ADR-AC Symposium May 12th, 2022

***fake* antigen – the *true* cause of drug induced anaphylaxis**

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Bern Switzerland

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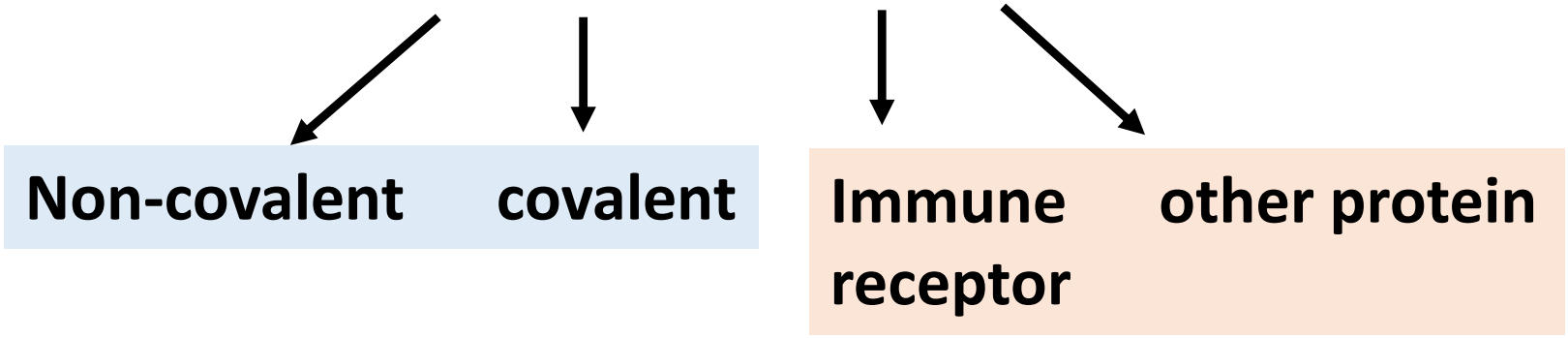
Adverse Drug Reactions
Analysis & Consulting

Conflict of interest statement

WJP has been involved in Consultations & Funding in 2020-2022 by following companies: argenX, Staten, Roche

To understand DRUG HYPERSENSITIVITY,
you have to start with the beginning…..
Is the drug acting as antigen or as drug?[1)]

It`s all about drug binding to proteins
how and where*



Pichler WJ, Hausmann O. Classification of Drug Hypersensitivity into Allergic, p-i, and Pseudo-Allergic Forms. Int Arch Allergy Immunol. 2016;171(3-4):166-179.

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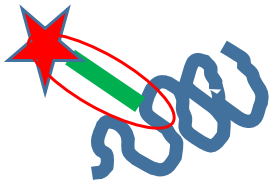
it`s all about drug binding to proteins: how and where

- ***Drugs:*** *most drugs do not bind by covalent bonds to proteins; but they bind constantly by non-covalent bindings to proteins like HSA, transferrin, Ig,..*

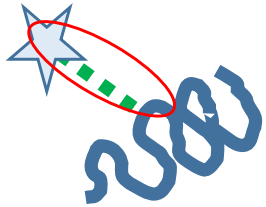
- **Some drugs are haptens:** *Haptens are relatively small molecules that can bind by covalent means to proteins. The new complex is stable & big enough to elicit an immune response*

- **Carrier/protein:** *e.g. human serum albumin (HSA), transferrin, cell surface proteins like ICAM, HLA, ….*

- **Covalent bonds**: *A covalent bond, also called a molecular bond, is a chemical bond that involves the sharing of electron pairs between atoms; very stable (–); new antigen*



- **Non-covalent interactions** *rely on electrostatic interactions, ion pairs, hydrogen pairs … Even though they are weak individually, the cumulative energy of molecular interactions is significant (Kd: $10^{-5 \text{ to } -7}$M) (····); may form "fake antigen"*



Drugs bind to proteins……

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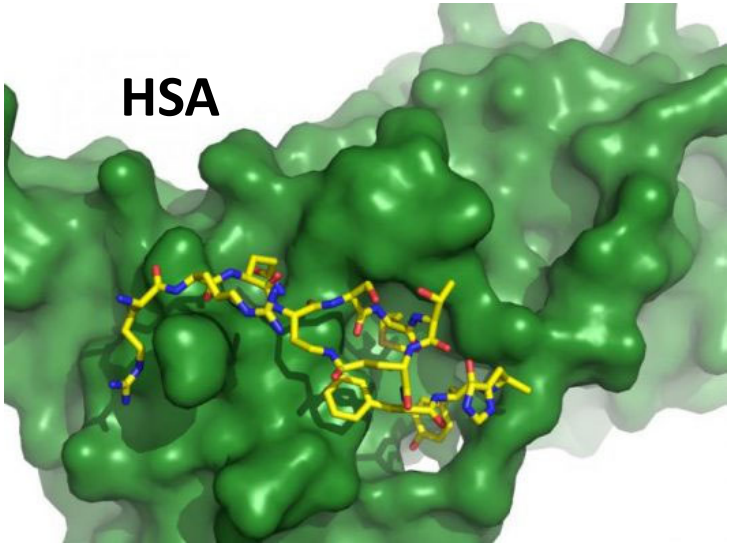
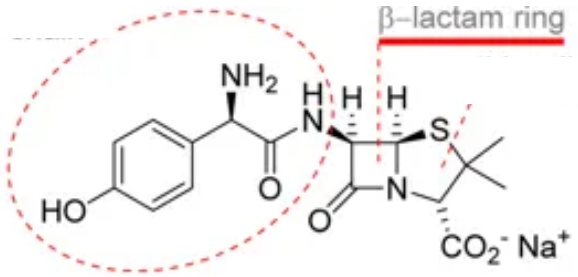
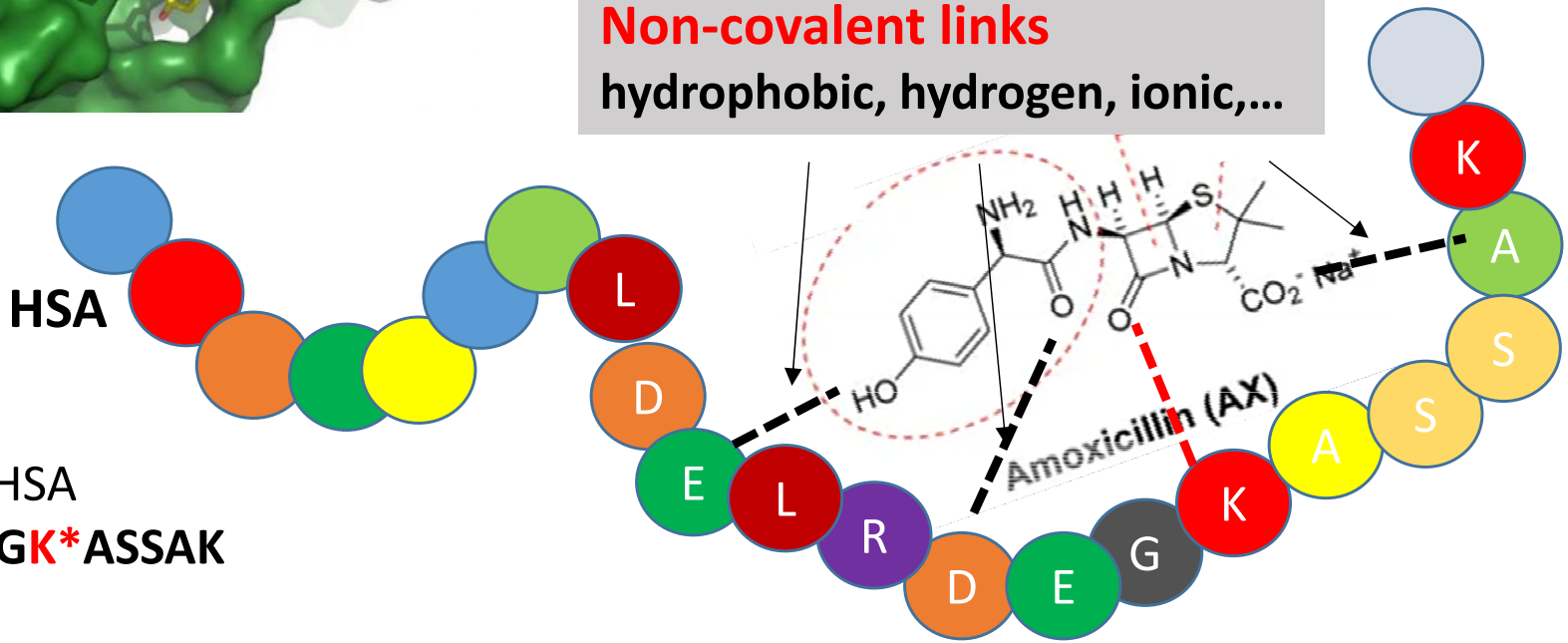
First	later
• Drugs bind to proteins constantly: **non-covalent bindings**	• **Haptens** are drugs able to bind to proteins by **covalent bonds**
• Labile	• Stable
• Fast, **seconds/minutes**	• Slow, **hours**
• Reversible	• Not-reversible
• **Ignored** by the immune system	• **New antigen** for immune system
• all drugs, including haptens / β-lactams	• Few drugs (e.g. β-lactams)





