Exanthematous drug eruptions

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Disclosure slide

• **Owner** of **adr-ac GmbH**, a company devoted to drug hypersensitivity analysis and research

• **Consultant** for Pfizer, Hoffmann LaRoche, Böhringer-Ingelheim, Novartis, Menarini, Aicuris, Swatch

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Exanthematous drug eruptions

- «rashes»
- Urticaria immediate reactions
- Delayed appearing exanthems with cell infiltration
  it is frequent - antibiotics (0.5 - 8% of treated)
    - antiepileptics
    - allopurinol, diuretics,
      antivirals, ........
indapamid
Drug allergy

1) What has happened?

2) Mechanism & how severe is the drug induced illness?

3) Which drug is responsible?

Danger signs:
- timing
- clinical
- laboratory

Skin testing
- lymphocyte stimulation tests
- provocation tests (?)

Factors:
- history
- drug exposure
- cofactors
- viral infection
- previous drug allergies
Delayed reaction: it is T-cell mediated

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>No symptoms</td>
<td>symptoms</td>
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Few precursor cells ... Expansion.... Symptoms arise if a certain amount of specific T-cells is homing to the tissue and exerts effector function
Maculo\textit{papular} drug exanthem

CD4$^+$

CD8$^+$
Delayed reaction

Symptoms arise if a certain amount of specific T-cells is homing to the tissue and exerts effector function.
maculopapular exanthema

- mild
- moderate
- massive, confluent

25% have liver involvement
The function of effector T cells determines clinical phenotype:

MPE pustular bullous

cytotoxic
IFNg
IL-13
IL-5
IL-8/GM-CSF

granzymeB+
perforin+
cytotoxic

precursor cells
effector cells
bullous Exanthem:
Perforin+ and GranzymB+
T cells infiltrate epidermis
Cytotoxic T cells kill keratinocytes

- Keratinocyte cell necrosis
- Hydropic degeneration
- Eosinophils
- Mononuclear cell infiltrate

Diagram:
- Keratinocyte
- Drug-specific CD4+ T (CD8+ T) cell
- MHC II
- TCR
- LFA-1
- iCD54
- Granzyme B
- Perforin

Cytotoxic T cells interact with keratinocytes through the MHC II and TCR pathway, inducing keratinocyte cell necrosis. Eosinophils and mononuclear cell infiltrate are also present in the tissue.
Acute generalized exanthematous pustulosis (AGEP)
AGEP
a T cell reaction recruiting PMN

FIRST T cells
T cell infiltration into epidermis (cytotoxic and IL-8/GM-CSF): vesicle

SECOND PMN accumulation pustule

later PMN

CD4  CD8  NEUTROPHIL ELASTASE

NEUTROPHIL ELASTASE
T-cells react with a drug, are stimulated and expand: they organize a certain pathology

**Drug, e.g. amoxicillin**

**bullous E**

MHC-I (+ MHC-II)

CD8+ > CD4+

cytotoxicity (CD8+)

IFNγ; IL-5

MPE

MHC-II

CD4+

cytotoxicity (CD4+)

IL-5; IFNγ

AGEP

MHC-II + I

CD4+ & CD8+

cytotoxicity

IL-8; IL-5
## Classification of drug-hypersensitivity reactions

<table>
<thead>
<tr>
<th>TYPE IV a</th>
<th>TYPE IV b</th>
<th>TYPE IV c</th>
<th>TYPE IV d</th>
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</thead>
<tbody>
<tr>
<td>Th1</td>
<td>Th2</td>
<td>Cytotoxic T cells</td>
<td>T cells</td>
</tr>
<tr>
<td>IFN-γ, TNF-α</td>
<td>IL-5, IL-4, IL-13, eotaxin</td>
<td>Perforin, granzyme B, FasL</td>
<td>CXCL-8, GM-CFS IL-17 (?)</td>
</tr>
<tr>
<td>Monocyte, Macrophage</td>
<td>Eosinophilic inflammation</td>
<td>Cytotoxic T cells</td>
<td>Neutrophils</td>
</tr>
<tr>
<td>Tuberkulin skin test, (Contact dermatitis)</td>
<td>Maculopapular exanthem with eosinophilia</td>
<td>Contact dermatitis Maculopapular, Bullous exanthema</td>
<td>Pustular exanthema</td>
</tr>
</tbody>
</table>
Time of appearance of delayed skin reactions

