugs

- **gvh disease**

- **therapy with checkpoint inhibitors (??)**

- **generalized drug hypersensitivity:**
  flare up reactions, multiple drug hypersensitivity
What is the functional consequence of p-i?

Direct allo-like immune stimulations,
Which is «outside» the «normal» rules of autologous immune-stimulation:

• «uncontrolled» immune reaction;
  dendritic cells are bypassed, with activation of both, naive and memory cells; polyclonal (oligoclonal) cytotoxic immune reaction

• some crossreactivity of allo-activated T cells with self HLA+peptides; persistent activation

→ SJS/TEN, DiHS/DRESS: if analyzed: p-i; HLA restriction; bizarre, gvhd like symptoms
  Often transient symptoms after drug was stopped;
  Sometimes persisting symptoms after stop of drug:
  multiple drug hypersensitivity (MDH)
**Multiple drug hypersensitivity (MDH)**

MDH is a syndrome that develops as a consequence of massive T cell stimulations (p-i, allo-like). It is characterized by new drug hypersensitivity reactions to novel drugs occurring months to years after acute DHR.

MDH develops in ca. 15% of all patients with DiHS/DRESS. It is a drug induced chronic disease, similar to *chronic* graft versus host disease (chronic gvhd). It is characterized by permanently activated, CD4+CD25^{dim}, CD38^+ & PD-1^+ expressing T cells in the circulation in absence of drug therapy.
Clinical course of MDH

1st drug or drug combination

DiHS/DRESS, severe exanthem, bullous IgA dermatosis, ........

2nd drug or drug combination

Exanthem, DiHS/DRESS, erythrodermia, agranulocytosis, fulminant hepatitis, necrotizing carditis, SJS/TEN.....

SJS/TEN are NOT! risk diseases for MDH
Epicutaneous skin tests: positive to distinct drugs in T cell assay

Clinic:
1. DRESS after phenytoin;
   Three months later:
2. Severe MPE and erythrodermia after amoxicillin

Reactive (48hr) to phenytoin and amoxicillin
<table>
<thead>
<tr>
<th>Drug</th>
<th>µg</th>
<th>Autol. Plasma</th>
<th>AB-Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SI</td>
<td>SI</td>
</tr>
<tr>
<td>Phenytoin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>1.7</td>
<td>11.1</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>3.0</td>
<td>50.2</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>7.1</td>
<td>48.6</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td></td>
<td>2.3</td>
<td>19.9</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>48.1</td>
<td>124.7</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>22.2</td>
<td>57.6</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>22.8</td>
<td>51.4</td>
</tr>
<tr>
<td>Zonisamide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td></td>
<td>1.1</td>
<td>0.9</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>2.9</td>
<td>2.4</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>4.8</td>
<td>11.8</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>11.4</td>
<td>24.7</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td></td>
<td>1.1</td>
<td>0.9</td>
</tr>
<tr>
<td>0.1</td>
<td></td>
<td>1.1</td>
<td>2.0</td>
</tr>
<tr>
<td>1.0</td>
<td></td>
<td>1.1</td>
<td>0.9</td>
</tr>
<tr>
<td>10.0</td>
<td></td>
<td>0.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Clonazepam</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td></td>
<td>1.1</td>
<td>2.0</td>
</tr>
<tr>
<td>0.1</td>
<td></td>
<td>0.9</td>
<td>2.1</td>
</tr>
<tr>
<td>1.0</td>
<td></td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>10.0</td>
<td></td>
<td>0.9</td>
<td>4.1</td>
</tr>
<tr>
<td>Tetanus pos. control</td>
<td>5</td>
<td>19.3</td>
<td>147.3</td>
</tr>
<tr>
<td>Neg. Control (cpm)</td>
<td>-</td>
<td>1455</td>
<td>402</td>
</tr>
</tbody>
</table>

Example of **T cell stimulation in vitro (LTT)** in a patient with sequential MDH: Recurrent DRESS with SJS overlap symptoms following therapy with anticonvulsants (Phen, CBZ) and **8 years** later massive vesicular/bullous exanthema to Zonisimide and Levofloxacine exposure.

**Legend:**
Result of LTT with drug stimulated cell-cultures supplemented with 10% autologous plasma or 10% AB-serum. Positive results are bold; SI: stimulation index: cpm with drug / cpm control.
Pathomechanism

In MDH, the activation of T cells becomes *permanent*, like in a chronic virus infection or chronic gvh disease.

In “normal” DH, no permanent activation measurable.

*Pichler w et al, IACI, 2016*

*Daubner B et al, Allergy 2012 Jan;67(1):58-66*
Summary and conclusion

• DHR can be classified into allergic-immune, p-i and pseudoallergic DHR.

• The same drug can elicit different DHR dependent on type of binding and type of affected protein: allergic or p-i or pseudo-allergic reactions. This results in distinct clinic.

• The pharmacology of p-i DHR reveals «usual» effects of drugs on receptors (signalling, blocking, allosteric effects). Unusual is the type of receptor, namely HLA and TCR: the immunological consequences of p-i are unusual immune stimulations, similar to direct allostimulations. The clinical result is a dangerous disease like DIHS/DRESS, SJS/TEN. Some of the immune changes can even lead to permanent diseases (MDH).

• *For the near future*, the new findings should have an impact on understanding, preventing and treating DHR

• *For the distant future*, the findings may result in a new area of immunology: «immunology of small molecules» and possibly in novel therapies.
Thank you very much