Amoxicillin (AX)

Non-covalent links
hydrophobic, hydrogen, ionic,…



Pos 190/HSA
LDELRDEGK*ASSAK

Non-covalent binding *is the dominant type of inter-molecular force in supra-molecular chemistry. These* ***non-covalent interactions*** *include - ionic* ***bonds*** *- hydrophobic* ***interactions*** *- hydrogen* ***bonds*** *- van der Waals forces and dipole–dipole* ***bonds.*** *Drugs – including those able to bind by covalent bonds – bind* ***first*** *via non covalent bonds to proteins*

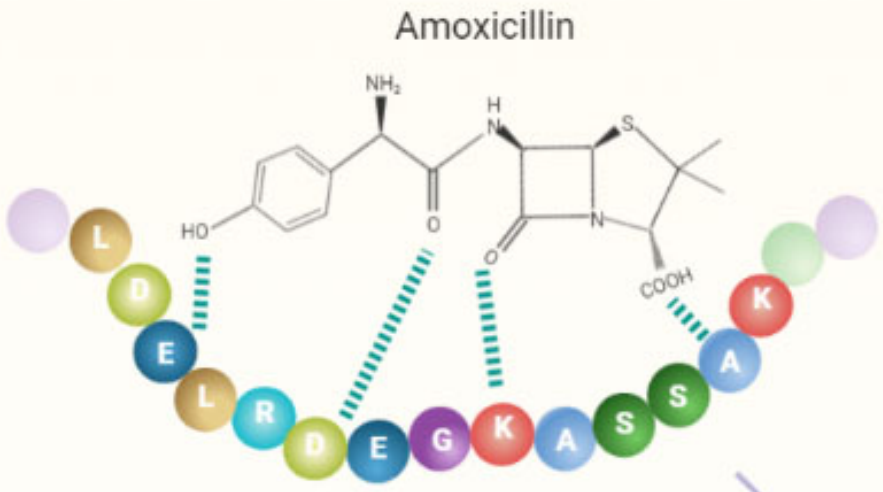
STEP 1
(fast)

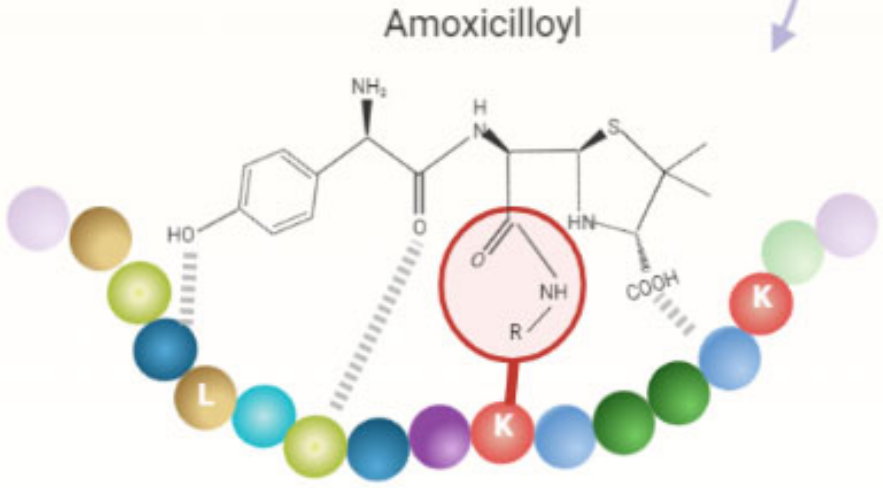
non-covalent binding

STEP 2
(slow)

covalent bond

———



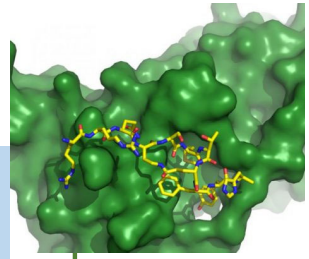


FAST

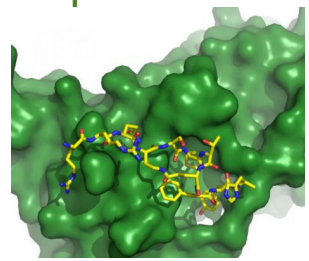
MINUTES

SLOW

HOURS



LOOK IDENTICAL



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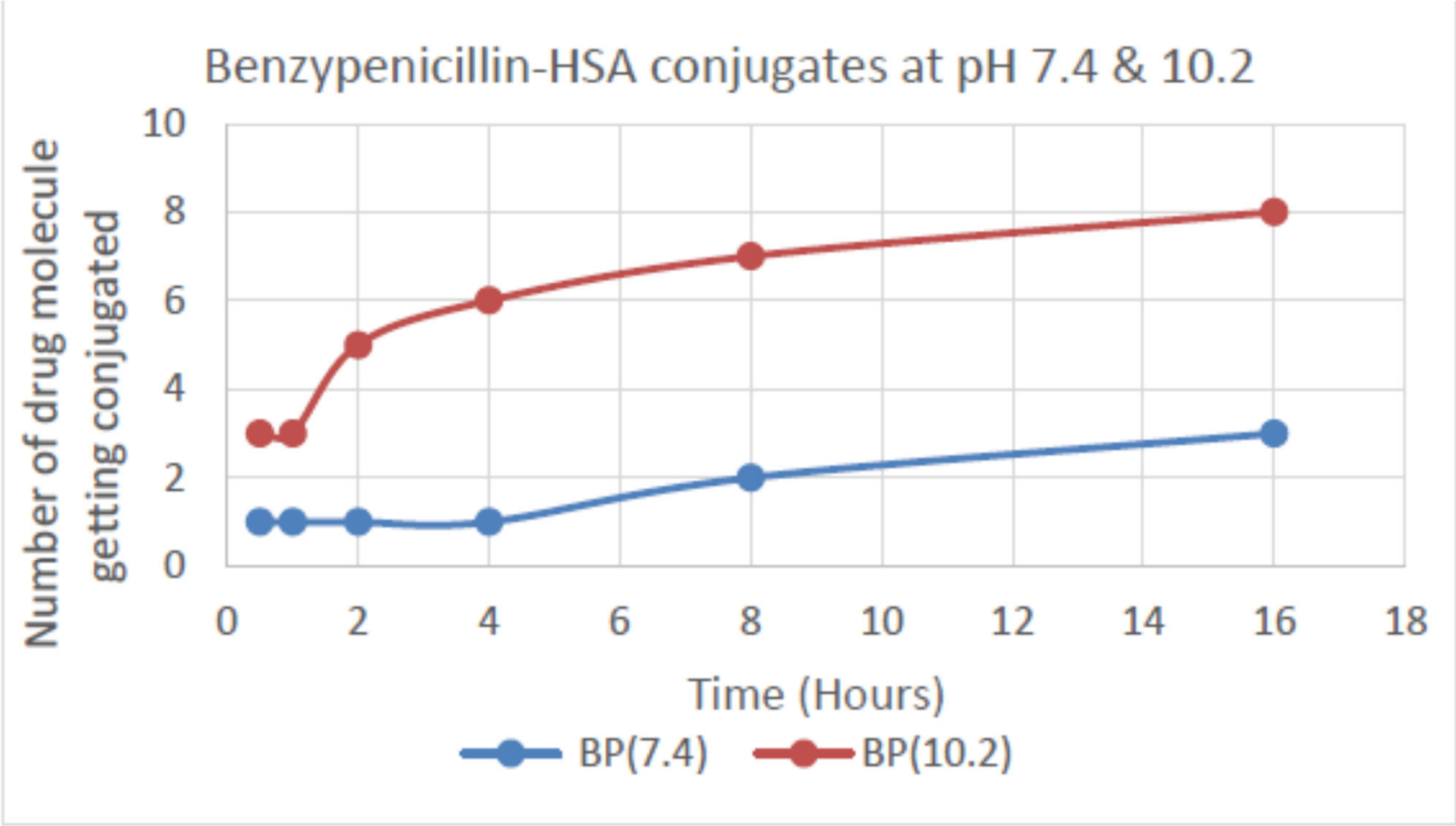
The formation of covalent bonds at pH 7,4 between β-lactams and carrier-protein is **SLOW.** It needs >4 hours (mass-spectrometry)