- **AGEP (amoxicillin)**: ~day 2-5
- **MPE**: day ~7-11
- **SJS/TEN**: day ~10 - >24 (allopurinol, SMX)
- **DRESS**: day ~12 - >50 (antiepileptics)
Severity? Danger signs – delayed reactions

Clinic
- widely spread exanthema
- induration, bullae, pustules
- erythrodermia
- pain in skin
- Nikolsky sign
- mucosal involvement
- lymphadenopathy
- fever
- general symptoms / malaise (liver, kidney, lung, pancreas)

Laboratory
- differential blood count (eosinophilia, activated lymphocytes)
- ALAT, ASAT, γGT, AP
- (CRP ↑~; Creatinine)

*delayed reactions: certain laboratory examinations are helpful and necessary*
Danger sign: facial edema, flash

DRESS

DRESS and haematophagocytic syndrome

TEN
Danger sign: mucosal involvement

Stomatitis
(SJS, TEN, DRESS)

Conjunctival involvement
SJS, TEN

Pictures by A Bircher, Basel
Severity? Danger signs – delayed reactions

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delayed reactions: certain laboratory examinations are helpful and necessary
Danger sign (laboratory): atypical lymphocytes, eosinophilia

„atypical lymphocytes“
(= activated CD8+ T-cells) in the blood at massive immune reactions
(e.g. generalised drug allergy, acute EBV und HIV-infection, acute Still syndrom...)

eosinophilia (>0,6G/l) is common (~50%) & typical for delayed drug hypersensitivity
SUMMARY: Exanthematous drug eruptions

1. Are T cells reactions

2. **Timing:** Appear between 2 d (AGEP) and >50 d (DRESS) of drug exposure

3. One **differentiates** papular [MPE], pustular [AGEP], bullous [SJS] (*and* macular / urticarial ....) exanthems

4. determine the **severity** of MPE by clinical and laboratory signs
HOW ARE DRUGS STIMULATING T CELLS?
Maculopapular drug eruption (MPE) - Immunohistology

T-cell infiltration into dermis, epidermis; cytotoxicity (killing of keratinocytes) recruitment of inflammatory cells

Cytotoxic CD4+ T cell
How are drugs stimulating T cells?

Cytotoxic T cell killing keratinocytes

T cell

TCR

peptide

HLA/ MHC

APC

HLA
pharmacological interaction with immune receptors (p-i) concept:

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

or

b) the drug binds to the HLA molecule (NOT to the presented peptide); the \{HLA-drug + peptide complex\} is recognized by the TCR
Pharmacological interaction with immune receptors = \textit{p-i concept}

It is a non-covalent binding of drugs to proteins functioning as immune receptors (TCR, HLA);

It explains an immune stimulation by a drug without postulating antigen-features of a drug!
p-i HLA: binding of drug to HLA molecule

Not the peptide (the «antigen»),

but the HLA molecule itself is modified
p-i concept: a drug fits into a particular HLA molecule

the drug binds to an allele-typic region in the HLA by van der Waals forces;
the \{HLA-peptide-drug\} complex is then recognized by the TCR

HLA-B*5701: binding groove
For abacavir

Illing et al, Nature 2012
p-i TCR: binding of drug to T cell receptor (TCR)
p-i TCR: T cell clones specific for sulfamethoxazole (SMX):

- **cross-reactivity**
- **inhibition** of SMX stimulation by other sulfanilamides \( (n = 11) \)
- **docking &**
- **dynamic modelling**
Two SMX specific T-cell clones «H13» & «1.3»