Sherin Vareeckal-Joseph, PhD thesis, Univ of SA, Adelaide, Australia:
The role of haptenation in penicillin and cephalosporin allergy

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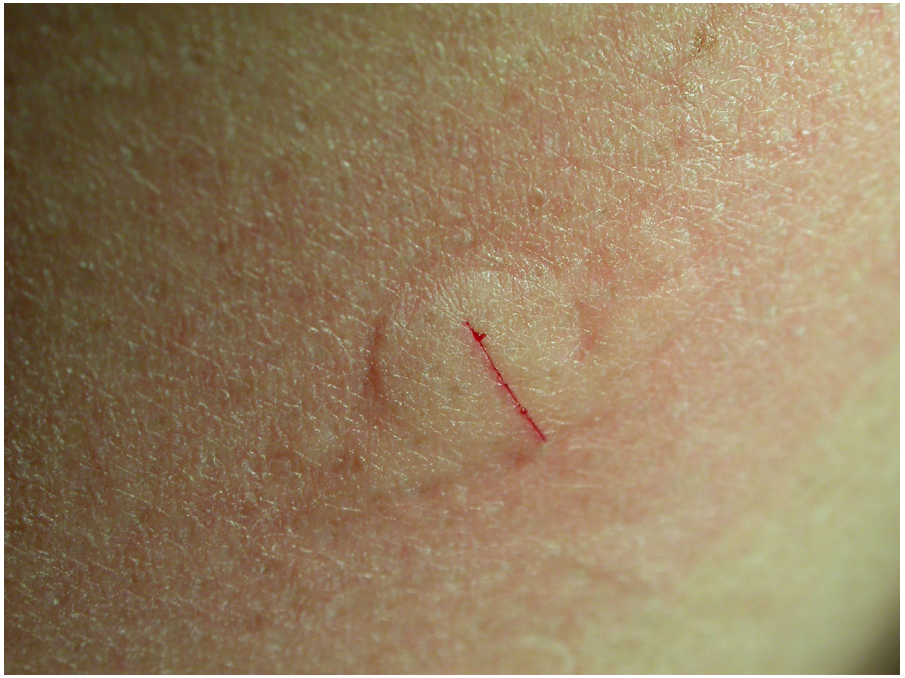
In real life……

Anaphylaxis to a beta-lactam (cefuroxim)

within minutes: -

- Anaphylaxis (cardiac arrest) to i.v. cefuroxim (perioperative, knee operation)
- pos. SPT
- pos. BAT

} IgE

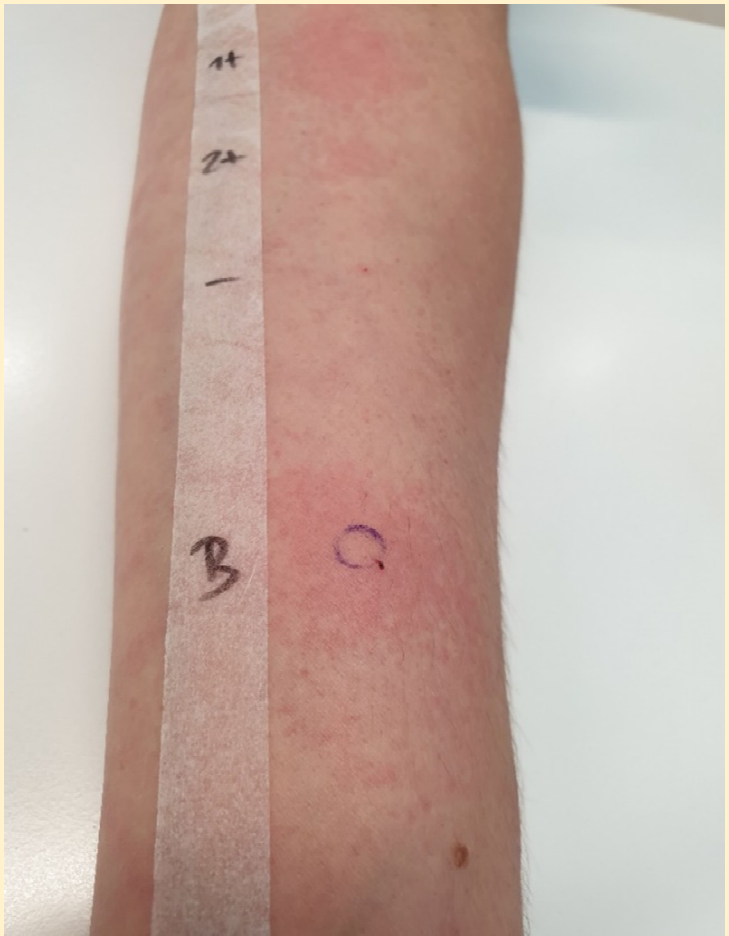


How is it possible that anaphylaxis, skin tests, BAT occur so rapidly (<2-15min), if formation of covalent bonds lasts hours????

→ It must work without covalent bonds!!!

IgE mediated reactions (e.g. anaphylaxis) to «inert» drugs (not haptens)

Acute generalized urticaria & brochospasm (**min**) after i.v. **Sulfamethoxazole** /Trimethoprim (Bactrim^{R}); pos. SPT and direct BAT to Bactrim^{R}



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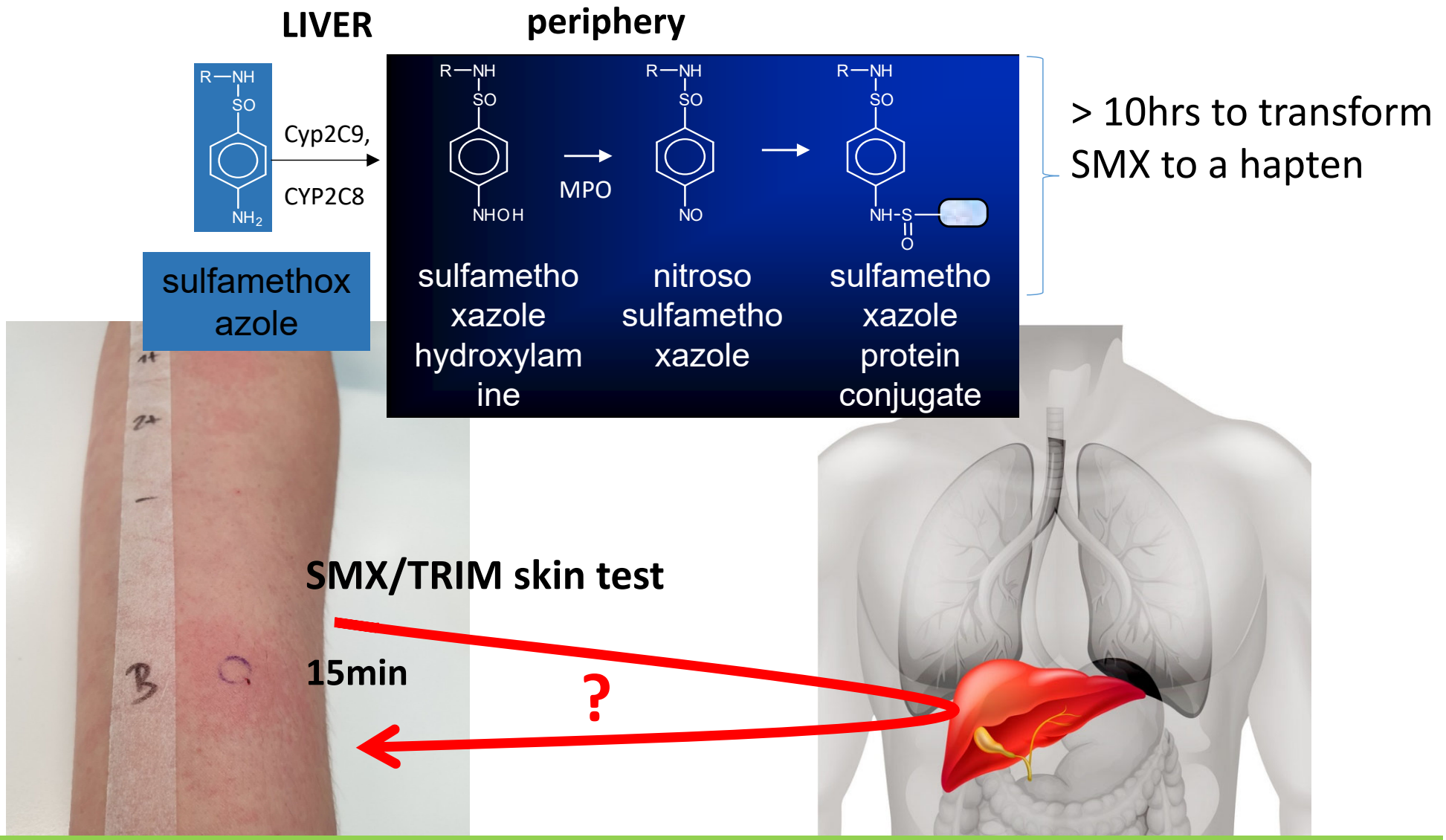
Pat K.-Sch. 1977: urticaria & bronchospasm after Bactrim monotherapy (**Sulfamethoxazole** /Trimetoprim)



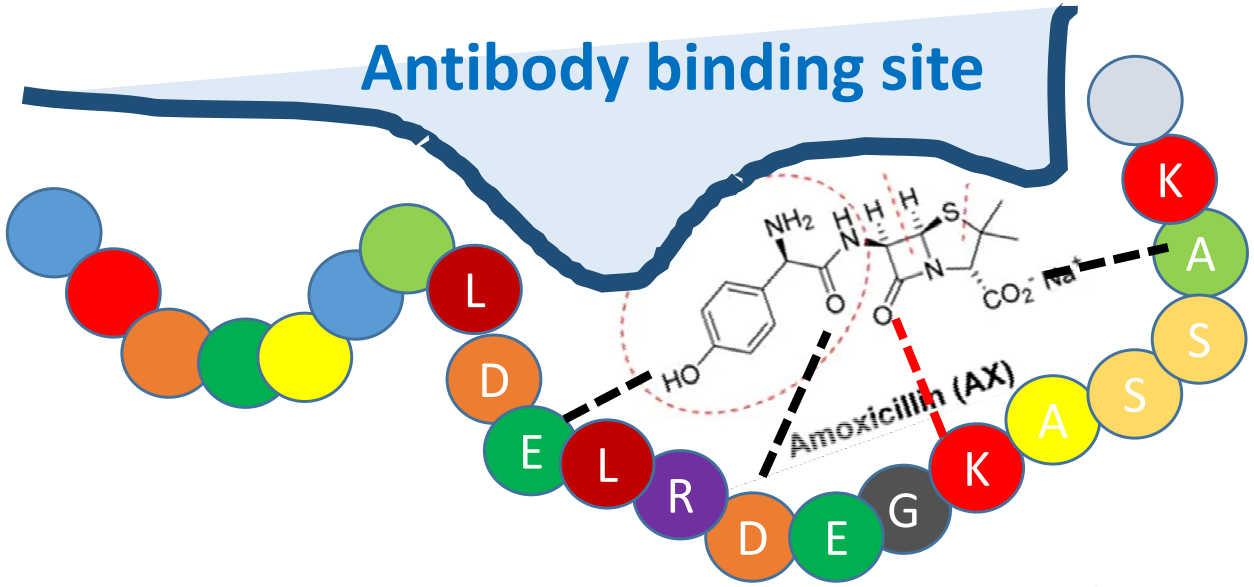
SMX from skin test site to liver and again back as SMX-NHOH/SMX-NO **???**

If one detects a positive skin test after 15min – ***it must be due to SMX itself***

MC bound IgE is interacting with drug-----protein complexes

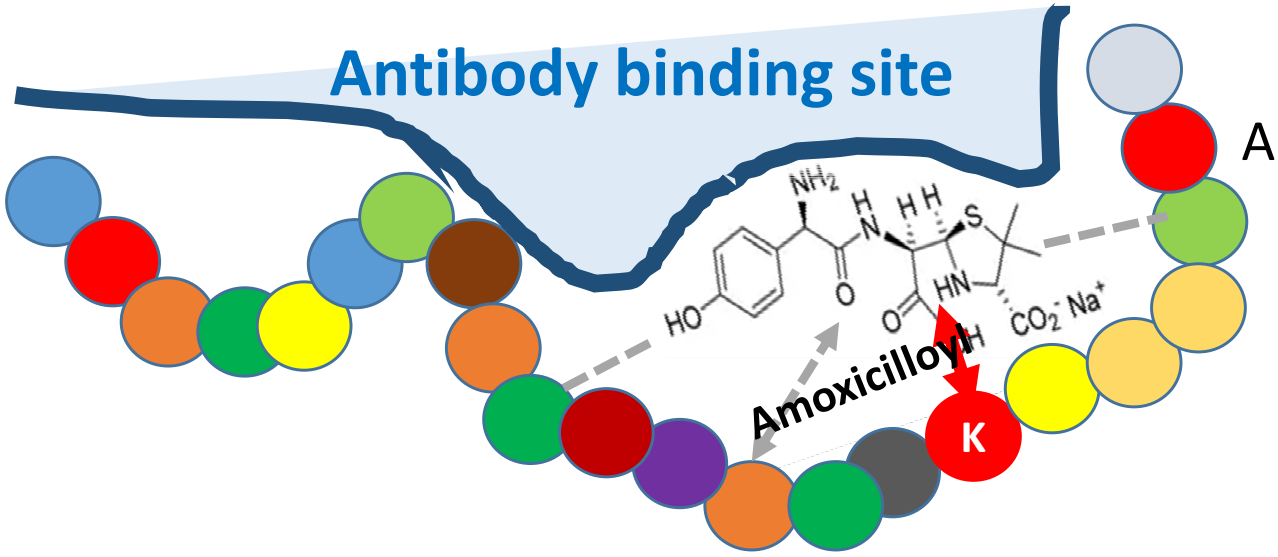
non-covalently

covalently



Amoxicillin (**non-covalent**)