1.3: only SMX; 11 other sulfanilamides (SA) not stimulatory

H13: SMX and 5 other SA stimulatory
SMX-specific Clone 1.3:

SMX binds to a **unique** site on the CDR3-α loop of the SMX specific TCR 1.3

*St. Watkins & WJ Pichler, OJI, 2013*
75% inhibition of SMX induced proliferation by the sulfanilamide SMT (sulfamethazole)

35% inhibition of SMX induced Ca++ influx by sulfanilamides
The TCR 1.3 showed CDR3α recognition of SMX.

The NH2 of SMX may contact the peptide.

This may explain the cross-reactivity of some TCC reactive with hapten (SMX-NO) and via p-i (SMX)*

SMX is bound to TCRVβ2 of TCR «H13», outside the HLA-peptide interaction site!

St Watkins & WJ Pichler: Sulfamethoxazole Induces a Switch Mechanism in T-Cell Receptors Containing TCRVβ20-1, by Altering pHLA Recognition; PLOS One, 2013
Drug (SMX) binding to the TCR-Vβ CDR2 loop. Only SMX and 5 of 11 other sulfanilamides fit into the pocket formed by the CDR2 region (TCR H13)

Stephan Watkins & Werner J. Pichler: Activating Interactions of Sulfanilamides with T Cell Receptors, Open J Immunology, 2013
Visualizing the H13 Binding Process

MD simulations of TCR H13 and HLA-DR*10:01 with or without SMX binding

The analysis of motions reveals a “switch”, where the TCR constant domain either sits on the TCRVβ (above), changing to the TCRVα and a change from mostly Vβ recognition of the HLA and peptide, to a Vα recognition of the HLA.
Gibbs Free Energy, $\Delta G$

- Free energy change is the most straightforward of the parameters

- For H13 it was shown SMX caused a **7 fold increase in affinity**, from -24 to -140 kcal/mol.

- This translates from a 2 $\mu$mol to a 0.79 $\mu$mol affinity.

* Normal TCR affinities are in the range of 5-1 $\mu$mol, however we know the H13 T cell only proliferates with SMX present.
Allosteric effect of SMX binding to CDR2-Vβ pocket of TCR H13

Stephan Watkins & Werner J. Pichler: Sulfamethoxazole Induces a Switch Mechanism in T cell Receptors Containing TCRVβ-20-1 Altering pHLA Recognition, PLOS ONE, in press
Two types of p-i TCR

A) A small molecule binds to a region on the TCR free from contact with the pHLA or other proteins-protein interfaces and the resulting complex can bind the pHLA through induced TCR conformations (Watkins S & Pichler WJ, Plos One 2013).

B) The CDR3α or β recognizes a small molecule, and the resulting complex can then bind the pHLA, with the small molecule acting as part of the TCR (Watkins S & Pichler WJ, Open J Immunol, 2013).

-In either, there is a dependence on a particular pHLA, but the effect is mediated by the TCR binding the small molecule.
SUMMARY II: p-i concept

1. p-i: pharmacological interaction of drugs with immune receptors
2. one differentiates between p-i TCR and p-i HLA
3. It explains T-cell reactivity to drugs without implying antigenic features of the drug
4. most severe reactions appear to be due to p-i, which is an off target activity of the drug on (selected) immune receptors (TCR, HLA)
5. In contrast to previous beliefs, an interaction of small molecules with the immune system is common, and needs to be better investigated
6th DRUG HYPERSENSITIVITY MEETING (DHM6)
in
BERN, SWITZERLAND
APRIL 9th – 13th, 2014
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Literature to drug eruptions


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Yun J, Marcaida MJ, Eriksson KK, Jamin H, Fontana S, Pichler WJ, Yerly D. Oxypurinol directly and immediately activates the drug-specific T cells via the preferential use of HLA-B*58:01. J. Immunol., under revision