or



Amoxicill**oyl** (**covalent**)

looks identical for the antibody binding site

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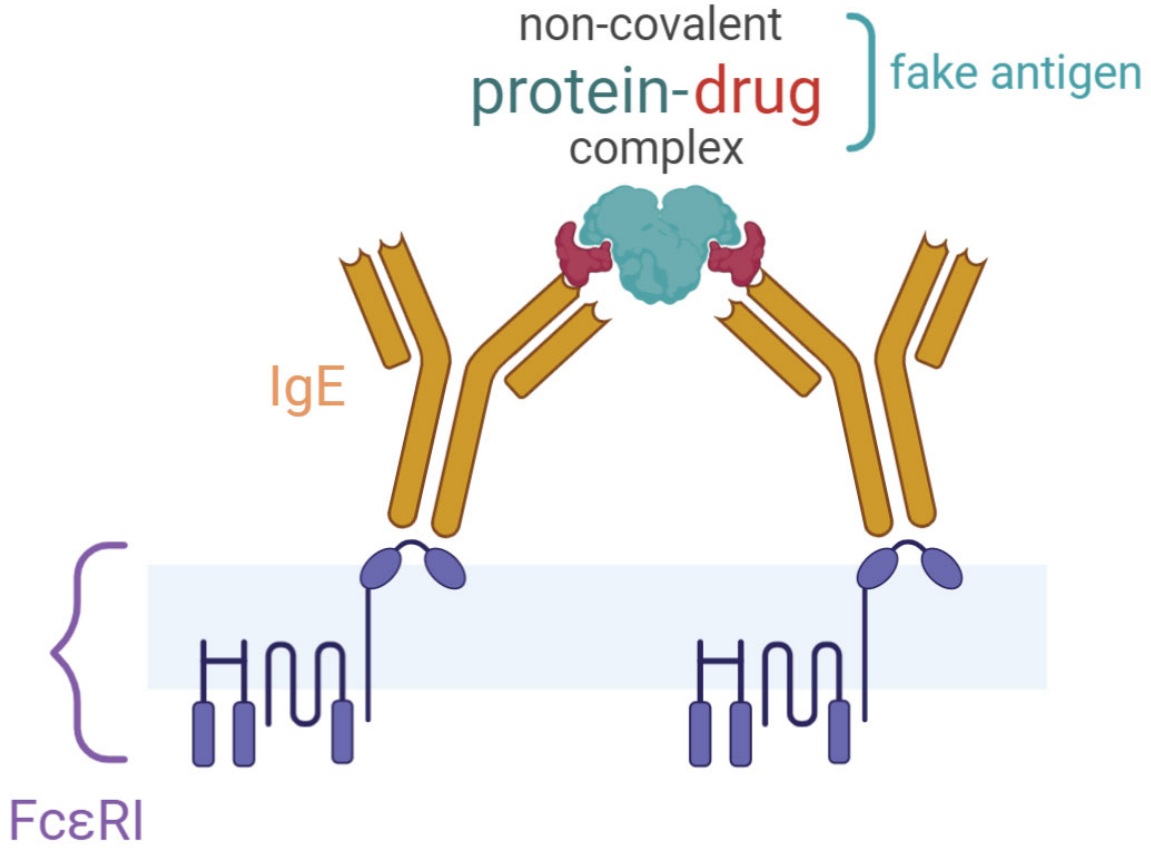
Fake antigen:

- rapidly (immediately, < 1min) formed drug-protein complexes
- this drug-protein complex is based on non-covalent bindings
- They are stable enough to interact with IgE & cross-link IgE bound to Fc-IgE-RI (=„fake antigen“)
- „fake antigen“ are not able to induce IgE
- (all?) immediate allergic reactions to drugs are based on these „fake antigens“ (clinic & tests) !!



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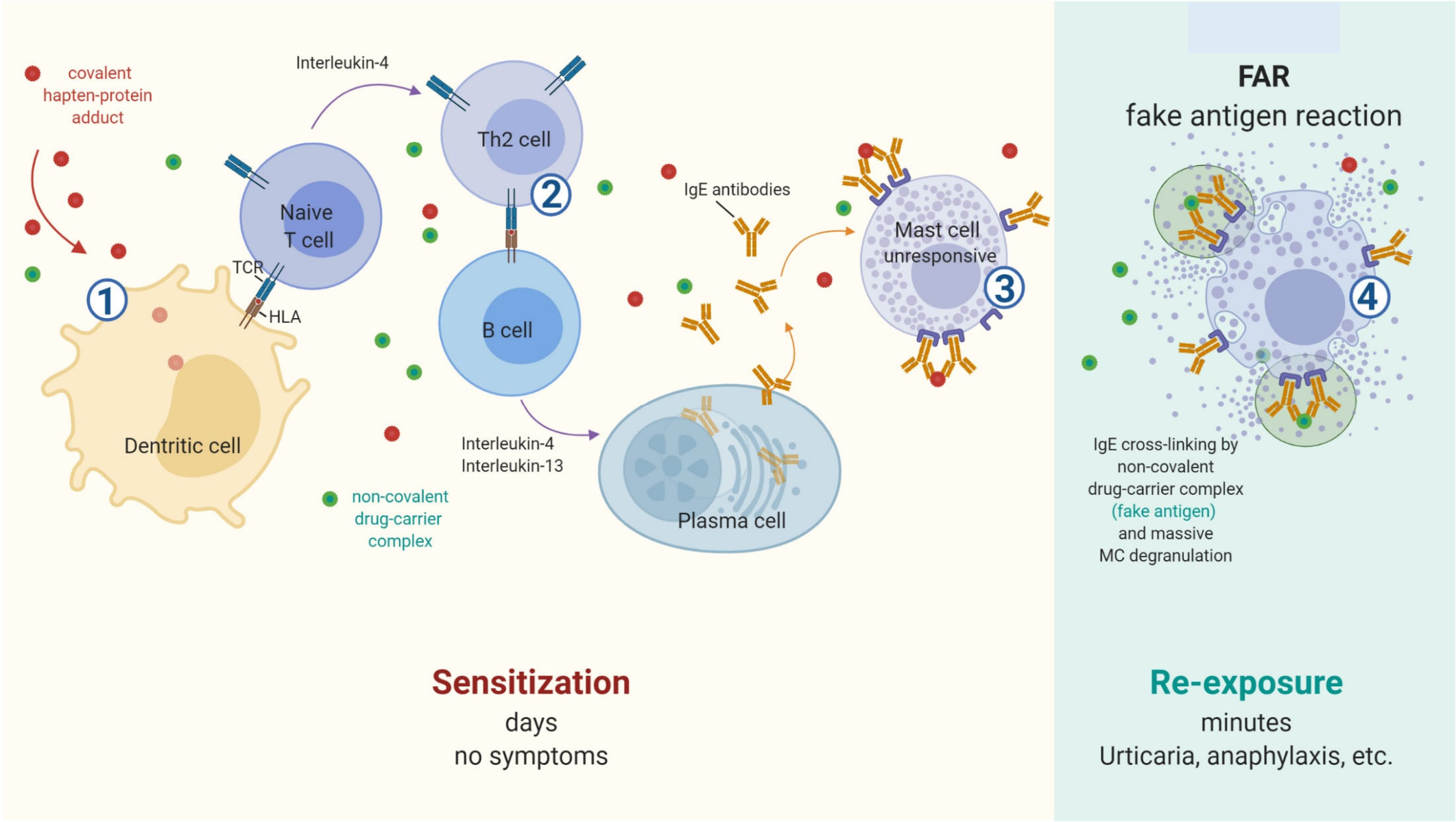
IgE-mediated Hypersensitivity



Pichler WJ. *Anaphylaxis to drugs: overcoming mast cell unresponsiveness by fake antigens. Allergy. 2021 May;76(5):1340-1349*

Overcoming mast cell unresponsiveness by «fake antigen»

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These **fake antigens** (immediately formed, high amounts) encounter IgE-armed MC *fine tuned (tolerant)* for *low* antigen concentrations

1. **Anaphylaxis** to iv (or oral) drugs, which can occur <5min after injection, first signs start <1min….
2. **Positive skin prick tests** (within 15min) to soluble drugs (amoxicillin, cefuroxim, RCM, ……..) *before* stable drug-carrier complexes can be formed in vivo
3. **Positive SPT/i.d. to drugs** (15min) **like SMX**, which need **metabolism** to generate reactive metabolites (SMX-NHOH/SMX-NO, >10hrs). The IgE was induced to the metabolite SMX-NO and is cross-reacting with SMX
4. The positive **in vitro BAT** to drugs, which occur fast, without in vitro metabolism (cefuroxim, SMX, PPI…)
5. **This concept has an enormous impact on:** Risk assessment of drugs, clincal interpretation, cross-reactivity, prevention, diagnosis…..

SPEED

NO METABOLISM

SEVERITY

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REVIEW ARTICLE

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

Anaphylaxis to drugs: Overcoming mast cell unresponsiveness by fake antigens

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Abstract

Our understanding of IgE-mediated drug allergy relies on the hapten concept, which is well established in *inducing adaptive* reactions of the immune system to small molecules like drugs. The role of hapten-carrier adducts in *re-challenge* reactions leading to mast cell degranulation and anaphylaxis is unclear. Based on clinical observations, the speed of adduct formation, skin and in vitro tests to inert drug molecules, a dif-

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(Specific) mast cell unresponsiveness

features & *function*

A normal and natural process supplementing IgE synthesis to an allergen

- Specific (IgE, allergen)
  - reaction by MC to other allergens/IgE not
- very sensitive
  - amount: low - high allergen
    interactions/cross-linking
  - speed of sensing in
    interactions/cr
- purpose: th of MC unresponsiveness
  preven ulation to those allergen
  co , which induced IgE;
  re served: MC reactivity is ready to suddenly
  ased concentrations

When IgE-sensitization occurs, simultaneously the MC/basophils are becoming unresponsive to the allergen (exposed concentration)

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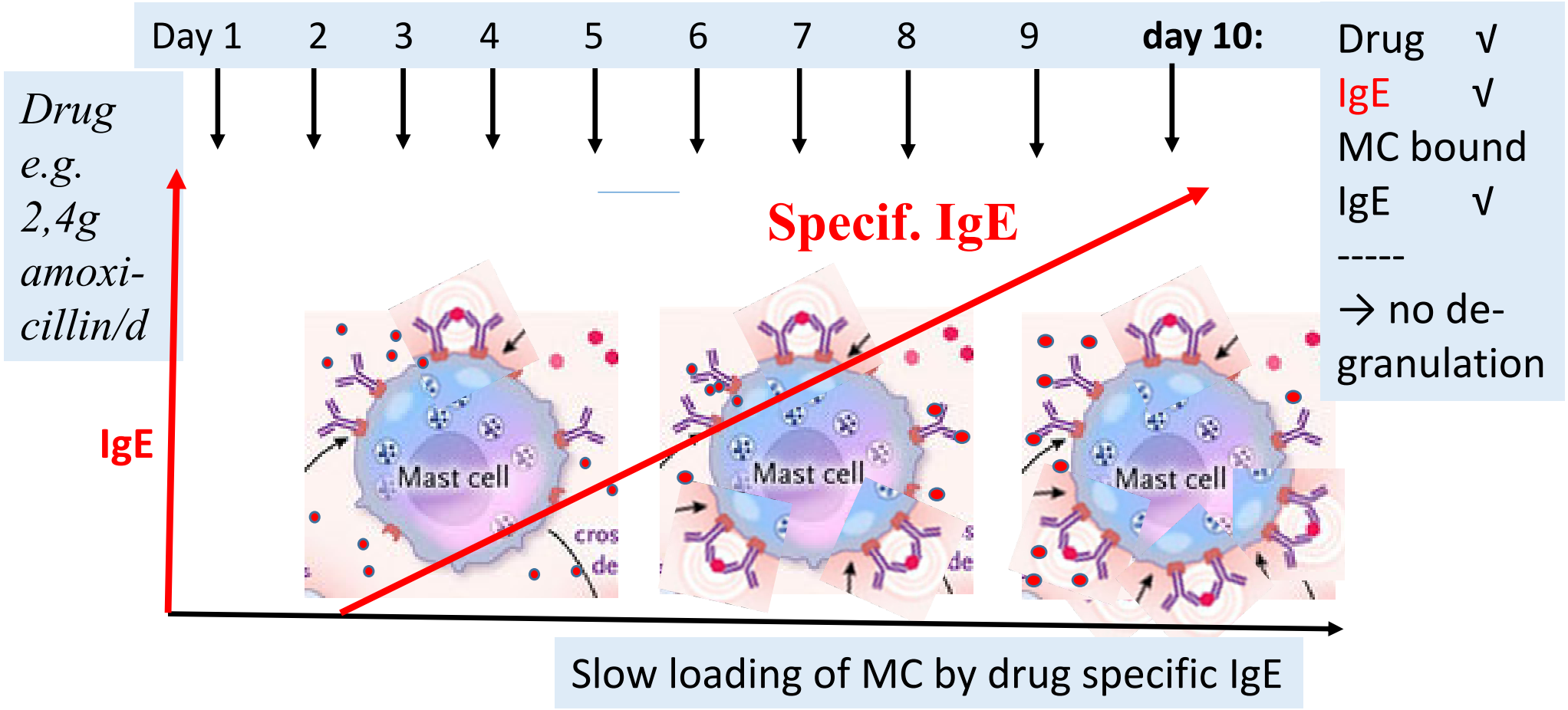
IgE-SENSITIZATION OCCURS IN PRESENCE OF DRUGS:
it leads to **slow** loading of Fc-IgE-RI on MC with IgE & IgE interactions with drug-protein (IgE-immune complexes)



MC unresponsiveness is a natural process supplementing IgE synthesis; it avoids inapprobriate MC degranulation

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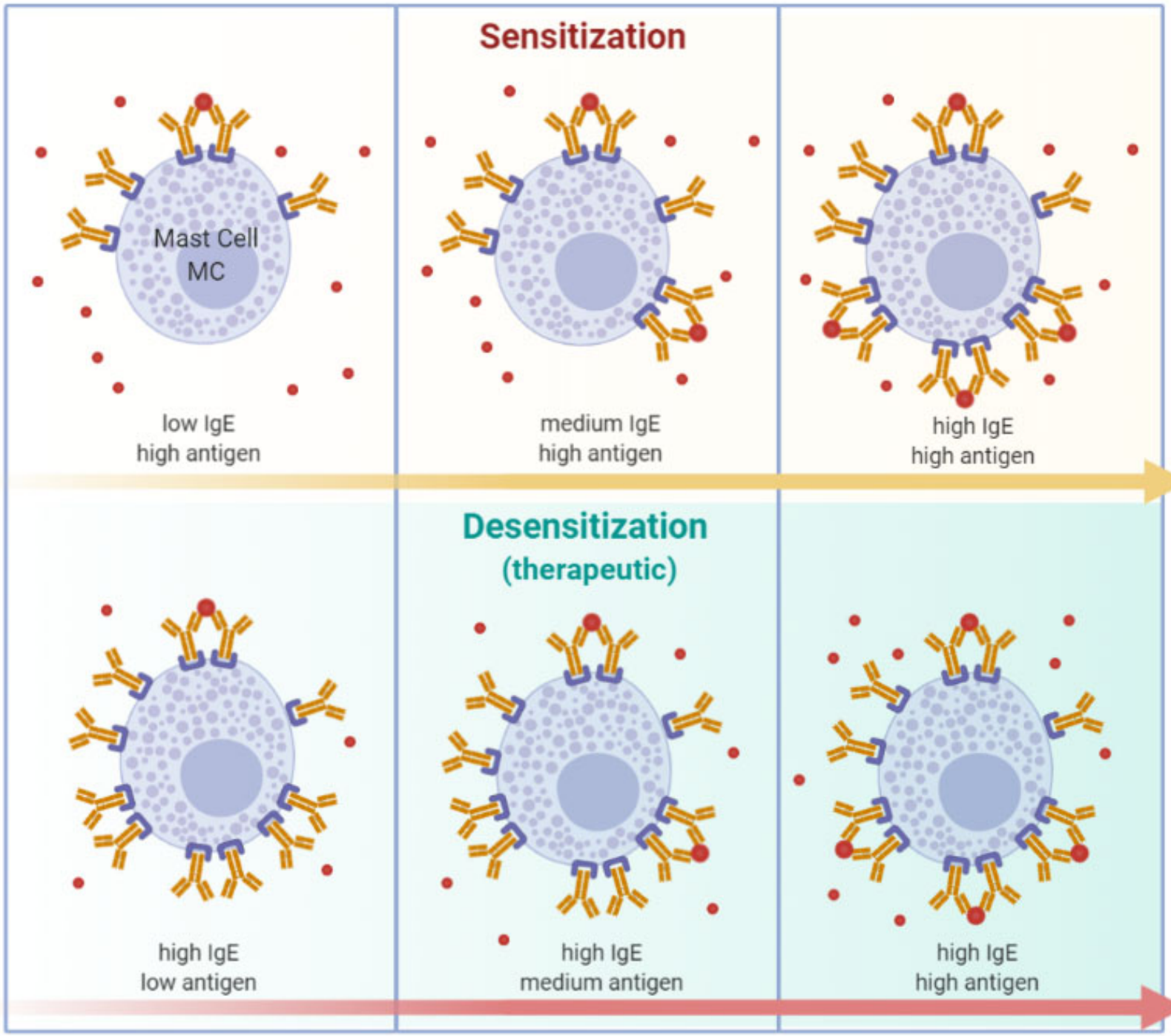

MC unresponsiveness by antigen-IgE-complexes and Fc-IgE-RI-cross-linking is induced

1) in initial sensitization by a gradual increase of IgE

2) in therapeutic desensitization by a gradual increase of antigen

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Examples of mast cell unresponsiveness

NO REACTION IN THE PRESENCE OF IgE & ANTIGEN:

- During **sensitization** (IgE), a **desensitization** of MC reactivity to the drug/potein occurs; example: amoxicillin therapy with IgE synthesis
- **Bee keepers do often carry IgE to bee venom:**
  They react in spring to the first bee stings, after days and some more stings no more: MC reactivity «fine tuned»
- **Rush venom immunotherapy:**
  0.1 µg - 1 µg - 10 µg - 20 µg - 30 µg - 50 µg (50 µg) (>111µg) venom within 3,5 hr. Transient tolerance of 50-100 µg venom in IgE sensitized
- **Drug desensitizations:**
  multiple schemes of drug desensitizations lasting 4-6hrs; necessity to redo desensitization after ca. 4 weeks

(Specific) mast cell unresponsiveness

features & *function*

= A normal and natural process supplementing IgE synthesis to an allergen

- purpose: MC unresponsiveness prevents **systemic** MC degranulation to those systemic allergen concentrations, which induced IgE
- alarm preserved locally: MC reactivity is preserved to higher **local** allergen concentrations

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- Specific (IgE, allergen)
  - reaction by MC to other allergens/IgE not affected
- very sensitive *(allergen/IgE↔Fc-IgE-RI)*
  - amount: sensing low - high allergen concentrations (IgE interactions/cross-linking)
  - speed: sensing speed of increasing drug concentration (IgE interactions/cross-linking)

?, detailed mechanism, purpose

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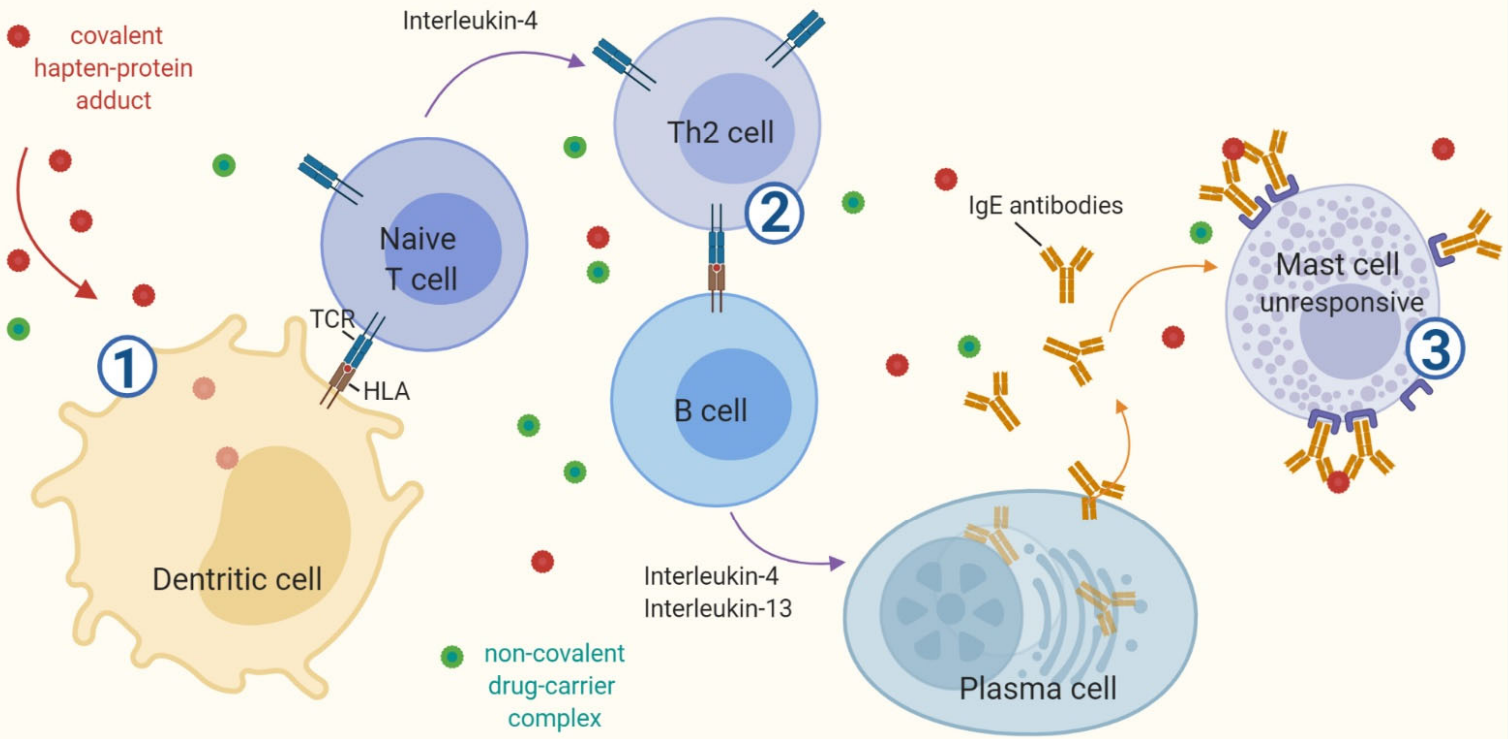
IgE-mediated Hypersensitivity



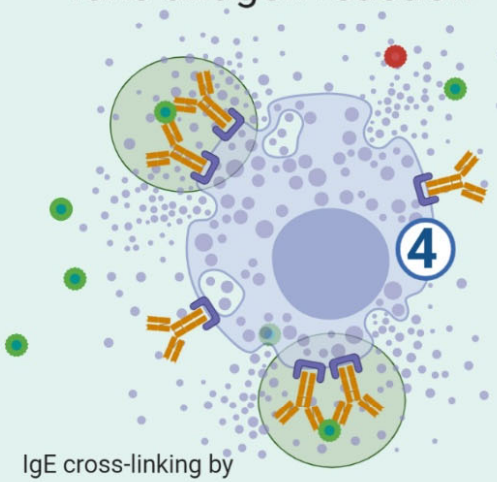
Sensitization

days

no symptoms

FAR

fake antigen reaction



IgE cross-linking by
non-covalent
drug-carrier complex
(fake antigen)
and massive
MC degranulation

Re-exposure

minutes

Urticaria, anaphylaxis, etc.

Explanations/Nomenclature

fake antigen can interact with IgE; they are based on non-covalent binding between drug and protein; such complexes are formed & available rapidly in high amounts

Covalent drug-protein complexes act like „true“ antigen (foreign protein); they are formed slowly and desensitize MC

MC unresponsiveness: during IgE-sensitization MC acquire „tolerance“ to the maximal/last (?) concentration of recognized allergen

It is a specific (IgE-mediated) un-responsiveness of MC (& basophils) to this allergen

The MC can still degranulate to higher concentrations of the same allergen or many other allergens/IgE

What means unresponsive: no degranulation?, or only partly degranulation?

Fine tuning of MC: the allergen concentration during sensitization determines the degree of mast cell (un)responsiveness *in future/in life*

With high allergen concentrations at sensitization, the mast cells become unresponsive to high allergen concentrations: sensitized, but not allergic

If the allergen concentrations during sensitization are low, mast cell unresponsivenss is limited to low concentration alone: degranulation may already occur at medium concentrations of allergen (= allergy)

Thank you

and to my colleagues
Lukas Jörg, Daniel Yerly, Oliver Hausmann, Lester Thoo, Katja Martin, Florian Pichler, Ulrika Axius, Matteo Prezzi for input, discussions & support

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Supplementing information

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Pichler WJ. Immune pathomechanism and classification of drug hypersensitivity. Allergy. 2019 Aug;74(8):1457-1471

Anaphylaxis is due to a sudden increased allergen concentration overcoming mast cell unresponsiveness

Anaphylaxis to drugs:
sensitization is due to true antigen (= covalent drug-protein complexes), which are formed slowly and induce MC-unresponsiveness to only low molar concentrations (compared to free drug).

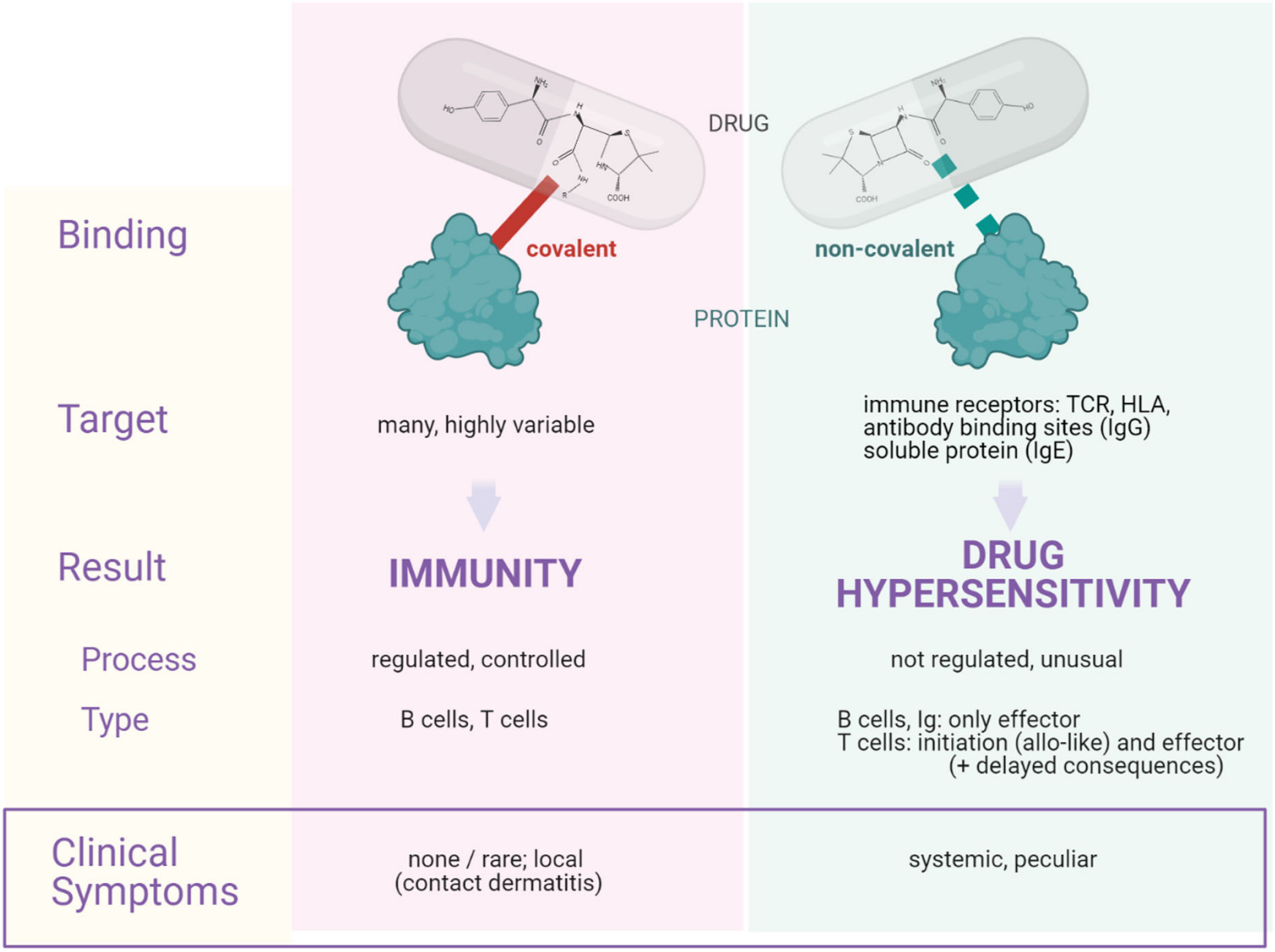
Anaphylaxis occurs due to the formation of **fake antigens:** these are formed rapidly, in high concentrations & are able to crosslink IgE/Fc-IgE-RI: this overcomes mast cell unresponsiveness

Anaphylaxis is due to a sudden increased allergen concentration overcoming mast cell unresponsiveness

Anaphylaxis (allergy) to protein antigens:
the sensitization occurs via small amounts of allergen, namely by percutaneous or respiratory allergen (= protein) uptake: This is sufficient to induce mast cell unresponsiveness to low allergen concentrations only.

Anaphylaxis occurs, when the MC are suddenly exposed to **high** allergen concentrations, as it is achieved by parental or oral allergen uptake: the high concentrations overcome MC unresponsiveness & allergic symptoms/anaphylaxis to protein allergen can occur.

Immune reactions to drugs are determined by drug binding to proteins: how and where



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